SODIUM AND POTASSIUM HYDROXIDES #103
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SODIUM AND POTASSIUM HYDROXIDES

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Rules and Regulations

Title 29—LABOR

Chapter I—National Labor Relations Board

PART 102—RULES AND REGULATIONS, SERIES 8

Subpart I—Service and Filing of Papers

PROOF OF SERVICE

Section 102.112 is amended to read as follows:

§ 102.112 Same; by parties; proof of service.

Service of papers by a party on other parties shall be made by registered mail, or by certified mail, or in any manner provided for the service of papers in a civil action by the law of the State in which the hearing is pending. Except for charges, petitions, exceptions, briefs, and other papers for which a time for both filing and response has been otherwise established, service on all parties shall be made in the same manner as that utilized in filing the paper with the Board, or in a more expeditious manner; however, when filing with the Board is accomplished by personal service the other parties shall be promptly notified of such action by telephone, followed by service of a copy by mail or telegraph. When service is made by registered mail, or by certified mail, the return post office receipt shall be proof of service. When service is made in any manner provided by the law of a State, proof of service shall be made in accordance with such law.

This section shall become effective upon date of publication (8-13-71).

OGDEN W. FIELDS,
Executive Secretary.

[FR Doc.71-11719 Filed 8-12-71;8:50 am]

Chapter XVII—Occupational Safety and Health Administration, Department of Labor

PART 1910—OCCUPATIONAL SAFETY AND HEALTH STANDARDS

Miscellaneous Amendments

On May 29, 1971, occupational safety and health standards were promulgated (36 F.R. 10466). Subsequent review of the standards, and comments and requests for information from the public have pointed up the need for some clarifications, corrections, and changes in effective dates of standards.

Reconsideration of "additional delay in effective date" provisions in various parts of 29 CFR Part 1910 has shown the scope of the provisions is too broad, and includes standards for which no delay is necessary for compliance. The purpose of some of the amendments is to limit the additional delay provisions to the specific standards that need additional time, so that the other standards may become effective sooner. On the other hand, Subpart J of Part 1910 is amended to give for the first time an additional delay to specified provisions.

Section 1910.179(b)(2) has been revised in order to give some time for the modification of existing overhead and gantry cranes, and to reflect accurately the American National Standards Institute (ANSI) standard from which the provision is derived. Similar changes have been made in § 1910.180 (crawler, locomotive, and truck cranes) and in § 1910.181 (derricks), for the same reasons.

Section 1910.217(a)(2) has been revised in order to give 36 months for the modification of presently installed mechanical power presses. The delay is given in the ANSI standard adopted in the section.

Section 1910.93 (air contaminants) has been revised in its entirety, in the interest of greater intelligibility and accuracy.

Several different dates have been changed to August 31, 1971, in order to achieve greater uniformity throughout Part 1910. Other amendments are made to correct mistakes, supply omissions, and to provide greater clarity.

Accordingly, pursuant to authority in section 6(a) and 8(g) of the Williams-Steiger Occupational Safety and Health Act of 1970 (84 Stat. 1593, 1600; 29 U.S.C. 655, 357), 29 CFR Part 1910 is hereby amended as set forth below. The provisions of 5 U.S.C. 553 concerning notice of proposed rule making, public participation therein, and delay in effective date are inapplicable by virtue of the exception to 5 U.S.C. Chapter 5 provided in section 6(a) of the Act. In addition, notice and public procedure are unnecessary because of the nature of the amendments, and impracticable, because of the need to give as much advance notice as possible of the changes before August 27, 1971, when many standards in Part 1910 become effective.

Part 1910 is amended as follows:

1. Section 1910.93 of Subpart G is revised to read as follows:

§ 1910.93 Air contaminants.

An employee's exposure to any material listed in table G-1, G-2, or G-3 of this section shall be limited in accordance with the requirements of the following paragraphs of this section.

(a) Table G-1:

(1) *Materials with names preceded by "C"—Ceiling Values.* An employee's exposure to any material in table G-1, the name of which is preceded by a "C" (e.g., C Boron trifluoride), shall at no time exceed the ceiling value given for that material in the table.

(2) *Other materials—8-hour time weighted averages.* An employee's exposure to any material in table G-1, the name of which is not preceded by "C", in any 8-hour work shift of a 40-hour work week, shall not exceed the 8-hour time weighted average given for that material in the table.

(b) Table G-2:

(1) *8-hour time weighted averages.* An employee's exposure to any material listed in table G-2, in any 8-hour work shift of a 40-hour work week, shall not exceed the 8-hour time weighted average limit given for that material in the table.

(2) *Acceptable ceiling concentrations.* An employee's exposure to a material listed in table G-2 shall not exceed at any time during an 8-hour shift the acceptable ceiling concentration limit given for the material in the table, except for a time period, and up to a concentration not exceeding the maximum duration and concentration allowed in the column under "acceptable maximum peak above the acceptable ceiling concentration for an 8-hour shift".

(3) *Example.* During an 8-hour work shift, an employee may be exposed to a concentration of Benzene above 25 p.p.m. (but never above 50 p.p.m.) only for a maximum period of 10 minutes. Such exposure must be compensated by exposures to concentrations less than 10 p.p.m. so that the cumulative exposure for the entire 8-hour work shift does not exceed a weighted average of 10 p.p.m.

(c) Table G-3: An employee's exposure to any material listed in table G-3, in any 8-hour work shift of a 40-hour work week, shall not exceed the 8-hour time weighted average limit given for that material in the table.

(d) Computation formulae:

(1) (i) The cumulative exposure for an 8-hour work shift shall be computed as follows:

$$E=\frac{C_aT_a+C_bT_b+\ \ldots\ C_nT_n}{8}$$

where:

E is the equivalent exposure for the working shift.

C is the concentration during any period of time T where the concentration remains constant.

T is the duration in hours of the exposure at the concentration C.

The value of E shall not exceed the 8-hour time weighted average limit in table G-1, G-2, or G-3 for the material involved.

(ii) To illustrate the formula prescribed in subdivision (i) of this subparagraph, note that isomyl acetate has an 8-hour time weighted average limit of 100 p.p.m. (table G-1). Assume that an

employee is subject to the following exposure:

Two hours exposure at 150 p.p.m.
Two hours exposure at 75 p.p.m.
Four hours exposure at 50 p.p.m.

Substituting this information in the formula, we have

$$\frac{2\times150+2\times75+4\times50}{8}=81 \text{ p.p.m.}$$

Since 81 p.p.m. is less than eight, the 8-hour time weighted average limit, the exposure is acceptable.

(2) (i) In case of a mixture of air contaminants an employer shall compute the equivalent exposure as follows:

$$E_m=\frac{C_1}{L_1}+\frac{C_2}{L_2}+\ldots\frac{C_n}{L_n}$$

Where:

E_m is the equivalent exposure for the mixture.

C is the concentration of a particular contaminant.

L is the exposure limit for that contaminant, from table G-1, G-2, or G-3.

The value of E_m shall not exceed unity (1).

(ii) To illustrate the formula prescribed in subdivision (i) of this subparagraph, consider the following exposures:

Material	Actual concentration of 8-hour exposure	8-hour time weighted average exposure limit
Acetone (Table G-1)	500 p.p.m.	1,000 p.p.m.
2-Butanone (Table G-1)	45 p.p.m.	200 p.p.m.
Toluene (Table G-2)	40 p.p.m.	200 p.p.m.

Substituting in the formula, we have:

$$E_m=\frac{500}{1,000}+\frac{45}{200}+\frac{40}{200}$$

$$E_m=0500+0225+0200$$

$$E_m=0925$$

Since E_m is less than unity (1), the exposure combination is within acceptable limits.

(e) To achieve compliance with paragraph (a) through (d) of this section, administrative or engineering controls must first be determined and implemented whenever feasible. When such controls are not feasible to achieve full compliance, protective equipment or any other protective measures shall be used to keep the exposure of employees to air contaminants within the limits prescribed in this section. Any equipment and technical measure use for this purpose must be approved for each particular use by a competent industrial hygienist or other technically qualified person. Whenever respirators are used, their use shall comply with § 1913.134 of this chapter.

TABLE G-1

Substance	p.p.m.[a]	mg./M³ [b]
Acetaldehyde	200	360
Acetic acid	10	25
Acetic anhydride	5	20
Acetone	1,000	2,400
Acetonitrile	40	70
Acetylene dichloride, see 1, 2-Dichloroethylene		
Acetylene tetrabromide	1	14
Acrolein	0.1	0.25
Acrylamide—Skin		0.3
Acrylonitrile—Skin	20	45
Aldrin—Skin		0.25
Allyl alcohol—Skin	2	5
Allyl chloride	1	3
**C Allylglycidyl ether (AGE)	10	45
Allyl propyl disulfide	2	12
2-Aminoethanol, see Ethanolamine		
2-Aminopyridine	0.5	2
**Ammonia	50	35
Ammonium sulfamate (Ammate)		15
n-Amyl acetate	100	525
sec-Amyl acetate	125	650
Aniline—Skin	5	19
Anisidine (o, p-isomers)—Skin		0.5
Antimony and compounds (as Sb)		0.5
ANTU (alpha naphthyl thiourea)		0.3
Arsenic and compounds (as As)		0.5
Arsine	0.05	0.2
Azinphos-methyl—Skin		0.2
Barium (soluble compounds)		0.5
p-Benzoquinone, see Quinone		
Benzoyl peroxide		5
Benzyl chloride	1	5
Biphenyl, see Diphenyl		
Bisphenol A, see Diglycidyl ether		
Boron oxide		15
C Boron trifluoride	1	3
Bromine	0.1	0.7
Bromoform—Skin	0.5	5
Butadiene (1, 3-butadiene)	1,000	2 200
Butanethiol, see Butyl mercaptan		
2-Butanone	200	590
2-Butoxy ethanol (Butyl Cellosolve)—Skin	50	240
Butyl acetate (n-butyl acetate)	150	710
sec-Butyl acetate	200	950
tert-Butyl acetate	200	950
Butyl alcohol	100	300
sec-Butyl alcohol	150	450
tert-Butyl alcohol	100	300
C Butylamine—Skin	5	15
C tert-Butyl chromate (as CrO_3)—Skin		0.1
n-Butyl glycidyl ether (BGE)	50	270
*Butyl mercaptan	10	35
p-tert-Butyltoluene	10	60
Calcium arsenate		1
Calcium oxide		5
*Camphor	2	
Carbaryl (Sevin®)		5
Carbon black		3.5
Carbon dioxide	5,000	9,000
Carbon monoxide	50	55
Chlordane—Skin		0.5
Chlorinated camphene—Skin		0.5
Chlorinated diphenyl oxide		0.5
*Chlorine	1	3
Chlorine dioxide	0.1	0.3
C Chlorine trifluoride	0.1	0.4
C Chloroacetaldehyde	1	3
α-Chloroacetophenone (phenacylchloride)	0.05	0.3
Chlorobenzene (monochlorobenzene)	75	350
o-Chlorobenzylidene malononitrile (OCBM)	0.05	0.4
Chlorobromomethane	200	1,050
2-Chloro-1,3-butadiene, see Chloroprene		
Chlorodiphenyl (42 percent Chlorine)—Skin		1
Chlorodiphenyl (54 percent Chlorine)—Skin		0.5
1-Chloro,2,3-epoxypropane, see Epichlorhydrin		
2-Chloroethanol, see Ethylene chlorohydrin		
Chloroethylene, see Vinyl choride		
C Chloroform (trichloromethane)	50	240
1-Chloro-1-nitropropane	20	100
Chloropicrin	0.1	0.7
Chloroprene (2-chloro-1,3-butadiene)—Skin	25	90

TABLE G-1—Continued

Substance	p.p.m.[a]	mg./M³ [b]
Chromium, sol. chromic, chromous salts as Cr		0.5
Metal and insol. salts		1
Coal tar pitch volatiles (benzene soluble fraction) anthracene, BaP, phenanthrene, acridine, chrysene, pyrene		0.2
Cobalt, metal fume and dust		0.1
Copper fume		0.1
Dusts and Mists		1
Cotton dust (raw)		1
Crag® herbicide		15
Cresol (all isomers)—Skin	5	22
Crotonaldehyde	2	6
Cumene—Skin	50	245
Cyanide (as CN)—Skin		5
Cyclohexane	300	1,050
Cyclohexanol	50	200
Cyclohexanone	50	200
Cyclohexene	300	1,015
Cyclopentadiene	75	200
2, 4-D		10
DDT—Skin		1
DDVP, see Dichlorvos		
Decaborane—Skin	0.05	0.3
Demeton®—Skin		0.1
Diacetone alcohol (4-hydroxy-4-methyl-2-pentanone)	50	240
1,2-diaminoethane, see Ethylenediamine		
Diazomethane	0.2	0.4
Diborane	0.1	0.1
Dibutylphthalate		5
C o-Dichlorobenzene	50	300
p-Dichlorobenzene	75	450
Dichlorodifluoromethane	1,000	4,950
1,3-Dichloro-5,5-dimethyl hydantoin		0.2
1,1-Dichloroethane	100	400
1,2-Dichloroethylene	200	790
C Dichloroethyl ether—Skin	15	90
Dichloromethane, see Methylenechloride		
Dichloromonofluoromethane	1,000	4,200
C 1,1-Dichloro-1-nitroethane	10	60
1,2-Dichloropropane, see Propylenedichloride		
Dichlorotetrafluoroethane	1,000	7,000
Dichlorvos (DDVP)—Skin		1
Dieldrin—Skin		0.25
Diethylamine	25	75
Diethylamino ethanol—Skin	10	50
Diethylether, see Ethyl ether		
Difluorodibromomethane	100	860
C Diglycidyl ether (DGE)	0.5	2.8
Dihydroxybenzene, see Hydroquinone		
Diisobutyl ketone	50	290
Diisopropylamine—Skin	5	20
Dimethoxymethane, see Methylal		
Dimethyl acetamide—Skin	10	35
Dimethylamine	10	18
Dimethylaminobenzene, see Xylidene		
Dimethylaniline(N-dimethylaniline)—Skin	5	25
Dimethylbenzene, see Xylene		
Dimethyl 1,2-dibromo-2,2-dichloroethyl phosphate, (Dibrom)		3
Dimethylformamide—Skin	10	30
2,6-Dimethylheptanone, see Diisobutyl ketone		
1,1-Dimethylhydrazine—Skin	0.5	1
Dimethylphthalate		5
Dimethylsulfate—Skin	1	5
Dinitrobenzene (all isomers)—Skin		1
Dinitro-o-cresol—Skin		0.2
Dinitrotoluene—Skin		1.5
Dioxane (Diethylene dioxide)—Skin	100	360
Diphenyl	0.2	1
Diphenylmethane diisocyanate (see Methylene bisphenyl isocyanate (MDI)		
Dipropylene glycol methyl ether—Skin	100	600
Di-sec, octyl phthalate (Di-2-ethylhexylphthalate)		5
Endrin—Skin		0.1
Epichlorhydrin—Skin	5	19
EPN—Skin		0.5
1,2-Epoxypropane, see Propyleneoxide		
2,3-Epoxy-1-propanol, see Glycidol		
Ethanethiol, see Ethylmercaptan		

See footnotes at end of table.

TABLE G-I—Continued

Substance	p.p.m.[a]	mg./M³ [b]
Ethanolamine	3	6
2-Ethoxyethanol—Skin	200	740
2-Ethoxyethylacetate (Cellosolve acetate)—Skin	100	540
Ethyl acetate	400	1,400
Ethyl acrylate—Skin	25	100
Ethyl alcohol (ethanol)	1,000	1,900
Ethylamine	10	18
Ethyl sec-amyl ketone (5-methyl-3-heptanone)	25	130
Ethyl benzene	100	435
Ethyl bromide	200	890
Ethyl butyl ketone (3-Heptanone)	50	230
Ethyl chloride	1,000	2,600
Ethyl ether	400	1,200
Ethyl formate	100	300
C Ethyl mercaptan	10	25
Ethyl silicate	100	850
Ethylene chlorohydrin—Skin	5	16
Ethylenediamine	10	26
Ethylene dibromide, see 1,2-Dibromoethane		
Ethylene dichloride, see 1,2-Dichloroethane		
C Ethylene glycol dinitrate and/or Nitroglycerin—Skin	[d] 0.2	1
Ethylene glycol monomethyl ether acetate, see Methyl cellosolve acetate		
Ethylene imine—Skin	0.5	1
Ethylene oxide	50	90
Ethylidine chloride, see 1,1-Dichloroethane		
N-Ethylmorpholine—Skin	20	94
Ferbam		15
Ferrovanadium dust		1
Fluoride (as F)		2.5
Fluorine	0.1	0.2
Fluorotrichloromethane	1,000	5,600
Formic acid	5	9
Furfural—Skin	5	20
Furfuryl alcohol	50	200
Glycidol (2,3-Epoxy-1-propanol)	50	150
Glycol monoethyl ether, see 2-Ethoxyethanol		
[illegible]ion, ® see Azinphos-		
[illegible]pyl		0.5
[illegible]um		0.5
[illegible]chlor—Skin		
[illegible]ptane (n-heptane)	500	2,000
Hexachloroethane—Skin	1	10
Hexachloronaphthalene—Skin		0.2
Hexane (n-hexane)	500	1,800
2-Hexanone	100	410
Hexone (Methyl isobutyl ketone)	100	410
sec-Hexyl acetate	50	300
Hydrazine—Skin	1	1.3
Hydrogen bromide	3	10
C Hydrogen chloride	5	7
Hydrogen cyanide—Skin	10	11
Hydrogen peroxide (90%)	1	1.4
Hydrogen selenide	0.05	0.2
Hydroquinone		2
C Iodine	0.1	1
Iron oxide fume		10
Isoamyl acetate	100	525
Isoamyl alcohol	100	360
Isobutyl acetate	150	700
Isobutyl alcohol	100	300
Isophorone	25	140
Isopropyl acetate	250	950
Isopropyl alcohol	400	980
Isopropylamine	5	12
Isopropylether	500	2,100
Isopropyl glycidyl ether (IGE)	50	240
Ketene	0.5	0.9
Lead arsenate		0.15
Lindane—Skin		0.5
Lithium hydride		0.025
L.P.G. (liquified petroleum gas)	1,000	1,800
Magnesium oxide fume		15
Malathion—Skin		15
Maleic anhydride	0.25	1
C Manganese		5
Mesityl oxide	25	100
Methanethiol, see Methyl mercaptan		
Methoxychlor		15
2-Methoxyethanol, see Methyl cellosolve		
Methyl acetate	200	610
Methyl acetylene (propyne)	1,000	1,650
Methyl acetylene-propadiene mixture (MAPP)	1,000	1,800
Methyl acrylate—Skin	10	35
Methylal (dimethoxymethane)	1,000	3,100
Methyl alcohol (methanol)	200	260
Methylamine	10	12
[illegible]yl amyl [illegible], see [illegible]thyl isobutyl carbinol		

TABLE G-I—Continued

Substance	p.p.m.[a]	mg./M³ [b]
Methyl (n-amyl) ketone (2-Heptanone)	100	465
C Methyl bromide—Skin	20	80
Methyl butyl ketone, see 2-Hexanone		
Methyl cellosolve—Skin	25	80
Methyl cellosolve acetate—Skin	25	120
Methyl chloroform	350	1,900
Methylcyclohexane	500	2,000
Methylcyclohexanol	100	470
o-Methylcyclohexanone—Skin	100	460
Methyl ethyl ketone (MEK), see 2-Butanone		
Methyl formate	100	250
Methyl iodide—Skin	5	28
Methyl isobutyl carbinol—Skin	25	100
Methyl isobutyl ketone, see Hexone		
Methyl isocyanate—Skin	0.02	0.05
C Methyl mercaptan	10	20
Methyl methacrylate	100	410
Methyl propyl ketone, see 2-Pentanone		
C α Methyl styrene	100	480
C Methylene bisphenyl isocyanate (MDI)	0.02	0.2
Molybdenum:		
Soluble compounds		5
Insoluble compounds		15
Monomethyl aniline—Skin	2	9
C Monomethyl hydrazine—Skin	0.2	0.35
Morpholine—Skin	20	70
Naphtha (coaltar)	100	400
Naphthalene	10	50
Nickel carbonyl	0.001	0.007
Nickel, metal and soluble cmpds, as Ni		1
Nicotine—Skin		0.5
Nitric acid	2	5
Nitric oxide	25	30
p-Nitroaniline—Skin	1	6
Nitrobenzene—Skin	1	5
p-Nitrochlorobenzene—Skin		1
Nitroethane	100	310
Nitrogen dioxide	5	9
Nitrogen trifluoride	10	29
Nitroglycerin—Skin	0.2	2
Nitromethane	100	250
1-Nitropropane	25	90
2-Nitropropane	25	90
Nitrotoluene—Skin	5	30
Nitrotrichloromethane, see Chloropicrin		
Octachloronaphthalene—Skin		0.1
*Octane	500	2,350
*Oil mist, mineral		*5
Osmium tetroxide		0.002
Oxalic acid		1
Oxygen difluoride	0.05	0.1
Ozone	0.1	0.2
Paraquat—Skin		0.5
Petrole m distillates (naptha)	500	2,000
Propane	1,000	1,800
Silver, metal and soluble compounds		0.01
Sodium fluoroacetate (1080)—Skin		0.05
Sodium hydroxide		2
Stibine	0.1	0.5
*Stoddard solvent	500	2,950
Strychnine		0.15
Sulfur dioxide	5	13
Sulfur hexafluoride	1,000	6,000
Sulfuric acid		1
Sulfur monochloride	1	6
Sulfur pentafluoride	0.025	0.25
Sulfuryl fluoride	5	20
Systox, see Demeton ®		
2,4,5T		10
Tantalum		5
TEDP—Skin		0.2
Tellurium		0.1
Tellurium hexafluoride	0.02	0.2
TEPP—Skin		0.05
C Terphenyls	1	9
1,1,1,2-Tetrachloro-2,2-difluoroethane	500	4,170
1,1,2,2-Tetrachloro-1,2-difluoroethane	500	4,170
1,1,2,2-Tetrachloroethane—Skin	5	35
Tetrachloroethylene, see Perchloroethylene		
Tetrachloromethane, see Carbon tetrachloride		
Tetrachloronaphthalene—Skin		2
Tetraethyl lead (as Pb)—Skin		0.075
Tetrahydrofuran	200	590
Tetramethyl lead (as Pb)—Skin		0.075
Tetramethyl succinonitrile—Skin	0.5	3
Tetranitromethane	1	8

TABLE G-I—Continued

Substance	p.p.m.[a]	mg./M³ [b]
Tetryl (2,4,6-trinitrophenylmethylnitramine)—Skin		1.5
Thallium (soluble compounds)—Skin as Tl		0.1
Thiram		5
Tin (inorganic cmpds, except oxides		2
Tin (organic cmpds)		0.1
C Toluene-2,4-diisocyanate	0.02	0.14
o-Toluidine—Skin	5	22
Toxaphene, see Chlorinated camphene		
Tributyl phosphate		5
1,1,1-Trichloroethane see Methyl chloroform		
1,1,2-Trichloroethane—Skin	10	45
Parathion—Skin		0.1 1
Pentaborane	0.005	0.0
Pentachloronaphthalene—Skin		0.5
Pentachlorophenol—Skin		0.5
*Pentane	1,000	2,950
2-Pentanone	200	700
Perchloromethyl mercaptan	0.1	0.8
Perchloryl fluoride	3	13.5
Phenol—Skin	5	19
p-Phenylene diamine—Skin		0.1
Phenyl ether (vapor)	1	7
Phenyl ether-biphenyl mixture (vapor)	1	7
Phenylethylene, see Styrene		
Phenyl glycidyl ether (PGE)	10	60
Phenylhydrazine—Skin	5	22
Phosdrin (Mevinphos ®)—Skin		0.1
Phosgene (carbonyl chloride)	0.1	0.4
Phosphine	0.3	0.4
Phosphoric acid		1
Phosphorus (yellow)		0.1
Phosphorus pentachloride		1
Phosphorus pentasulfide		1
Phosphorus trichloride	0.5	3
Phthalic anhydride	2	12
Picric acid—Skin		0.1
Pival ® (2-Pivalyl-1,3-indandione)		0.1
Platinum (Soluble Salts) as Pt		0.002
Propargyl alcohol—Skin	1	
n-Propyl acetate	200	840
Propyl alcohol	200	500
n-Propyl nitrate	25	110
Propylene dichloride	75	350
Propylene imine—Skin	2	5
Propylene oxide	100	240
Propyne, see Methylacetylene		
Pyrethrum		5
Pyridine	5	15
Quinone	0.1	0.4
RDX—Skin		1.5
Rhodium, Metal fume and dusts, as Rh		0.1
Soluble salts		0.001
Ronnel		10
Rotenone (commercial)		5
Selenium compounds (as Se)		0.2
Selenium hexafluoride	0.05	0.4
Titaniumdioxide		15
Trichloromethane, see Chloroform		
Trichloronaphthalene—Skin		5
1,2,3-Trichloropropane	50	300
1,1,2-Trichloro 1,2,2-trifluoroethane	1,000	7,600
Triethylamine	25	100
Trifluoromonobromomethane	1,000	6,100
2,4,6-Trinitrophenol, see Picric acid		
2,4,6-Trinitrophenylmethylnitramine, see Tetryl		
Trinitrotoluene—Skin		1.5
Triorthocresyl phosphate		0.1
Triphenyl phosphate		3
Turpentine	100	560
Uranium (soluble compounds)		0.05
Uranium (insoluble compounds)		0.25
C Vanadium:		
V_2O_5 dust		0.5
V_2O_5 fume		0.1
Vinyl benzene, see Styrene		
**C Vinyl chloride	500	1,300
Vinylcyanide, see Acrylonitrile		
Vinyl toluene	100	480
Warfarin		0.1
Xylene (xylol)	100	435
Xylidine—Skin	5	25
Yttrium		1
Zinc chloride fume		1
Zinc oxide fume		5
Zirconium compounds (as Zr)		5

See footnotes at end of table.

*1970 Addition.

[a] Parts of vapor or gas per million parts of contaminated air by volume at 25° C. and 760 mm. Hg pressure.

[b] Approximate milligrams of particulate per cubic meter of air.

(No footnote "c" is used to avoid confusion with ceiling value notations.)

[d] An atmospheric concentration of not more than 0.02 p.p.m., or personal protection may be necessary to avoid headache.

[e] As sampled by method that does not collect vapor.

[f] For control of general room air, biologic monitoring is essential for personnel control.

TABLE G-2

Material	8-hour time weighted average	Acceptable ceiling concentration	Acceptable maximum peak above the acceptable ceiling concentration for an 8-hour shift.	
			Concentration	Maximum duration
Benzene (Z37.4-1969)	10 p.p.m	25 p.p.m	50 p.p.m	10 minutes.
Beryllium and beryllium compounds (Z37.29-1970).	2 μg./M³	5 μg./M³	25 μg./M³	30 minutes.
Cadmium fume (Z37.5-1970)	0.1 mg./M³	3 mg./M³		
Cadmium dust (Z37.5-1970)	0.2 mg./M³	0.6 mg./M³		
Carbon disulfide (Z37.3-1968)	20 p.p.m	30 p.p.m	100 p.p.m	Do.
Carbon tetrachloride (Z37.17-1967)	10 p.p.m	25 p.p.m	200 p.p.m	5 minutes in any 4 hours.
Ethylene dibromide (Z37.31-1970)	20 p.p.m	30 p.p.m	50 p.p.m	5 minutes.
Ethylene dichloride (Z37.21-1969)	50 p.p.m	100 p.p.m	200 p.p.m	5 minutes in any 3 hours.
Formaldehyde (Z37.16-1967)	3 p.p.m	5 p.p.m	10 p.p.m	30 minutes.
Hydrogen fluoride (Z37.28-1969)	do			
Fluoride as dust (Z37.28-1969)	2.5 mg./M³			
Lead and its inorganic compounds (Z37.11-1959).	0.2 mg./M³			
Methyl chloride (Z37.18-1969)	100 p.p.m	200 p.p.m	300 p.p.m	5 minutes in any 3 hours.
Methylene Chloride (Z37.3-1969)	500 p.p.m	1,000 p.p.m	2,000 p.p.m	5 minutes in any 2 hours.
Organo (alkyl) mercury (Z37.30-1969)	0.01 mg./M³	0.04 mg./M³		
Styrene (Z37.15-1969)	100 p.p.m	200 p.p.m	600 p.p.m	5 minutes in any 3 hours.
Trichloroethylene (Z37.19-1967)	do	do	300 p.p.m	5 minutes in any 2 hours.
Tetrachloroethylene (Z37.22-1967)	do	do	do	5 minutes in any 3 hours.
Toluene (Z37.12-1967)	200 p.p.m	300 p.p.m	500 p.p.m	10 minutes.
Hydrogen sulfide (Z37.2-1968)		20 p.p.m	50 p.p.m	10 minutes once only if no other measurable exposure occurs.
Mercury (Z37.8-1971)		1 mg./10M³		
Chromic acid and chromates (Z37.7-1971)		do[h]		

TABLE G-3—MINERAL DUSTS

Substance	Mppcf[a]	Mg/M³[b]
Silica:		
Crystalline:		
Quartz (respirable)	250[d]	10mg/M³[e]
	%SiO₂+5	%SiO₂+2
Quartz (total dust)		30mg/M³
		%SiO₂+2
Cristobalite: Use ½ the value calculated from the count or mass formulae for quartz.		
Tridymite: Use ½ the value calculated from the formulae for quartz.		
Amorphous, including natural diatomaceous earth	20	80mg/M³
		%SiO₂
Tremolite	5	20mg/M³
		%SiO₂
Silicates (less than 1% crystalline silica):		
Asbestos--12 fibers per milliliter greater than 5 microns in length[i] or	2	
Mica	20	
Soapstone	20	
Talc	20	
Portland cement	50	
Graphite (natural)	15	
Coal dust (respirable fraction less than 5% SiO₂)		2.4mg/M³ or
For more than 5% SiO₂		10mg/M³
		%SiO₂+2
Inert or Nuisance Dust:		
Respirable fraction	15	5mg/M³
Total dust	50	15mg/M³

NOTE: Conversion factors—
mppcf×35.3=million particles per cubic meter
=particles per c.c.

[a] Millions of particles per cubic foot of air, based on impinger samples counted by light-field technics.

[b] The percentage of crystalline silica in the formula is the amount determined from air-borne samples, except in those instances in which other methods have been shown to be applicable.

[c] As determined by the membrane filter method at 430 × phase contrast magnification.

[d] Both concentration and percent quartz for the application of this limit are to be determined from the fraction passing a size-selector with the following characteristics:

Aerodynamic diameter (unit density sphere)	Percent passing selector
2	90
2.5	75
3.5	50
5.0	25
10	0

The measurements under this note refer to the use of an AEC instrument. If the respirable fraction of coal dust is determined with a MRE the figure corresponding to that of 2.4 Mg/M³ in the table for coal dust is 4.5 Mg/M³

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and the regulations in this part for the promulgation of a regulation.

(b) "Reasonable grounds" shall include an explanation showing wherein the person has a substantial interest in such regulation and an assertion of facts (supported by data if available) showing that new information exists with respect to the food additive or that new uses have been developed or old uses abandoned, that new data are available as to toxicity of the chemical, or that experience with the existing regulation or exemption may justify its amendment or repeal. New data should be furnished in the form specified in § 121.51 for submitting petitions.

§ 121.75 Exemption for investigational use and procedure for obtaining authorization to market edible products from experimental animals.

A food additive, including one that is a new drug or antibiotic, or foods containing such a food additive, intended for investigational use by qualified experts shall be exempt from the requirements of section 409 of the act under the following conditions:

(a) If intended for investigational use in vitro or with laboratory research animals, it bears a label which states prominently, in addition to other information required by the act:

Caution—Contains a new food additive (or new drug, or antibiotic) for investigational use only in laboratory research animals, or for tests in vitro. Not for use in humans.

(b) If intended for clinical investigational use in animals other than laboratory research animals, and the edible products of the animals are to be marketed as food, permission for the marketing of the edible products as food has been requested by the sponsor, and authorization has been granted by the Food and Drug Administration in accordance with §130.3a of this chapter, or by the Department of Agriculture in accordance with § 309.20 of Title 9, Code of Federal Regulations, and it bears a label which states prominently, in addition to other information required by the act, the following:

Caution—Contains a new food additive (or new drug, or antibiotic) for use only in investigational animals in clinical trials. Not for use in humans.

Edible products of investigational animals are not to be used for food unless authorization has been granted by the U.S. Food and Drug Administration or by the U.S. Department of Agriculture.

[31 F.R. 4891, Mar. 24, 1966]

Subpart B—Exemption of Certain Food Additives From the Requirement of Tolerances

§ 121.101 Substances that are generally recognized as safe.

(a) It is impracticable to list all substances that are generally recognized as safe for their intended use. However, by way of illustration, the Commissioner regards such common food ingredients as salt, pepper, sugar, vinegar, baking powder, and monosodium glutamate as safe for their intended use. The lists in paragraph (d) of this section include additional substances that, when used for the purposes indicated, in accordance with good manufacturing practice, are regarded by the Commissioner as generally recognized as safe for such uses.

(b) For the purposes of this section, good manufacturing practice shall be defined to include the following restrictions:

(1) The quantity of a substance added to food does not exceed the amount reasonably required to accomplish its intended physical, nutritional, or other technical effect in food; and

(2) The quantity of a substance that becomes a component of food as a result of its use in the manufacturing, processing, or packaging of food, and which is not intended to accomplish any physical or other technical effect in the food itself, shall be reduced to the extent reasonably possible.

(3) The substance is of appropriate food grade and is prepared and handled as a food ingredient. Upon request the Commissioner will offer an opinion, based on specifications and intended use, as to whether or not a particular grade or lot of the substance is of suitable purity for use in food and would generally be regarded as safe for the purpose intended, by experts qualified to evaluate its safety.

(c) The inclusion of substances in the list of nutrients does not constitute a finding on the part of the Department that the substance is useful as a supplement to the diet for humans.

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Glycerin.
Guar gum.
Invert sugar.
Iron, reduced.
Locust bean gum (carob bean gum).
Magnesium carbonate.
Magnesium chloride.
Magnesium hydroxide.
Magnesium sulfate.
Methyl and ethyl acrylate.
Mono- and diglycerides from glycerolysis of edible fats and oils.
Oleic acid.
Oxides of iron.
Potassium sorbate.
Propionic acid.
Propylene glycol.
Silicon dioxides.
Pulps from wood, straw, bagasse, or other natural sources.
Soap (sodium oleate, sodium palmitate).
Sodium aluminate.
Sodium carbonate.
Sodium chloride.
Sodium hexametaphosphate.
Sodium hydrosulfite.
Sodium hydroxide.
Sodium phosphoaluminate.
Sodium silicate.
Sodium sorbate.
Sodium sulfate.
Sodium thiosulfate (additive in salt).
Sodium tripolyphosphate.
Sorbitol.
Soy protein, isolated.
Sulfamic acid.
Sulfuric acid.
Starch, acid modified.
Starch, pregelatinized.
Starch, unmodified.
Sucrose.
Talc.
Urea.
Vanillin.
Zinc hydrosulfite.
Zinc sulfate.

(i) Substances migrating to food from cotton and cotton fabrics used in dry food packaging that are generally recognized as safe for their intended use, within the meaning of section 409 of the act, are as follows:

Acacia (gum arabic).
Acetic acid.
Beef tallow.
Calcium chloride.
Carboxymethylcellulose.
Coconut oil, refined.
Corn dextrin.
Cornstarch.
Fish oil (hydrogenated).
Gelatin.
Guar gum.
Hydrogen peroxide.
Japan wax.
Lard.
Lard oil.
Lecithin (vegetable).
Locust bean gum (carob bean gum).
Oleic acid.
Peanut oil.
Potato starch.
Sodium acetate.
Sodium bicarbonate.
Sodium carbonate.
Sodium chloride.
Sodium hydroxide.
Sodium sulfate.
Sodium silicate.
Sodium tripolyphosphate.
Sorbose.
Soybean oil (hydrogenated).
Stearic acid.
Talc.
Tall oil.
Tallow (hydrogenated).
Tallow flakes.
Tapioca starch.
Tartaric acid.
Tetrasodium pyrophosphate.
Urea.
Wheat starch.
Zinc chloride.

(Secs. 201(s), 409, 701(a), 52 Stat. 1055, 72 Stat. 1784, 1785 et seq., as amended; 21 U.S.C. 321(s), 348, 371(a)) [30 F.R. 15345, Dec. 23, 1965, as amended at 33 F.R. 5619, Apr. 11 1968; 34 F.R. 17064, Oct. 21, 1969; 35 F.R. 1049, Jan. 27, 1970]

§ 121.102 Adjuvants for pesticide chemicals.

Adjuvants, identified and used in accordance with § 120.1001 (c) and (d) of this chapter, which are added to pesticide use dilutions by a grower or applicator prior to application to the raw agricultural commodity, are exempt from the requirement of tolerances under section 409 of the act.

(Sec. 409, 72 Stat. 1735; 21 U.S.C. 348)

Subpart C—Food Additives Permitted in Feed and Drinking Water of Animals or for the Treatment of Food-Producing Animals

AUTHORITY: The provisions of this Subpart C issued under sec. 409, 72 Stat. 1735; 21 U.S.C. 348, unless otherwise noted.

§ 121.200 Definitions and interpretations applicable to Subpart C.

(a) Regulations prescribing conditions under which additives may be safely used in animal feed, animal feed supplements, concentrates, or premixes or in animals intended for food use shall not be construed to relieve such additives from the provisions of sections 505 and

Cite this Code CFR

thus: 9 CFR 1.1

Class of substance	Substance	Purpose	Products	Amount
	Sodium or potassium nitrite (supplies of sodium nitrite and potassium nitrite and mixtures containing them must be kept securely under the care of a responsible employee of the establishment. The specific nitrite content of such supplies must be known and clearly marked accordingly).	To fix color	do	2 lbs. to 100 gals. pickle at 10 percent pump level; 1 oz. to 100 lbs. meat (dry cure); ¼ oz. to 100 lbs. chopped meat and/or meat byproduct. The use of nitrites, nitrates, or combination shall not result in more than 200 parts per million calculated as sodium nitrite, in finished product.
Denuding agents; may be used in combination. Must be removed from tripe by rinsing with potable water.	Lime (calcium oxide, calcium hydroxide).	To denude mucous membrane.	Tripe	Sufficient for purpose.
	Sodium carbonate	do	do	Do.
	Sodium gluconate	do	do	Do.
	Sodium hydroxide	do	do	Do.
	Sodium metasilicate	do	do	Do.
	Sodium persulfate	do	do	Do.
	Trisodium phosphate	do	do	Do.
Emulsifying agents.	Acetylated monoglycerides.	To emulsify product.	Shortening	Do.
	Diacetyl tartaric acid esters of mono and diglycerides.	do	Rendered animal fat or a combination of such fat with vegetable fat.	Do.
	Glycerol-lacto stearate, oleate, or palmitate.	do	do	Do.
	Lecithin	To emulsify product (also as antioxidant).	Oleomargarine, shortening.	Do.
	Mono and diglycerides (glycerol palmitate, etc.).	To emulsify product.	Rendered animal fat or a combination of such fat with vegetable fat.	Sufficient for purpose in lard and shortening; 0.5 percent in oleomargarine.
	Polyglycerol esters of fatty acids (polyglycerol esters of fatty acids are restricted to those up to and including the decaglycerol esters and otherwise meeting the requirements of § 121.1120 (a) of the Food Additive Regulations).	do	Rendered animal fat or a combination of such fat with vegetable fat when use is not precluded by standards of identity or composition.	Sufficient for purpose.
	Polysorbate 80 (polyoxyethylene (20) sorbitan monooleate).	do	Shortening for use in nonstandardized baked goods, baking mixes, icings, fillings, and toppings and in the frying of foods.	1 percent when used alone. If used with polysorbate 60 the combined total shall not exceed 1 percent.
	Propylene glycol mono and diesters of fats and fatty acids.	do	Rendered animal fat or a combination of such fat with vegetable fat.	Sufficient for purpose.
	Polysorbate 60 (polyoxyethylene (20) sorbitan monostearate).	do	Shortening for use in nonstandardized baked goods, baking mixes, icings, fillings, and toppings and in the frying of foods.	1 percent when used alone. If used with polysorbate 80 the combined total shall not exceed 1 percent.
	Steryl-2-lactylic acid	do	Shortening to be used for cake icings and fillings.	3.0 percent.
	Steryl monoglyceridyl citrate.	do	Shortening	Sufficient for purpose.

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Class of substance	Substance	Purpose	Products	Amount
	Sodium sulfate	do	do	Do.
	Sodium tripolyphosphate.	do	do	Do.
	Sucrose	do	do	Do.
	Trisodium phosphate.	do	do	Do.
Miscellaneous	Potassium sorbate	To retard mold growth.	Dry sausage	2.5 percent in water solution may be applied to casings after stuffing or casings may be dipped in solution prior to stuffing.
		To preserve product and to retard mold growth.	Oleomargarine or margarine.	0.1 percent by weight of the finished oleomargarine or margarine.
	Calcium disodium, EDTA[2] (calcium disodium ethylenediaminetetraacetate).	To preserve product and to protect flavor.	do	75 parts per million by weight of the finished oleomargarine or margarine.
	Propyl paraben (propyl p-hydroxybenzoate).	To retard mold growth.	Dry sausage	3.5 percent in water solution may be applied to casings after stuffing, or casings may be dipped in solution prior to stuffing.
	Sodium bicarbonate	To neutralize excess acidity, cleaning vegetables.	Rendered fats, soups, curing pickle.	Sufficient for purpose.
	Calcium propionate	To retard mold growth.	Pizza crust	0.32 percent alone or in combination based on weight of the flour used.
	Sodium propionate	do	do	
	✓ Sodium hydroxide	To decrease amount of cooked out juices.	Cured hams, pork shoulder picnics and loins, canned hams and pork shoulder picnics, and products covered by § 319.101(d); chopped ham and bacon.	May be used only in combination with phosphates in ratio of four parts phosphate to one part sodium hydroxide; the combination shall not exceed 5 percent in pickle at 10 percent pump level; 0.5 percent in product.
Phosphates	Disodium phosphate	do	do	5 percent of phosphate in pickle at 10 percent pump level; 0.5 percent of phosphate in product (only clear solution may be injected into product).
	Monosodium phosphate.	do	do	Do.
	Sodium hexametaphosphate.	do	do	Do.
	Sodium tripolyphosphate.	do	do	Do.
	Sodium pyrophosphate.	do	do	Do.
	Sodium acid pyrophosphate.	do	do	Do.
Proteolytic enzymes.	Aspergillus oryzae	To soften tissues	Beef cuts	Solutions consisting of water, salt, monosodium glutamate, and approved proteolytic enzymes applied or injected into cuts of beef shall not result in a gain of more than 3 percent above the weight of the untreated product.
	Aspergillus flavusoryzae group.	do	do	Do.
	Bromelin	do	do	Do.
	Ficin	do	do	Do.
	Papain	do	do	Do.

Class of substance	Substance	Purpose	Products	Amount
Refining agents (must be eliminated during process of manufacturing).	Acetic acid	To separate fatty acids and glycerol.	Rendered fats	Sufficient for purpose.
	Bicarbonate of soda	do	do	Do.
	Carbon (purified charcoal).	To aid in refining of animal fats.	do	Do.
	✓ Caustic soda (sodium hydroxide).	To refine fats	do	Do.
	Diatomaceous earth; Fuller's earth.	do	do	Do.
	Sodium carbonate	do	do	Do.
	Tannic acid	do	do	Do.
Rendering agents	Tricalcium phosphate	To aid rendering	Animal fats	Do.
	Trisodium phosphate	do	do	Do.
Artificial sweeteners.	Saccharin	To sweeten product	Bacon	0.01 percent.
Synergists (used in combination with antioxidants).	Citric acid	To increase effectiveness of antioxidants.	Lard and shortening	0.01 percent alone or in combination with antioxidants in lard or shortening.
			Dry sausage	0.003 percent in dry sausage in combination with antioxidants.
			Fresh pork sausage	0.01 percent on basis of fat content, in combination with antioxidants.
			Dried meats	0.01 percent on basis of total weight in combination with antioxidants.
	Malic acid	do	Lard and shortening	Do.
	Monoisopropyl citrate.	To increase effectiveness of antioxidants.	Lard, shortening, oleomargarine, fresh pork sausage, dried meats.	0.02 percent.
	Phosphoric acid	do	Lard and shortening.	0.01 percent.
	Monoglyceride citrate.	do	Lard, shortening, fresh pork sausage, dried meats.	0.02 percent.

[1] These are proprietary products, and a list thereof can be obtained from the Technical Services Division, Consumer and Marketing Service, U.S. Department of Agriculture, Washington, D.C. 20250.

[2] Information as to the specific products for which use of this substance is approved may be obtained upon inquiry addressed to the Director, Laboratory Services Division, Consumer and Marketing Service, U.S. Department of Agriculture, Washington, D.C. 20250.

(d) No substance may be used in or on any product if it conceals damage or inferiority or makes the product appear to be better or of greater value than it is. Therefore:

(1) Paprika or oleoresin paprika may not be used in or on fresh meat, such as steaks, or comminuted fresh meat food products, such as chopped and formed steaks or patties; or in any other meat food products consisting of fresh meat (with or without seasoning), except chorizo sausage and Italian brand sausage, and except other meat food products in which paprika or oleoresin paprika is permitted as an ingredient in a standard of identity or composition in Part 319 of this subchapter.

(2) Sorbic acid, calcium sorbate, sodium sorbate, and other salts of sorbic acid may not be used in cooked sausage or any other product; sulfurous acid and salts of sulfurous acid may not be used in or on any product and niacin or nicotinamide may not be used in or on fresh product; except that potassium sorbate, propylparaben (propyl p-hydroxybenzoate), calcium propionate, sodium propionate, benzoic acid, and sodium benzoate may be used in or on any product only as provided in the chart in § 318.7 (c) (4) or as approved by the Administrator in specific cases.

[35 F.R. 15586, Oct. 3, 1970, as amended at 36 F.R. 11903, June 23, 1971; 36 F.R. 12003, 12004, June 24, 1971; 36 F.R. 23720, Dec. 14, 1971]

EFFECTIVE DATE: At 36 F.R. 23720, Dec. 14, 1971, effective 30 days after publication, the table listing for sodium or potassium nitrate in § 318.7(c) (4) was revised. For the convenience of the user, the superseded text is set forth below.

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Food and Drugs

PARTS 10 TO 129

Revised as of April 1, 1973

CONTAINING
A CODIFICATION OF DOCUMENTS
OF GENERAL APPLICABILITY
AND FUTURE EFFECT

AS OF APRIL 1, 1973

With Ancillaries

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§ 121.1031 Food starch-modified.

Food starch-modified as described in this section may be safely used in food. The quantity of any substance employed to effect such modification shall not exceed the amount reasonably required to accomplish the intended physical or technical effect, nor exceed any limitation prescribed. To insure safe use of the food starch-modified, the label of the food additive container shall bear the name of the additive "food starch-modified" in addition to other information required by the act. Food starch may be modified by treatment prescribed as follows:

(a) Food starch may be acid-modified by treatment with hydrochloric acid or sulfuric acid or both.

(b) Food starch may be bleached by treatment with one or more of the following:

	Limitations
Active oxygen obtained from hydrogen peroxide and/or peracetic acid, not to exceed 0.45 percent of active oxygen.	----------
Ammonium persulfate, not to exceed 0.075 percent and sulfur dioxide, not to exceed 0.05 percent.	----------
Chlorine, as sodium hypochlorite, not to exceed 0.0082 pound of chlorine per pound of dry starch.	----------
Potassium permanganate, not to exceed 0.2 percent.	Residual manganese (calculated as Mn), not to exceed 50 parts per million in food starch-modified.
Sodium chlorite, not to exceed 0.5 percent.	----------

(c) Food starch may be oxidized by treatment with chlorine, as sodium hypochlorite, not to exceed 0.055 pound of chlorine per pound of dry starch.

(d) Food starch may be esterified by treatment with one of the following:

	Limitations
Acetic anhydride	Acetyl groups in food starch-modified not to exceed 2.5 percent.
Adipic anhydride, not to exceed 0.12 percent, and acetic anhydride.	Do.
Monosodium orthophosphate.	Residual phosphate in food starch-modified not to exceed 0.4 percent calculated as phosphorus.
1-Octenyl succinic anhydride, not to exceed 3 percent.	----------
1-Octenyl succinic anhydride, not to exceed 2 percent, and aluminum sulfate, not to exceed 2 percent.	----------
Phosphorus oxychloride, not to exceed 0.1 percent.	----------
Sodium trimetaphosphate.	Residual phosphate in food starch-modified not to exceed 0.04 percent, calculated as phosphorus.
Sodium tripolyphosphate and sodium trimetaphosphate.	Residual phosphate in food starch-modified not to exceed 0.4 percent calculated as phosphorus.

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	Limitations
Succinic anhydride, not to exceed 4 percent.	----------
Vinyl acetate	Acetyl groups in food starch-modified not to exceed 2.5 percent.

(e) Food starch may be etherified by treatment with one of the following:

	Limitations
Acrolein, not to exceed 0.6 percent.	----------
Epichlorohydrin, not to exceed 0.3 percent.	----------
Epichlorohydrin, not to exceed 0.1 percent, combined with propylene oxide, not to exceed 10 percent.	Residual propylene chlorohydrin not more than 5 parts per million in food starch-modified.
Propylene oxide, not to exceed 25 percent.	Do.

(f) Food starch may be esterified and etherified by treatment with one of the following:

	Limitations
Acrolein, not to exceed 0.6 percent and vinyl acetate, not to exceed 7.5 percent.	Acetyl groups in food starch-modified not to exceed 2.5 percent.
Epichlorohydrin, not to exceed 0.3 percent, and acetic anhydride.	Acetyl groups in food starch-modified not to exceed 2.5 percent.
Epichlorohydrin, not to exceed 0.3 percent, and succinic anhydride, not to exceed 4 percent.	----------
Phosphorus oxychloride, not to exceed 0.1 percent, and propylene oxide, not to exceed 10 percent.	Residual propylene chlorohydrin not more than 5 parts per million in food starch-modified.

(g) Food starch may be modified by treatment with one of the following:

	Limitations
Chlorine, as sodium hypochlorite, not to exceed 0.055 pound of chlorine per pound of dry starch; 0.45 percent of active oxygen obtained from hydrogen peroxide; and propylene oxide, not to exceed 25 percent.	Residual propylene chlorohydrin not more than 5 parts per million in food starch-modified.
Sodium hydroxide, not to exceed 1 percent.	----------

(h) Food starch may be modified by a combination of the treatments prescribed by paragraphs (a) and/or (b) of this section and any one of the treatments prescribed by paragraph (c), (d), (e), (f), or (g) of this section, subject to any limitations prescribed by the paragraphs named.

[30 F.R. 15845, Dec. 23, 1965, as amended at 31 F.R. 14351, Nov. 8, 1966; 32 F.R. 8359, June 10, 1967; 34 F.R. 1771, Feb. 6, 1969; 34 F.R. 6778, Apr. 23, 1969]

§ 121.1032 Yellow prussiate of soda.

(a) The food additive yellow prussiate of soda (sodium ferrocyanide decahydrate; $Na_4Fe(CN)_6 \cdot 10H_2O$) contains a minimum of 99 percent by weight of sodium ferrocyanide decahydrate.

(b) The additive is used or intended for use as an anticaking agent in salt and as an adjuvant in the production of dendritic crystals of salt in an amount needed to produce its intended effect but not in excess of 13 parts per million calculated as anhydrous sodium ferrocyanide.

37 F.R. 5490, Mar. 16, 1972]

§ 121.1034 BHT.

The food additive BHT (butylated hydroxytoluene), alone or in combination with other antioxidants permitted in this Subpart D may be safely used in or on specified foods, as follows:

(a) The BHT meets the following specification: Assay (total BHT) 99 percent minimum.

(b) The BHT is used alone or in combination with BHA, as an antioxidant in foods, as follows:

Food	Limitations (total BHA and BHT) parts per million
Dehydrated potato shreds	50
Dry breakfast cereals	50
Emulsion stabilizers for shortenings	200
Potato flakes	50
Potato granules	10
Sweetpotato flakes	50

(c) To assure safe use of the additive:

(1) The label of any market package of the additive shall bear, in addition to the other information required by the act, the name of the additive.

(2) When the additive is marketed in a suitable carrier, in addition to meeting the requirement of subparagraph (1) of this paragraph, the label shall declare the percentage of the additive in the mixture.

[30 F.R. 15845, Dec. 23, 1965, as amended at 34 F.R. 6977, Apr. 26, 1969]

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Treatment of Caustic Alkali Poisoning

JAY M. ARENA, M.D.

From the Department of Pediatrics and the Poison Control Center, Duke University Medical Center, Durham, North Carolina

CAUSTIC ALAKLI POISONING may result from the ingestion of many household products. Treatment for this type of poisoning will vary to a certain extent with the agent ingested. Thus, while some alkalis require vigorous and immediate attention, others, such as chlorinated alkalis, are far less dangerous.

ALKALIS

Sodium and potassium hydroxide (caustic soda or potash), sodium and potassium carbonates, peroxides and oxides are used in the manufacture of soap, chemical synthesis, washing powders, household drain pipe cleaning agents [lye, Drano (new liquid form contains trichloroethane instead of sodium hydroxide), Pronto, etc.] and in glycosuria testing tablets (Clinitest Tablets). They combine with protein to form proteinates and with fats to form soap, thus producing deep caustic and penetrating burns on contact with tissues. Eight out of eleven ingestions of Clinitest Tablets resulted in esophageal strictures, some after the ingestion of only one tablet.

Symptoms of alkali ingestion are burning pain from mouth to stomach. Swallowing is difficult at first and then impossible. Mucous membranes are soapy and white, but become brown, edematous, and ulcerated; vomitus is bloody and may contain shreds of mucous membrane. The pulse is feeble and rapid; respiration is increased and collapse may ensue. Death, due to shock, asphyxia from glottic edema, or intercurrent infection, pneumonia, or mediastinitis may occur in 48–72 hours in severe poisoning. Within weeks or months and sometimes years, esophageal stricture develops. It is a paradox that corrosive acid ingestion produces fewer permanent effects on the esophagus than do the alkalis, and it is not unusual for these individuals to develop pyloric stenosis instead of esophageal strictures.

Alkali coming into contact with the skin may produce first, second, or even third degree burns depending on concentration of alkali and

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duration of contact. Eye involvement may cause severe conjunctivitis, as well as corneal destruction. The white, thick liquid or paste in the core of some golf balls may contain sodium hydroxide, and the accidental splashing of this material into the eye may cause serious injury.

Treatment

Esophagus

Diluted vinegar, lemon or orange juice should be given immediately. Do *not* use gastric lavage or emetics since the small amount of alkali that usually reaches the stomach is rapidly neutralized by the acid gastric juice. Olive oil or other demulcents ease pain. Esophagoscopy should be done within 48 hours by an experienced endoscopist to determine future management of such patients. The absence of oral burns should never preclude this examination, as esophageal injury may be present with little or no mouth involvement.

Steroids are effective in diminishing fibroelastic activity and act as anti-inflammatory agents in preventing esophageal stenosis in rabbits after the ingestion of lye. These effects suggested the use of steroids for the prevention of stenosis in children who have swallowed a caustic alkali. Recently, similar favorable results were observed in cats whose esophagus more closely resembles that of a child. Clinical reports in humans, though often favorable, have occasionally given equivocal results with this type of therapy. Nevertheless, the use of corticosteroids is rational therapy in that it is less painful, less time consuming, less expensive, and probably more effective in preventing strictures than the older procedure of bougienage as developed by Bokay and Salzer. For children 1–4 years old, divided doses of 60 mg./day of prednisolone are given for the first 4 days; 40 mg./day is given for the next 4 days, and finally 20 mg./day is given until the esophagus is healed, usually in 3 weeks. The standard dose for older children and adults is 60–100 mg./day with corresponding decreases in dosage during the treatment program. A broad-spectrum antibiotic suspension is given orally as long as steroids are continued. Large doses of penicillin are administered parenterally for mediastinal or pulmonary complications.

As an alternate method of treatment bougienage may be used. On the third or fourth day, after the inflammation and edema of the esophageal mucosa has subsided, and daily for 2 weeks, a rubber eyeless catheter filled with mercury or small shot should be passed into the stomach. The catheter size should be gradually increased

until a number 32 or 34 enters easily. Dilatation should be continued for 4 weeks. If the dilator passes with difficulty and partial stricture is suspected or demonstrated by barium swallow or esophagoscopy, dilatation should be continued periodically for at least 1 year. Seventy-five per cent of esophageal strictures can be prevented by this method, but cooperation is usually so poor that this figure is seldom attained. Combined therapy of steroids and bougienage are used in some centers.

Skin

Areas of the skin that come in contact with alkali should be thoroughly cleansed with running water until skin is free of alkali as indicated by disappearance of soapiness. In second or third degree burns, 2% acetic acid wet dressings should be used to complete neutralization of the alkali in deeper tissues.

Eyes

Alkalis produce some of the most severe chemical eye injuries. The increase in hydroxyl ion concentration beyond the limits of tissue protein stability results in the formation of gel-like alkaline proteinates. In addition, the reaction with fats to form soaps produces considerable damage to the structure of the cell membranes and thus allows the alkali to penetrate rapidly into the tissues. The speed of penetration is responsible for the capacity of alkalis to cause great ocular damage. Treatment consists of the quick, thorough irrigation of the eye with water at the nearest source of supply for 5 minutes. After instillation of a local anesthetic, irrigation with water or normal saline is continued for half an hour. Any attempt to treat a chemical eye injury with a specific neutralizing-agent is now considered detrimental. An opthalmologist should be consulted for degree of damage and specific therapy.

CHLORINATED ALKALIS

Bleaches are the second most common compounds (household agent or drug) ingested by children. As a matter-of-fact, ingestion of bleaches represents approximately 5 per cent of all poisoning in children under 5 years of age reported to the poison control centers (second only to aspirin). (See Table 1 for contents of household bleaches.)

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These agents are chlorinated alkalis or hypochlorites (Clorox, and bleaches of many varieties) that are corrosive with resulting caustic actions depending not only on their variable degrees of alkalinity but also on their potent oxidizing effects producing "available chlorine." Systemic effects rarely occur, and though harsh to mucous membranes with occasional alarming irritation and edema of the pharynx and larynx, local effects are usually insignificant.

Table 1. Contents of Common Household Bleaches

Bleach	Contents
Sodium hypochlorite solutions	Liquid bleaches commonly referred to as chlorine bleach generally contain about 5% sodium hypochlorite. Packets sold in vending machines may be more concentrated (about 10%). They contain small amounts of free alkali.
Sodium perborate bleaches	Generally dry products containing sodium perborate (tetrahydrate) or sodium perborate monohydrate in the range of 15–85%. Other ingredients present may include sodium carbonate, sodium tripolyphosphate, sodium sulfate, sodium silicate, or other common laundry detergent ingredients. These products, therefore, can be strongly alkaline in addition to their borate toxicity.
Dichloroisocyanurates and trichloroisocyanurates	Generally dry bleach powders or tablets containing 20–30% of the chloroisocyanurate with phosphates, carbonates, or other ingredients listed above under sodium perborate bleaches. These products may be strongly irritating.
Optical bleaches	These are additives present in some laundry products as fabric brighteners in amounts up to 0.2%. They vary in toxicity from around 2–20 gm./kg.
Hair bleaches	These include hydrogen peroxide 6% (20 volume), ammonium hydroxide 1%, vinegar and lemon rinses.
Hair neutralizers	Cold wave neutralizers are oxidizing agents and include hydrogen peroxide 6%, sodium perborate about 80–90%, and in some cases sodium bromate about 12–15%.

In a survey of two separate reports of 339 and 129 cases of Clorox ingestion, only 2 patients showed any evidence of esophageal injury (6). These were mild and of such a nature as to exclude the possibility of the late development of stricture. Routine esophagoscopy for evaluation is apparently not indicated unless considerable corrosion of the oropharynx is noted or if unusually large amounts had been ingested.

There have been incidents documented in which housewives have mixed acid-type toilet bowl cleaners with sodium hypochlorite bleaches that resulted in toxic fumes. The mixture produces chlorine gas which, when inhaled, may cause respiratory irritation (rarely

pulmonary edema) with coughing and labored breathing, along with inflammation of the eyes and other mucous membranes.

Treatment is symptomatic. Sips of milk or other demulcents to allay the burning sensation of the oral mucous membranes is usually all that is necessary. Although these preparations are alkaline, on contact with acid gastric juice or acid solutions they release hypochlorous acid which is extremely irritating. Buffering the acid by administration of alkali, paradoxic as it may seem, reduces the irritative effect. Vigorous anti-alkali therapy however is rarely indicated. Milk of magnesia (1 oz.) is a useful antacid, adsorbent, demulcent, and cathartic. Sodium bicarbonate should be avoided because it releases carbon dioxide which may produce considerable gas with gastric distention.

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Carcinoma of the Esophagus With A History of Lye Ingestion

JACK E. ARRANTS, M.D., HUMBERTO ALBUERNE,* M.D., M. J. JURKIEWICZ, M.D.

From the Surgical Service, College of Medicine, University of Florida, Gainesville

SINCE THE report by Catell in 1896[1] the management of the patient with a history of lye ingestion has continued to constitute a major medical problem. Lye is usually taken for suicidal purposes or is accidently ingested. The subsequent clinical course is largely dependent upon the amount ingested, and the time it remains in contact with the mucous membranes of the esophagus. When a large quantity is taken a fatal outcome can be predicted. Death is secondary to massive destruction of the esophagus and stomach. When only a small amount is ingested, stricture formation may occur and the problem then becomes management of the stricture. Early vigorous treatment with steroids, endoscopy and dilitation are of prime importance to prevent late stricture formation with a resultant alimentary cripple. Unfortunately treatment is often unsuccessful and a chronic stricture develops. In such patients carcinomatous degeneration of the cicatrical formation may occur.

Case Report

A 67-year-old Negro woman was first admitted to Duval Medical Center on April 4, 1961. She had been unable to swallow for the last 5 days. She gave a history of lye ingestion at age 3 and since that time stated that she has had an occasional episode of mild dysphagia. At the time of admission she had lost approximately 10 lbs. She stated, however, that she had never required any esophageal dilitations.

Physical examination on admission was unremarkable except that the individual was undernourished. Barium swallow (Fig. 1) revealed a narrowed area in the midportion of the esophagus with also two areas in the cervical region. At esophagoscopy there was noted an elliptical-shaped band approximately 4 cm. below the hyoid bone. The esophagoscope could not be passed beyond this point. Biopsy was reported to be negative for tumor. Esophageal washings taken revealed a Papanicolaou smear of Class IV. Because of the possibility of cancer the patient underwent a right thoracocotomy. At the time of operation the entire esophagus was mobilized from the level of the cricoid cartilage to the cardia. There was no evidence of any overt stricture formation, nor any carcinomatous change. Because of the inability to find any gross pathology it was elected to treat her with periodic dilitations.

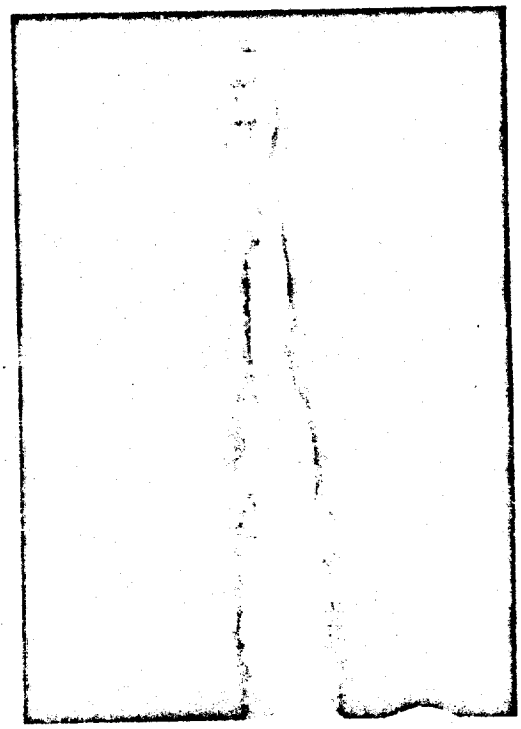

FIG. 1. Contrast roentgenogram demonstrating [illegible] stenosis in the midesophagus; done in 1961.

* Surgical Service, Duval Medical Center, Jacksonville, Florida.

Submitted October 2, 1964.

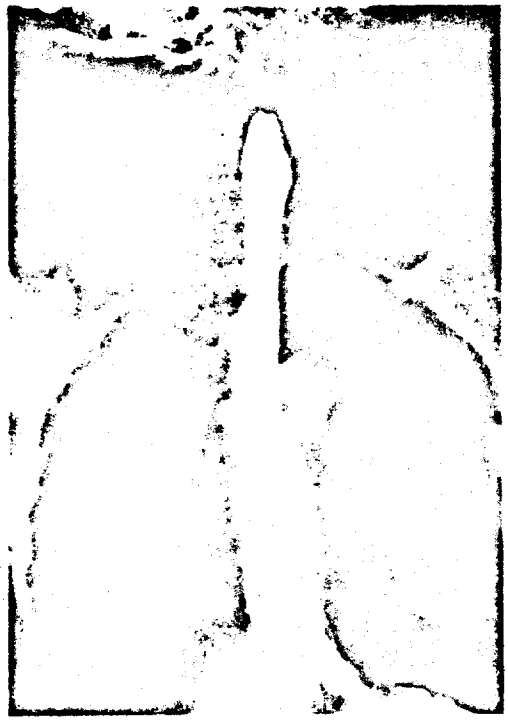

FIG. 2. Barium swallow in 1963 again demonstrating area of stricture in midthoracic esophagus with almost complete obliteration of the lumen at that point.

The patient was re-admitted to the hospital one year later because of a 6-week history of some difficulty in swallowing. At the time of admission she could only swallow fluids. Esophagoscopy again was done and the findings noted were similar to the previous examinations. The elliptical-shaped band below the cricoid was noted. Again it was elected to treat her with periodic dilitations.

The patient was admitted to the hospital one month later because of the same difficulty. Barium swallow at this time (Fig. 2) again revealed three areas of narrowing: two in the cervical region and one just below the bifurcation of the trachea. Esophagoscopy was done and the scope could be introduced 25 cm. At this level a tight stricture approximately 3 mm. in diameter was noted which could not be dilated from above. She therefore had a gastrostomy with retrograde dilitations. The patient did well until 9 months later when she required another gastrostomy because she had removed the tube from the stomach.

Two months later she came to the hospital with a 2-week history of coughing up liquids which she had immediately taken by mouth. She also reported one to two episodes of hemoptysis. Attempt at barium swallow was done, and at flouroscopy a broncho-esophageal fistula was noted on the left. Bronchoscopy was done and in the main left-end bronchus there was an area of granulation tissue approximately 2 cm. below the carino that apparently represented the site of the broncho-esophageal fistula. Biopsy of this revealed only muscular tissue with bronchial epithelium. The patient was prepared for operation and a left exploratory thoracotomy was done; there was noted to be dense scar tissue and stricture of the esophagus extending from just below the arch of the aorta distally for approximately 5 cm. The actual site of the bronchial-esophageal fistula could not be ascertained. Because of the poor condition of the patient no definitive procedure was done and a gastrostomy tube was again placed into the stomach for retrograde dilitations. Following operation the patient did poorly and expired on the fifth postoperative day.

At the time of the postmortem examination the posterior mediastinum contained a large necrotic mass. There was a large defect in the

FIG. 3. Gross examination of the esophagus at postmortem showing huge esophageal defect and carcinomatous change.

FIG. 4. Low-power photomicrograph of esophageal wall demonstrating the infiltrating tumor.

esophagus (Fig. 3) which was irregular and measured 6 cm. along its greatest diameter. Microscopically the esophagus showed severe inflammatory inflammation and fibrosis with an infiltrating carcinoma which involved the entire wall (Fig. 4). The tumor invaded the immediate adjacent trachea and bronchus and there were metastases to the lungs.

Discussion

Although the etiology of primary carcinoma of the esophagus remains obscure there are several factors which may be considered as predisposing the development of neoplastic degeneration. It has been recognized for many years that cancer of the esophagus is more prone to occur at the three anatomic zones of constriction. These zones are the superior end of the esophagus, the bifurcation of the trachea and the passage of the esophagus through the diaphragm. If one considers chronic irritation as an etiologic factor in the development of cancer, it is at these three zones where the damaging effects of hot liquids, alcohol, spicy foods and large bolus of ingested substances occur. Carcinoma has also been noted within structures such as diverticula and the large dilated pouch above an area of cardiospasm. Lye ingestion with secondary stricture formation can be indirectly considered an etiologic agent.

Clinically these patients are quite similar to those with pure cancer of the esophagus. There are, however, several salient differences. First, one can usually get a history of lye ingestion with continued or intermittent mild dysphagia. There is a striking difference in the age group. Patients with symptoms secondary to stenosis are usually several decades younger than those with typical epidermoid carcinoma of the esophagus. The ratio of idiopathic cancer of the esophagus in males to females is 4 or 5 to 1; however with lye strictures it is 3 to 2.

Except for the case report of Catell[4] in which lye ingestion probably was not a factor because there was only several months between ingestion and appearance of cancer, the interval between ingestion of lye and the appearance of carcinoma is between 22 and 54 years, with an average age of 46.

Dysphasia is present in all patients with anorexia and weight loss is common. Pain and bleeding are ominous signs in a patient with lye ingestion. Even though intermittent dysphasia may be present the appearance of dysphasia after a long asymptomatic interval must be considered neoplastic until proved otherwise.

Most of the strictures occur in the upper one third of the esophagus. Pathologically

lye burn is quite similar to a thermal burn. There is first ulceration with edema, hyperemia and intense cellular reaction. Later fibrosis with scar formation and resultant stricture develops. Also a severe peri-esophageal reaction occurs which results in the fixation of the esophagus to the surrounding structures. This is an important consideration if operation is to be considered at a later date. Histologically all of the cancers are epidermoid with typical microscopic appearance. By the time of diagnosis many have invaded the adjacent structures. Carboni and Virno[3] reported an incidence of 37.5 percent with left broncho-esophageal fistula, as noted in the present case.

Diagnosis can be exceedingly difficult. Repeated x-rays and endoscopic examinations are mandatory. Negative biopsy findings do not rule out cancer. These individuals must be followed periodically for an indefinite period of time.

Treatment of the individual with lye ingestion is somewhat controversial. If after the acute inflammatory reactions subside, no stricture occurs, then only continued observation is necessary. However this is found infrequently and it is in the treatment of chronic strictures that arguments arise.

In the past the usual treatment has been repeated dilitations without direct surgical intenvention. However with the definite possibility of malignancy, with an incidence ranging from 0.2 per cent reported by Pack and Livingston[6] to 5 per cent reported by Benedict,[2] other types of treatment have been undertaken. Carboni and Virno[3] recommend early operation with esophageal resection and intrathoracic esophagogastrectomy. Lortat-Jacobs and Richard[5] recommend bypass without esophagectomy, even small strictures. They state they have never seen esophageal cancer in a nonfunctioning esophagus. Alvarez and Colbert[1] utilized a gastric tube following the procedure of Heimlich for reconstruction of continuity. They likewise did esophageal resection. Whatever type of procedure is used, the one common feature is that the esophagus with the stricture is removed from contact with ingested substances.

Summary

A case of a patient with lye ingestion with resultant carcinoma of the esophagus is reported. The difficulties concerning diagnosis and treatment are mentioned. Because of the distinct possibility of malignancy it is our contention that any chronic stricture should have a bypass operation to prevent repeated irritation to the stenosed area. Treatment of carcinoma developing on a lye stricture is identical to treatment of idiopathic carcinoma of the esophagus.

Acknowledgement

We wish to thank Dr. Thaddeus M. Moseley, III, Chief of Surgical Service at Duval Medical Center, Jacksonville, Florida, for permission to use the clinical case in this report.

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Baker Chemical Co.

Company Code [redacted]

Sub. No. 0192

PRODUCT LISTING

Comm. No. 3726

Date Issued 3-17-66

Primary Listing: * Sodium Hydroxide, 'Baker Analyzed' REAGENT, Low in Carbonate, Special Pellets

Secondary Listing:

Formula: NaOH

F W 39.99717

Specifications:

Meets A. C. S. Specifications

Assay (NaOH)	98.0	%
Sodium Carbonate (Na_2CO_3)	0.50	%
Chloride (Cl)	0.005	%
Nitrogen Compounds (as N)	0.001	%
Phosphate (PO_4)	0.001	%
Sulfate (SO_4)	0.003	%
Ammonium Hydroxide Precipitate	0.020	%
Heavy Metals (as Ag)	0.002	%
Iron (Fe)	0.001	%
Nickel (Ni)	0.001	%
Potassium (K)	0.020	%

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Carcinoma of the Esophagus at the Site of Lye Stricture

Report of a Case

EDGARD BARBOSA, M.D., R. G. RATE, M.D., F.A.C.S., F.I.C.S., D.A.B.*
AND V. E. CHESKY, M.D., F.A.C.S., F.I.C.S., D.A.B.**
HALSTEAD, KANSAS

CARCINOMA of the esophagus at the site of a lye stricture is extremely rare. Only a few well documented cases could be found in the literature. To these we wish to add 1 more case, in which esophageal carcinoma developed in a 37-year-old man, thirty-six years after accidental ingestion of lye.

REPORT OF CASE

V. D. F., a 37-year-old white man, was admitted to the Halstead Hospital in October 1955 because of increasing difficulty in swallowing foods, considerable soreness in the chest, fever and coughing accompanied by a large amount of thick purulent sputum. At the age of 1 year he had swallowed lye, which resulted in complete obstruction of the esophagus and required a gastrostomy for feeding purposes. As a result of the lye burn a severe stricture developed, which required esophageal dilatations at two-year intervals to keep the esophageal lumen patent. Prior to his most recent admission to the hospital he was able to drink liquids and eat semisolid and solid foods such as potatoes, fish and ground meat.

Physical examination gave negative results except for an area of dullness and tubular breath sounds in the right upper pulmonary field. A well-healed gastrostomy scar was noted in the midepigastrium.

A roentgen film of the chest taken at the time of admission disclosed an abscess in the upper lobe of the right lung. During a therapeutic bronchoscopic procedure, purulent material was aspirated from the bronchial orifice of the right upper lobe. Esophagoscopic study on May 1, 1956, revealed an obstruction and stricture at the 26 cm. level. Retained foods and fluids were aspirated. The esophagus was dilated to the diameter of a No. 30 French

Fig. 1.—Cross section of the gross specimen, showing a firm white tumor invading the adjacent structures.

*Thoracic Surgery, the Hertzler Clinic, Halstead.
**Hertzler Clinic, Hertzler Research Foundation and Halstead Hospital, Halstead.
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Fig. 2.—Low power photomicrograph of the tumor, showing squamous cell carcinoma originating from the surface and growing in strands. Note keratinization and variation in size of the nuclei.

catheter at the site of stricture. A biopsy specimen taken at the site of esophageal stricture disclosed squamous cell carcinoma, Grade II.

Pathologic Report (C. A. Hellwig, M.D.).—Gross:* The specimen consisted of three hard pieces of grayish red tissue, one 1 mm. and the others between 3 and 4 mm. in diameter. *Microscopic:* The tumor grew in strands originating from the surface. There was marked variation in the size of the nuclei. Some cells were very large and showed keratinization. A few horn pearls were observed.

At bronchoesophagoscopic study on May 18 the peripheral portion of the esophagus was narrowed and contracted by scar tissue. A cauliflower-like carcinomatous growth encircling the esophageal lumen was visualized. Biopsies confirmed the earlier diagnosis of squamous cell carcinoma, Grade II. Bougies were passed to dilate the lumen to the diameter of a No. 40 French catheter. Bronchoscopic study at this time disclosed a healthy orifice of the right upper lobe, from which no purulent material could be aspirated.

A right thoracotomy was performed on June 1. The midesophageal lesion had invaded the mediastinum, the trachea and the pulmonary hilus. Palliative resection of the esophageal lesion with an end-to-end anastomosis was done, a 10-cm. vascular pedicle graft from the transverse colon being used.

COMMENT

Teleky, in 1904, reported a case of carcinoma of the esophagus at the site of stricture, twenty years after the ingestion of lye. In Bigger and Vinson's case the interval between the ingestion of lye and the occurrence of carcinoma was forty-two years. In Henniger's case, reported in 1932, the interval was sixteen years—the shortest on record. In our case the existence of carcinoma was confirmed by biopsy thirty-six years after the ingestion of lye. The carcinomatous change usually occurs between thirty and forty years after lye is ingested. Hardin recently reported a ten-year analysis of 71 patients with acute caustic burns, in which no carcinoma had yet developed. On the other hand, Wurnig, in 1955, reported 7 cases of carcinoma following ingestion of lye. The age at which the accident occurred was between the ages of 2 and 14 years, and the carcinoma developed between the ages of 42 and 62 years.

Fatti and his co-workers, in February 1956, reported the treatment employed in 147 cases of caustic stricture of the esophagus in a period of six years. According to the authors, 99, or 70 per cent, of the patients have been successfully treated by esophageal dilatation. After a follow-up of perhaps forty years, carcinomatous degeneration may be predicted. Biopsies should be done more frequently to assure the endoscopist that he is not treating an

*Pathologist, Hertzler Clinic.

already developed carcinoma by dilating the esophageal lumen!

Not a single case of adenocarcinoma in this connection has been found in the literature. In all cases reported the tumors have been of the squamous cell type, which indicates degeneration of the surface epithelium.

Treatment.—The triad of events after ingestion of a corrosive agent (in the case here reported, lye) is as follows: First, a severe stricture is created, owing to burn and necrosis of the esophageal mucosa. Second, after an interval of years, malignant degeneration develops at the site of the stricture. Finally, the carcinoma invades the trachea or the bronchial tree by direct extension, initiating a bronchoesophageal fistula. To prevent the aforementioned complications, early resection of the esophageal stricture with primary anastomosis is in our opinion the treatment of choice. It cures the stricture, prevents the development of carcinoma and restores to society a healthy, normal person, instead of leaving an "esophageal cripple" whose span of life may be no longer than forty-five years. With the current progress of modern surgery, the mortality rate of segmental esophageal resection for stricture is low; an occasional failure is a small price to pay if one envisions the miserable lives led by those who accidentally ingest caustics at an age when they can barely speak or stand on their own feet!

SUMMARY

1. A case of carcinomatous degeneration of the squamous cell type at the site of lye stricture, thirty-six years after the ingestion of lye, is reported.

2. A triad of complications takes place after ingestion of lye: (a) severe esophageal stricture; (b) carcinomatous degeneration at the site of the stricture, and (c) bronchoesophageal fistula.

3. The treatment of choice is early segmental resection of the esophageal stricture in order to restore the continuity of the esophagus and to prevent development of carcinoma at the stricture site in later years.

ZUSAMMENFASSUNG

1. Es wird über einen Fall krebsiger Degeneration vom Plattenepithelypus einer Speiseröhrenverengung 36 Jahre nach Verschlucken einer Lauge berichtet.

2. Nach dem Trinken von Lauge tritt eine Trias von Komplikationen auf: a) schwere Verengung der Speiseröhre, b) krebsige Entartung an der Stelle der Striktur und c) Fistelbildung zwischen Speiseröhre und Bronchus.

3. Die Behandlung der Wahl besteht in frühzeitiger Resektion des verengten Speiseröhrenabschnitts, um eine unbehinderte Passage herzustellen und der Entwicklung eines Krebses an der Stelle der Verengung vorzubeugen.

SUMARIO

1. O áutor apresenta um caso de degeneração carcinomatosa de tipo de célula escamosa, no local de estreitamento, ocorrendo 36 anos após a ingestão de soda cáustica.

2. Uma tríade de complicações tem lugar após a ingestão de soda cáustica, (a) grave estreitamento esofageal; (b) degeneração carcinomatosa no local do estreitamento, e (c) fístula bronquioesofageal.

3. O tratamento de escolha é a ressecção segmental precoce do estreitamento esofageal afim de restaurar a continuidade do tracto gastrointestinal e impedir o desenvolvimento do carcinoma no local do estreitamento.

TOXICITY EXPERIMENTS WITH FISH IN REFERENCE TO TRADE WASTE POLLUTION

DAVID L. BELDING

From the Evans Memorial and Boston University School of Medicine

I. THE PROBLEM OF WATER POLLUTION

Of the various problems confronting our fisheries, the one which demands the most immediate attention is the water pollution. If allowed to increase, it means the serious depletion and even ruin of a great part of our inland and coastal fisheries, and the longer it remains unregulated, the more difficult will become its ultimate control. Already it has made such inroads that the restoration of many heavily polluted streams is practically impossible. The water pollution evil is not a mere local issue; its scope is national, and its elimination requires concerted action. The battle for unpolluted streams must be fought simultaneously throughout the country by a well organized campaign.

OBSTACLES.—The elimination of water pollution means overcoming many obstacles, especially in organizing a cooperative campaign among states where local conditions, different laws, and divergent forms of pollution present numerous complications. The antagonism of the manufacturing interests must be overcome and indifferent public opinion aroused by a campaign of education. Laws must be carefully drawn and must be sufficiently broad in their application, as well as rigid in their enforcement to facilitate the handling not only of transitory pollution, but also of cases where polluting material is surreptitiously emptied into a stream under the pretense of a nominally operating disposal system. The present lack of definite knowledge as to the quantity and kind of pollution which will injure fish life is a serious handicap. Since the cost of freeing badly polluted streams in heavy manufacturing centers is prohibitive, it may be advisable to allow them to continue to serve as sewers for waste disposal, but in border line cases which demand a careful consideration of all conditions, proper standards for determining whether or not a stream should be cleared of pollution are badly needed.

EFFECT ON FISH LIFE.—Two kinds of water pollution affect fish life, sewage and trade wastes. Sewage enters the stream from private toilets and cesspools, and in the form of effluents from municipal systems. Owing to its prominence as a public health factor, methods of disposal have been devised by which

it will eventually be controlled. If unregulated, it damages water supplies and manufacturing interests, endangers public health through contamination of edible shellfish, and is injurious to fish environment. Trade wastes include all forms of waste material from industrial sources. Fish preservation is concerned chiefly with this important type of pollution, which directly and indirectly destroys fish life.

Acting directly, pollution reduces the oxygen supply, renders fish unfit for food, drives them from their habitat, and causes death or predisposition to disease. Indirectly, it may cause destruction of eggs and young, reduction of the spawning grounds, changes in bottom and vegetation, and limitation of food supply. The effect of severe recent or transitory pollution in flagrant cases is conspicuously indicated by the presence of dead or dying fish, but unappreciable continuous pollution may be fully as harmful due to a cumulative effect over a long period.

REMEDIAL MEASURES.—Constructive work, in order to avoid strong opposition, must be undertaken gradually. In the first place, new pollution must be prevented; secondly, small streams with few sources of pollution must be cleared; and finally, through the cooperation of manufacturers, the pollution in the larger streams must be eliminated or diminished. The manufacturers can be influenced by the diplomatic but forceful handling of each case. As a last resort in refractory cases where moral suasion is of no avail, rigid enforcement of laws must be invoked. To insure such action simple, direct, readily enforceable laws are necessary. Favorable public opinion is absolutely necessary for the successful enforcement of laws. It may be obtained by making an appeal through the medium of pamphlets, posters, magazine and newspaper articles, and lectures on the basis of fair play, loss of outdoor enjoyment, and danger to public health.

INVESTIGATIONS.—The ultimate solution of the problem of water pollution involves certain biological investigations: (1) Suitable means must be found for eliminating various types of trade wastes. (2) Standards for judging as to when a stream is polluted must be determined. These standards must be applicable to different types of streams and to various kinds and concentrations of water pollution, in order to ascertain the maximum amount each stream may handle with safety. (3) The direct and indirect effect of the various constituents of water pollution upon fish life must be observed.

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Since the factors involved in these biological problems are so complicated, the immediate and ultimate effect of various types of trade waste pollution must be carefully studied before we shall be in a position to determine the exact amount of pollution fish can survive or to formulate standards for judging the amount of pollution any given stream can handle.

II. FACTORS INFLUENCING EXPERIMENTAL STUDIES UPON THE EFFECT OF WATER POLLUTION ON FISH

The chief obstacle in obtaining accurate data relative to the effect of various inorganic and organic wastes upon fish life is the difficulty of controlling the various complicating factors which enter into an experiment. Unless their influence is recognized, errors of interpretation are inevitable. As a rule the conditions, under which these experiments are made, are far from ideal. Field tests must be performed in an environment in which exact laboratory technique is impossible. Necessarily, all experimental work of this nature should be most throughly scrutinized before acceptance.

1. FISH

The selection of the fish for experimental work is of primary importance. All possible safeguards should be taken to secure healthy, normal individuals, since disease and weakness render them susceptible to changes in environment.

INDIVIDUAL VARIATION.—Individual fish, particularily if diseased or weak, vary considerably as to the lethal dose, and unless an ample number of suitable controls are used results may prove untrustworthy. Even healthy fish of the same size, weight, and appearance may show several hours differance in the onset of symptoms and time of death.

FAMILY.—Certain families are more resistant than others, e. g., Cyprinidae vs. Salmonidae. Hardy fish exist where the more delicate cannot survive. With unfavorable changes in environment, the more susceptible fish are the first to perish, although if destruction through natural agencies is limited to a habitat frequented by certain species, it may appear otherwise. Certain families may be resistant to one form of trade waste pollution and susceptible to another, e.g., perch and bass. Obviously, it is impossible to make adequate comparisons as

to the toxic effect of various chemicals unless tests are made with the fish from the same family. Even then the lethal dose for one species may not correspond to that for another.

SPECIES.—A difference in susceptibility between members of the same family is shown by the action of copper sulphate upon Chinook salmon, brook trout, and rainbow trout, which were held in the same pool. The solution which was sufficient to cause the death of all the salmon and seriously effect the brook trout, producing marked symptoms and causing a few deaths, had no apparent effect upon the rainbow trout. The well known resistant qualities of the rainbow trout and the susceptibility of the salmon hold true in protection against chemical poisons.

AGE.—Observations upon fingerlings and adult brook trout indicate that there is a difference between young and old in their resistance to disease and to toxic substances. Brook trout fry are relatively immune to infection with B. salmonicida, whereas adult fish are extremely susceptible. Fry in the yolk sac stage withstand certain trade wastes better than older fry or adult fish. Young fish are more resistant than older fish to some chemicals and less resistant to others, e. g., fingerling trout appear more resistant to carbolic acid, a corrosive poison, and more susceptible to hydrochloric acid than adult trout.

SIZE AND WEIGHT.—The difference in the resistance of old and young fish may be due in part to age and in part to size, weight, and greater surface area which produce greater demands upon the body metabolism. A comparison of the oxygen requirements of forty-one 19 cm. and eighteen 26 cm. trout with the same combined weight illustrates the latter point. The larger trout showed earlier symptoms of distress and consumed during the first two hours nearly twice the quantity of dissolved oxygen used by the smaller fish.

VITALITY.—Experimental results depend upon the general condition of the fish. Vitality varies at different seasons. It is lowest immediately after spawning, during extremely warm weather, and during periods of food scarcity. Although fish reared under artificial conditions have not the vitality of the wild stock, they undergo confinement better and are preferable for experimental work of this character. The active, darting, nervous species tend to perish when closely confined.

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II. ENVIRONMENT

WATER.—The constituents of the water may alter the effect of certain chemicals, e. g., the lethal dose of copper sulphate has been observed by Marsh to vary in different waters from 1:400,000 to 1:6,500,000. In certain waters the presence of carbonic acid may change ammonia to the less toxic ammonium carbonate, sulfids may be more quickly oxidized to sulphates, and buffer action may influence the hydrogen-ion concentration. The waters favorable to fish life will produce a more sturdy species than an unfavorable environment.

Toxicity experiments in a new environment are apt to record an abnormal susceptibility on the part of the test fish, e. g., the transfer of alewives or even the hardy bullhead to cold spring water rapidly lowers their resistance.

CONTAINERS.—The ratio of the size and number of the fish to the container is important. Goldfish, which are accustomed to aquarium life, may be successfully tested in small containers for long periods, whereas large fish die rapidly because of oxygen depletion, increased carbonic acid, and injury in the cramped quarters.

FLOW OF WATER.—Tests with chemicals are most satisfactory when made without change of water. The solution should be of uniform strength before the fish are added. If tests are made with flowing water some method of producing a constant uniform concentration must be devised. Mechanical injury, such as forcing weakened fish against the screen at the lower end of the pool must be avoided.

OXYGEN.—The amount of dissolved oxygen in the water has a direct bearing on toxicity tests. Each species of fish requires a minimum oxygen level for existence and a higher one for normal existence. Unless the surface area of the container is sufficient to supply the necessary oxygen during the course of the experiment, the influence of diminished oxygen must be counteracted by some form of aeration. With trade wastes which have an oxygen demand sufficient to reduce the dissolved oxygen below the point of tolerance, it is difficult to separate the effect of oxygen depletion from chemical poisoning, e. g., with hydrogen sulfid, the dissolved oxygen is decreased with the concentration of the gas and a double effect is produced, hydrogen sulfid poisoning and diminished oxygen.

TEMPERATURE.—Cold water fish can stand a comparatively high temperature when the change is gradual, but sudden

changes may prove destructive. Brook trout are killed by a rapid transfer from 55 to 80 degrees Fahrenheit. Extreme fluctuation in night and day temperature has been observed to cause a high death rate in brook trout fingerlings. Increased temperature, by lowering the vitality of the fish and favoring the growth of the infecting organism, plays an important role in fish epidemics. Similarly it produces more rapid destruction of fish by chemical pollution; e. g., the lethal dose of hydrogen sulfid for carp at 40 degrees Fahrenheit was 34 p.p.m. and at 65 degrees 6 p.p.m.

III. TOXICITY STATISTICS

These variable factors indicate the difficulty of obtaining accurate results in field and laboratory studies of the effect of chemical wastes upon fish and explain the discrepancies in the results of different investigators. They emphasize the importance of carefully controlled tests and the necessity of determining the minimum lethal dose of each chemical for various species of fish in order to establish standards.

Our laboratory and field experiments deal with a single phase of the pollution problem, the immediate effect of certain chemical constituents of trade waste pollution upon young and adult fish. Observations are limited to the symptoms and mortality occurring within twenty-four hours. Although these crude tests determine the concentration which fish may withstand for twenty-four hours, they give no information as to the effect of weaker solutions acting over a longer period of time. For the sake of completeness we have compared our results with the statistics of previous investigators.

METHODS OF WORK.—The chemicals used in these tests comprised acids, bases, salts, and gases, either constituents of trade wastes or remedies used empirically by fish culturists in the treatment of fish diseases. Unless otherwise specified, the chemicals were of high grade purity. Brook trout, rainbow trout, chinook salmon, carp, goldfish, and suckers were used. Owing to their sensitivity to changes in environment brook trout proved the most satisfactory indicators, although carp and goldfish were more convenient to handle. For most tests two to three year old trout averaging about 26 cm. in length and 215 grams in weight were used. Carp, suckers, and goldfish were employed for tests with hydrogen sulfid and paper mill wastes.

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The trout were held without change of water in four wooden tanks containing 780, 646, 147, and 147 liters respectively. Five adults were placed in the larger tanks and two in the smaller. Control tests showed that this number survived without discomfort in these tanks for longer than twenty-four hours. The temperature of the water ranged from 53 to 60 degrees Fahrenheit, averaging 56. In a few instances tanks which received a slow seepage of spring water were used. Gasoline, petrol, leather board, paper mill, and wool scouring waste tests were conducted in tanks and in Chase hatching jars. For laboratory experiments with sodium and hydrogen sulfid, 50 liter glass aquaria supplied with air forced through a Berkefield filter were used. In field work with paper mill waste and hydrogen sulfid, large wooden wash tubs proved convenient.

SYMPTOMS.—Chemical poisons produce certain general symptoms, but the various substances differ qualitatively as to selectivity of symptoms and quantitatively in the rapidity of onset and course of development. The general type and specific characteristics of the chemical determine the symptomatology. At times the selective action of the poison is sufficiently defined as to be of diagnostic value. The early symptoms are more important from the standpoint of differential diagnosis, since the late symptoms are usually confused with those present in all dying fish.

In order to be available for ready reference, the characteristic symptoms produced by twice the minimum lethal dose have been tabulated for the various chemicals (Table 1). A description of the general symptoms in chronological order is given first, and under the individual chemicals, attention is called to those of diagnostic importance.

1. IRRITATION.—The first noticeable sign after the fish is immersed in the chemical bath is increased activity in the form of swimming, wavy movements and rubbing the body against sides of the tank. The degree of activity and rapidity of onset varies with the chemical. With the more irritating chemicals the fish becomes covered with a grayish mucus, evidently a protective reaction. Its absence in the salmon may explain the relative susceptibility of that species.

2. INACTIVITY.—The period of activity is followed ordinarily by a period of sluggishness, in which the fish lies inactive at the bottom of the tank. Response to stimulus during this stage is particularly poor and even during the active stage is below normal.

3. SWIMMING.—The fish first shows restlessness by incessant movement through the water. At times it apparently tries to get out of the noxious environment. Then its movements become more irregular and it aimlessly wanders around the tank, frequently striking the sides. It may glide quickly to the surface and occasionally leap from the water. Intervals of resting at the bottom and surface swimming follow, the movements becoming more and more incoordinate.

4. OXYGEN HUNGER.—Oxygen hunger is manifested by surface swimming and gulping air. It is due either to lack of dissolved oxygen or to interference with gill function.

5. EQUILIBRIUM.—With the approach of death the fish loses its powers of equilibrium. Suddenly it turns on its side and swims with a convulsive flutter. Sometimes it is able to right itself, but more often there is increasingly feeble and spasmodic swimming on the back with slow irregular respirations.

6. RESPIRATIONS.—The respiratory movements of normal trout range from 90 to 100, averaging about 95. Owing to individual variation, it is difficult to determine accurately changes in respiration. With some chemicals there is a temporary increase in the respiratory rate, with others no change or a slight decrease; but with the onset of marked symptoms of discomfort and with the approach of death practically all fish manifest some form of slow convulsive respiratory movement.

LETHAL DOSE.—The minimum lethal dose for the various chemicals is given in Table 2. It represents the lowest dilution capable of producing death within twenty-four hours. For comparison the statistics of other investigators have been included.

1. HYDROCHLORIC ACID.—With minor differences hydrochloric, nitric, and sulphuric acids produce the same general type of symptoms in trout and differ chiefly in respect to the lethal dose. The effect of an acid upon fish depends both upon ionization and upon inherent toxic properties. The toxic dilution of these acids ranges between a pH of 4 and 5, yet fish have been found in waters as acid as pH 4.6.

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Hydrochloric acid is used in plating and in the manufacture of chemicals and dyes. The characteristic symptoms are gradual onset, early surface swimming, lack of response to stimuli, and a mucous coating. There is terminal loss of equilibrium, irregular slow respiration, and frenzied rushing. The minimum lethal dose is 1:100,000, which is stronger than that obtained by other investigators.

2. NITRIC ACID.—Nitric acid is used in the manufacture of fertilizer, chemicals, munitions, etc. It acts more rapidly than hydrochloric and suggests the action of toxic factors other than ionization. The chief characteristics are marked irritation and activity, rapid formation of mucus, decreased forceful convulsive respiration, early loss of equilibrium, and frenzied rushings, particularly in the terminal stages.

3. SULPHURIC ACID.—Sulphuric acid is used in wool scouring, nail and iron works, and in the manufacture of munitions, chemicals, and starch. The onset of symptoms is slower, activity is less noticeable, and sluggishness is more marked than with hydrochloric and nitric acid. There is no frenzied rushing or convulsive actions. The fish, covered with mucus, swim slowly and blindly around the pool aimlessly bumping the sides.

4. ACETIC ACID.—The organic acids present greater diversity of action than the inorganic acids. Acetic acid is used by fish culturists in the treatment of diseased fish. Trout can survive for a short time immersion in a dilution of 1:200. The symptomatology presents no characteristic features; slow onset, moderately increased activity followed by sluggishness, and the formation of a protective covering of mucus.

5. CARBOLIC ACID.—Carbolic acid is characterized by its corrosive action. The most striking feature is its rapid and severe irritating effect even in high dilutions. The fish immediately endeavor to rub off the irritating substance by rolling over on the bottom and by rubbing against the sides of the pool. They show no evidence of oxygen hunger and seek the corners of the pool. In dilute solutions the fish soon lose their equilibrium and lie on their back or side for several hours before death with spasmodic twitching of head and tail. The respiratory movements are slightly increased, become irregular, forceful, and jerky. Dying trout show a general congestion of gills, liver, and viscera.

6. TANNIC ACID.—Tannic acid, an astringent derived from galls and closely related to gallic acid, is used in the manufacture of ink and leather. Trout gradually become uneasy, swimming restlessly to the surface with oxygen hunger and then lie quietly on the bottom with respiration noticeably quickened. Increasing signs of discomfort terminate in erratic swimming, surface jumping, loss of equilibrium, and death. The rapid respiratory rate and oxygen hunger indicate injury to the gills.

7. AMMONIUM HYDROXIDE.—The action of the three alkalis, ammonium, potassium and sodium hydroxide, differs only in degree of toxicity. Ammonium hydroxide is a product of gas and ammoniacal works, the effluents of which have been found to kill fish. The symptoms are rapid and fulminating. The fish dash wildly about, jumping and thrashing at the surface. With the minimum toxic dose signs of irritation appear in two and one-half hours and in three hours the fish turn on their backs with altered respiration and spasmodic struggling. Respirations are markedly decreased and death occurs after five hours. On removal to fresh water dying fish recover in six hours. The symptoms increase with the strength of the solution. In ten times the toxic dose there is immediately frenzied rushing, jumping out of the tank, and swimming on back in 15 minutes. The hydroxide is changed to the less injurious carbonate by free carbonic acid, and therefore the action of this chemical varies in different waters.

8. SODIUM HYDROXIDE.—Trade wastes containing sodium hydroxide are derived from a number of industrial processes. It produces an irritating effect upon the fish which rapidly become covered with mucus. In strong solutions the movements of the fish become sluggish. It comes to the surface gasping and blindly bumps the sides of the tank. In the minimum lethal solution it remains at first quietly at the bottom and then comes to the surface gasping. Later it lies on its back with periods of rapid dashing and leaping. The strong dilutions rapidly inhibit the respiration, but with the minimum lethal dilution no change is noticed for the first two hours, then the respiratory movements become slow and feeble and after the loss of equilibrium show marked irregularity.

9. POTASSIUM HYDROXIDE.—Potassium hydroxide produces the same train of symptoms as sodium hydroxide, namely, sluggishness, irritation and distress, oxygen hunger, decreased respiration and loss of equilibrium. With solutions of similar

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strength the action is less marked than that of sodium hydroxide.

10. ARSENATE OF LEAD.—The particular preparation used contained 12.5 per cent of As_2O_5, 31.5 per cent of $Pb\ O_2$, 0.75 per cent of water soluble arsenic and moisture not over 50 per cent. It is used for spraying trees. Its slow action produces no characteristic symptoms in fish.

11. CALCIUM HYPOCHLORITE.—The toxic action of calcium hypochlorite depends upon the free chlorine and therefore is transitory. With strong solutions the fish pass at once into a stage of irritation, followed by sluggishness and lack of response to stimuli. With dilute solution the onset is insidious and the two stages occur together. The respiratory movements are increased. The fish rubs its body against the sides of the pool and swims to the surface with increasing frequency, which finally culminates in rushing and leaping at the surface and spasmodic gasping. With loss of equilibrium the fish rests head down in the water, turning with a top-like motion. Then it settles to the bottom where it repeats from time to time the rushing tactics which each time become more feeble. The smaller trout were less affected than the larger. The extreme oxygen hunger, surface rushing, and peculiar head balancing are of diagnostic importance.

12. COPPER SULPHATE.—Copper sulphate is used as an algacide for water supplies. Owing to the extreme range of toxicity for different species of fish and for various waters, it is difficult to determine the quantity which may be used for algacidal purposes without injury to fish life. The fish first shows increased activity and then lies sluggishly on the bottom with increased spasmodic respiration. Occasionally it darts through the water rapidly, usually inclined slightly to the side. Loss of equilibrium follows, the fish remains quiet and unresponsive to stimuli, and occasionally struggles to the surface. The effect of the poison persists even after transfer to fresh water which does not save the life of the fish.

13. FERROUS SULPHATE.—Ferrous sulphate occurs in wastes from iron and nail works. With the exception of Clark and Adams, observers find that iron salts have a relatively low toxicity. There is nothing characteristic about the symptoms, which develop slowly.

14. MERCURIC CHLORID.—This chemical is used as an antiseptic. It acts as a tissue coagulant. At death the auricles are filled with blood and the gills pale. The fish, apparently unaffected at first, die suddenly without noticeable symptoms.

15. POTASSIUM PERMANGANATE.—Fish culturists use this antiseptic for the treatment of diseased fish. Fish immersed in strong solutions recover promptly when placed in fresh water. The skin acquires a yellow tinge from the chemical and the gills are deeply injected. The chief symptoms are surface swimming and leaping.

16. HYDROGEN SULFID.—This gas produces in man acute and fatal poisoning in chemical works and sewers through its action on the respiratory tract. It is one of the most toxic of the coal tar wastes. It is associated with decomposition of wood pulp and is formed in small amounts in nature. Since potassium and sodium sulfid hydrolize into hydrogen sulfid in the blood their action is similar. Small amounts are without perceptible effect but larger quantities paralyze respiration. Since it is oxidized rapidly in the body it has no cumulative action. The limit of tolerance of different species of fish has a wide range. Its action depends upon the concentration of the gas and the temperature of the water.

The chief symptoms are irritation, frenzied rushing, respiratory paralysis, partial recovery of respiration, stupor, loss of equilibrium, oxygen hunger, and recovery on removal. The respiratory changes are diagnostic. There is an initial lowering or suspension of respiratory movements followed by a partial recovery and subsequently a decline in rate with increasing irregularity.

TRADE WASTES.—The lethal dose of the trade wastes varies with their composition and dilution. In addition to their chemical constituents certain wastes tend to reduce the supply of dissolved oxygen in the water.

The sulphite liquor from paper pulp manufacture has a slow action. It produces no special symptoms beyond those usually observed in dying fish. Leather board waste acts in a similar manner. With wool scouring waste, which usually contains sulphuric acid and organic debris the fish manifests uneasiness, endeavors to leap out of the tank and shows oxygen hunger.

The writer wishes to acknowledge the able assistance of Mr. J. A. Kitson in conducting the field experiments.

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TABLE 1

SYMPTOMS

	INORGANIC ACIDS			ORGANIC ACIDS			ALKALIS				SALTS				GASES	
	Hydrochloric Acid	Nitric Acid	Sulphuric Acid	Acetic Acid	Tannic Acid	Carbolic Acid	Ammonium Hydroxide	Potassium Hydroxide	Sodium Hydroxide	Arsenate of Lead	Calcium Hypochlorite	Copper Sulphate	Ferrous Sulphate	Mercuric Chloride	Potassium Permanganate	Hydrogen Sulfid
1. Onset																
Rapid		+				+	+				+	+				+
Slow	+		+	+	+			+	+	+			+	+	+	
2. Irritation																
Restlessness	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rubbing movements		+				+					+					
Secretion of mucus	+	+	+	+				+	+							
3. In activity																
Sluggish movements	+	+		+	+	+		+	+	+	+	+	+	+		+
Lack of response to stimuli	+		+	+		+					+	+				+
4. Swimming																
Rapid movements	+	+					+				+	+			+	+
Irregular movements	+	+	+	+	+	+	+	+	+		+	+				+
Frenzied rushing and leaping		+			+	+	+				+				+	+
5. Equilibrium																
Early loss		+				+	+				+					+
Late loss	+		+	+	+			+	+	+		+	+	+	+	
6. Respiration																
Increased					+	+					+	+			+	
Decreased	+	+	+				+	+	+	+						+
Normal				+									+	+		
7. Oxygen Hunger					+		+	+	+		+					+

TABLE 2

LETHAL DOSE OF CERTAIN CHEMICALS FOR FISH

CHEMICAL	AUTHORITY	SPECIES	DILUTION: Killed within 24 hours	DILUTION: No effect
I Inorganic Acids				
Hydrochloric	Belding	Brook trout	100,000	
	Clark & Adams	Carp, shiners, suckers	274,000	
	Marsh	Mummichogs, sunfish	125,000*	
	Penny & Adams	Minnows, goldfish		50,000
Nitric	Belding	Brook trout	640,000	
	Penny & Adams	Minnows, goldfish	50,000*	
	Clark & Adams	Carp, goldfish, suckers, shiners		50,000
				174,000
Sulphuric	Belding	Brook trout	160,000	
	Penny & Adams	Minnows	50,000*	
		Goldfish		50,000
	Wells	Blue-gill sunfish	40,800	277,000
		Mummichogs and sunfish	83,000*	
	Marsh			
II Organic Acids				
Acetic	Belding	Brook trout	20,000	
	Penny & Adams	Minnows	8,750	
		Goldfish	3,500*	
Tannic	Belding	Brook trout	160,000	
	Penny & Adams	Minnows	14,000*	
		Goldfish	7,000	
Carbolic	Belding	Brook trout	160,000	
	Weigelt	Trout	200,000*	
	Penny & Adams	Minnows	70,000*	
		Goldfish	3,000*	7,000
III Alkalis				
Ammonium Hydroxide	Belding	Brook trout	160,000	
	Clark & Adams	Carp, shiners, suckers	77,000*	106,000
	Weigelt	Small fish	33,000*	
Potassium Hydroxide	Belding	Brook trout	20,000	
	Penny & Adams	Goldfish	7,000*	35,000
		Minnows	35,000*	
	Wells	Blue-gill sunfish	18,000	36,000
Sodium Hydroxide	Belding	Brook trout	40,000	
	Clark & Adams	Carp, shiners, suckers	14,000	18,000
IV Salts				
Arsenate of Lead	Belding	Brook trout	40,000	
Calcium Hypochlorite	Belding	Brook trout	Over 100,000	
	Mason	Trout	2,000,000	
	Waring	Tench	200,000*	
		Trout	125,000*	
Copper Sulphate	Belding	Brook trout	250,000	
	Moore & Kellermann	Trout		7,000,000
		Black bass		500,000
	Penny & Adams	Minnows, goldfish	100,000*	200,000
Ferrous Sulphate	Belding	Brook trout	7,500	
	Clark & Adams	Carp, shiners, suckers	157,000	200,000
	Mason	Trout	10,000	20,000
	Penny & Adams	Minnows & goldfish	10,000	

*Time not specified.

TABLE 1—Continued

LETHAL DOSE OF CERTAIN CHEMICALS FOR FISH

Chemical	Authority	Species	Dilution: Lethal within 24 hours	Dilution: No effect
[illegible]	Belding	Brook trout	30,000	
[illegible]	[illegible]	Trout	[illegible]	
Potassium Permanganate	Belding	Brook trout	[illegible]	
[illegible]	Belding	Carp	[illegible]	
		Sunfish (?)	[illegible]	
		Brook trout	[illegible]	
		Sunfish [illegible]	[illegible]	
		Sunfish [illegible]	[illegible]	
[illegible]	Shelford	Carp	[illegible]	
		Sunfish	[illegible]	
		Trout	[illegible]	
	[illegible]	[illegible]	[illegible]	
[illegible]	Belding	Brook trout	[illegible]	
[illegible]	Belding	Brook trout	[illegible]	
	[illegible]	[illegible]		200 (?)
	[illegible]	[illegible]	[illegible]	
	Thomas	Trout	[illegible]	[illegible]
[illegible]	Belding	Brook trout	[illegible]	

*Time not specified

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Discussion

Mr. J. B. Doze (Kansas): Did I understand you to say that the action of copper sulphate on the perch and on the trout was similar, that the trout would stand more than the scaled fish?

Dr. Belding: I have only used it on trout and salmon. The figures which I gave on perch, bass, sunfish, etc., were furnished by Moore and Kellerman back in 1905; and I believe Marsh also has certain figures showing the variation of different species. In the case of perch the dilution was one and one-half millions, and Marsh claims that in the case of trout it should be seven millions.

Mr. Doze: Carp?

Dr. Belding: In the case of carp he gave three millions; but I will be perfectly frank and say that I question its correctness.

Mr. Doze: I was going to question that, because we sometimes kill carp with it when it does not affect the bass. I questioned that particular experiment.

Dr. Belding: It is not my work; I simply quoted it.

Dr. Field: In experiments made with the State Board of Health of Massachusetts on Lake Massapoag for a determination of the quantities of copper sulphate, when one part to three million was used, I found that 550,000 white perch were killed right off the bat. That was the first thing it killed—and a very few bass, a very few suckers, and a very few pickerel, also a few shiners. The white perch was the least resistant of any of the species of fish in that pond.

President Titcomb: My experience is that the bass is the most resistant of the warm water fishes we have here. The carp is quite sensitive. The northern pike is another very resistant species, much more so than the common pickerel, reticulatus. I wanted to ask Dr. Belding whether in these experiments he had a time limit as to the exposures of these fish in each case?

Dr. Belding: Twenty-four hours—everything is based on twenty-four hours. If you did not set a time limit, your results would be very variable.

President Titcomb: You find that the effects of the copper sulphate in our largest natural lake, Bantam Lake, a comparatively shallow body of water, the maximum depth being only about twenty-eight feet, the copper sulphate had some action for a period of five days—very little, however, on the last day. I refer here to the clearing up of algae. Observations were taken daily, and it was found that during the first three days it was quite effective. I speak of that because I had got the impression that most of it was precipitated within twenty-four hours.

Dr. Field: Another element to be taken into consideration in the use of copper sulphate is that the algae floating in the water, such as Anabaena, and so on, will first take up the copper sulphate, thus reduc-

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ing the amount that reaches the fish and causes it to be in greater dilution than it would be if the water were clear.

PRESIDENT TITCOMB: If it is applied when the first stages of the algae begin to appear, a repetition of the dose may be avoided, and the algae is prevented from getting started so that it becomes very thick, as it does in certain waters of this state.

DR. BELDING: There are three factors that account for all these discrepancies. First, the water; because the conditions in it, such as mentioned by Dr. Field, and the chemical constituents differ, so that you cannot compare one body of water with another. Second, the species of fish vary in their resistance. Third, the method of administering the copper sulphate in the pond, whether it is distributed evenly so that the concentration is uniform and not more pronounced in certain parts of the pond than in others.

DR. DAVIS: In connection with Dr. Belding's results so far as tannic acid is concerned, the Bureau has had a rather interesting experience in one of its hatcheries, apparently due to that cause. At Hartsville, Massachusetts, for a number of years they have had very serious mortality among the brook trout fingerlings in the late winter or early spring. But that happens only in years when there is an excessively heavy rainfall, or when a warm spell comes on following a very heavy snowfall, in other words, when there is a good deal of surface water flowing into the hatchery supply. From further extended inquiry I am satisfied that the mortality among these trout only occurred since the chestnut blight. There was a hillside up above the hatchery which was covered with chestnut trees. Since the chestnut blight attacked the trees of course they have all died and the dead trees have fallen down on the hillside. As you know, chestnut bark is one of the richest in tannic acid, I think, of any of our native trees, and the evidence points rather conclusively to the fact that the tannic acid has got into the water carried down into the hatchery, thus resulting in mortality among the fish. Another interesting point there which coincides with Dr. Belding's experiment is the fact that the rainbow trout were not affected. The brook trout were almost entirely killed in several instances, while the rainbow trout were unaffected—fish of the same age, small fingerlings, still in the sac stage, and others which had been fed only two or three weeks.

DR. BELDING: Brown trout are even more resistant.

DR. DAVIS: There were none at this hatchery.

PRESIDENT TITCOMB: Dr. Stillman, in the survey down there where there was a cleaning out of the trout in one or two streams after the deforestation, was the chestnut a factor there?

DR. STILLMAN: Yes, it is a very common occurrence to find streams which have been devastated apparently affected by the acid from decaying tops. Oak and chestnut are the trees which seem usually to account for this condition, although we see a suggestion of organic pollution from such trees as produce the resins and oils, as, for instance, birch oil, but of course those trees are not so plentiful in lumbering operations. It would be very easy to pick out on a map a dozen streams that are not producing trout at the present time and where the cause seems to be entirely the organic acids from careless lumbering. Regulations and laws should be passed which would compel the lumbermen to clean up their tops after the cutting operations. It seems to affect all the fish life—possibly small pickerel less than other fish; but insect life suffers as well. I think that the blame is often laid on the removal of shade in those streams which fail after lumbering operations, when it is not always that; it is quite as often the organic pollution. Dr. Belding has shown what a small quantity of acid is necessary to poison water, and it must be remembered that when the streams are quite low in the summer we get a greater concentration of the acid in the water than when they are flowing strongly in the winter. I think most of the damage is done in ordinary lumbering under those conditions.

MR. A. H. DINSMORE (Vermont): Several years ago we had some landlocked salmon in a raceway under a steep hillside that had been burned over thirty years before. On the hillside there is now quite a heavy vegetable mould from blueberry bushes and other shrubs that have grown up since the fire. In the summer time, when the weather was intensely warm, we had a cloudburst—I was in the West at the time but these are the facts as they came to me. Almost immediately following that, the salmon, among which we had suffered no loss before, began to die in large numbers. We moved them to other waters, but they all died. I was speaking of that to Dr. Davis on one occasion when he was at our place and he told me of a case where a cloudburst coming in very hot weather produced the same effect. He said it was thought to be due to absorption of oxygen in the water—the surface water running in rapidly and the heat of the ground absorbing the oxygen.

DR. DAVIS: I have a number of instances on record of that sort of thing. Where there are very heavy rains during a hot spell, and there is also a great deal of debris and a large amount of organic matter washed in with the water, the supply of oxygen in the water is practically exhausted, and the resulting mortality has in many instances been laid to that. I do not believe that in any of these cases the cause has been actually demonstrated, but I have known of many cases where that would furnish a logical explanation.

MR. DINSMORE: We made a bulkhead back of that raceway with lumber, thinking we would cut out the surplus water. Last summer we got one of these cloudbursts, and we had in a cement bulkhead, a square cement box that supplies this same raceway, probably four or

five hundred trout ranging from four to six inches in length. The water coming into this bulkhead came directly from a pond of about twenty acres. At the time the temperature was high, ranging around 68 to 70 degrees. The trout had made splendid growth all summer, with absolutely no loss. Below that were three or four thousand trout fingerlings, in the raceway where we had lost the salmon—trout that were then from one and a half to two inches long. This shower occurred in the afternoon, and immediately following it, remembering what Dr. Davis had told me, I rushed down to the bulkhead to see what the effect had been on the five hundred fish in the cement bulkhead. I may say that they were not getting any drainage direct from the hillside; they were getting it through a long, narrow neck at the outlet of the pond. The water was rushing down the hillside into this outlet of the pond and then going around into the cement bulkhead. It had been no more than thirty minutes, probably less than that, since the rain started, and there were at least forty dead trout in the bulkhead. Many more were on their backs, and the fish in the raceway below were just beginning to be affected. I jumped into the pond at the head of the cement outlet, dug away some dirt and threw a tremendous flow of the pond water over the surface of the pond, whereupon the fish righted and were all saved. I am sure we would have lost practically all the fish in that bulkhead but for the turning through immediately of the surface waters from the pond.

MR. POWELL: With reference to the chestnut blight, how long would it take the bark of the dead tree to affect the streams?

DR. STILLMAN: We generally see no results the first year. In the cases I have noted personally, the deterioration begins the second year after the cut, and, according to the degree of pollution, it may last possibly six years. In one case I know of an eighteen year pollution, and it is still going on. Ordinarily, though, you can allow twelve to fourteen years to clear.

MR. POWELL: We have had this trouble in Maryland, but although the blight has been on for about seven years, it is just during the past three years that it has been affecting us.

DR. EMBODY: Possibly what I am going to say was covered by Dr. Belding; if it was I beg his pardon. I just want to call attention to two or three conditions resulting from the treatment of a pond with copper sulphate. They have been brought out before; it is nothing new at all, but I think it is well to emphasize them at this time.

First, when you treat a pond with copper sulphate—and you treat it primarily, as I understand, to kill the excessive algae in the pond—you kill not only the algae but also a great many of the food organisms; you kill certain microcrustacea and a great many insects such as naiads, nymphs, and some others. After these organisms are killed

their remains fall to the bottom and begin to decompose, in the process of which they use up the oxygen of the pond. So that you have not only the immediate effects of the copper sulphate but you also have the effects due to lack of oxygen in the pond. The way to overcome that, of course, is, after the treatment with copper sulphate, to run a good supply of water into the pond; you will then overcome that secondary reaction and have only the reaction due to the copper sulphate alone. In killing a large part of the food material or the forage in the pond you are doing something that the fish culturists would not want done, something that the owners of the pond would not want to see brought about in any pond they thought anything of.

MR. DOZE: What experiment would more nearly approach the toxic effect of leaf fall? We have a great leaf fall that kills a great many of our fish. Do you know of any quick remedy for that condition?

DR. BELDING: I do not know exactly what organic acids come from the leaves. I also do not know what the direct action of the acids would be. I do know that in certain of the ponds in Massachusetts in very warm weather, the organic acids and hydrogen-sulphide which are always present to a limited extent in nature, may become detrimental to fish life.

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Chemical Dehulling of Dent Corn[1]

C. W. BLESSIN, W. L. DEATHERAGE, and G. E. INGLETT, Northern Regional Research Laboratory, USDA, Peoria, Illinois

ABSTRACT

A process has been developed that rapidly dissolves the pericarp (hull or bran) of dent corn with alkali and leaves the endosperm, aleurone layer, and germ intact. Various times (2, 4, and 6 min.), temperatures (140°, 160°, and 180°F.), and concentrations (10, 15, and 20% w./w.) were investigated with aqueous solutions of sodium hydroxide to determine its effectiveness as a dehulling agent. Three dent corns (Bear C-14, Bear X-800, and a commercial sample of U.S. No. 2 yellow) were used in this study. In general, a 15% solution of sodium hydroxide at 160°F. essentially dissolved the pericarp in 3 to 4 min. when agitation was adequate. Residual alkali was neutralized with acetic acid. Average yield of the dehulled corn from this treatment was 93%. Microscopic observation showed that the major portion of the aleurone layer was not removed under these conditions. Alkali dehulling had little measurable effect on ash, ether extract, or protein content. Fiber and total niacin levels were reduced to 50% of their original value.

A number of procedures have been proposed for dehulling or peeling cereal grains, including corn, with various chemicals. Pomeranz (1) reviewed treatment of

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grains with sulfate ions, cellulose derivatives, gums, surface-active agents, fat solvents, and strong acid and alkalis. Morgan et al. (2,3) obtained yields of approximately 85 to 90% when wheat was dehulled with 25% sodium hydroxide at 180°F. for 3 min. However, Subba Rao et al. (4) reported a 35% loss in weight when corn was boiled in 10% sodium hydroxide for a period of 4 min. The data indicate that a considerable quantity of the germ and endosperm was removed by the alkali. In contrast, our procedure, when used with proper time, temperature, and alkali conditions, rapidly dissolves the pericarp and leaves the endosperm, aleurone layer, and germ intact. The dehulled product is similar to ordinary corn in appearance, taste, and hardness.

MATERIALS AND METHODS

Materials

Three yellow dent corn samples were used in this investigation. Bear C-14 and Bear X-800 were grown in the vicinity of Decatur, Ill. These two samples of corn were dried at air temperatures not exceeding 140°F. The third sample, of unknown origin and identity, came from a local elevator and was graded No. 2 yellow. Drying and handling conditions used with this commercial sample are not known.

Methods

Various combinations of alkali concentrations (10, 15, and 20%, w./w., aqueous solutions of sodium hydroxide), times (2, 3, 4, and 6 min.), and temperatures (140°, 160°, and 180°F.) were investigated in this work.

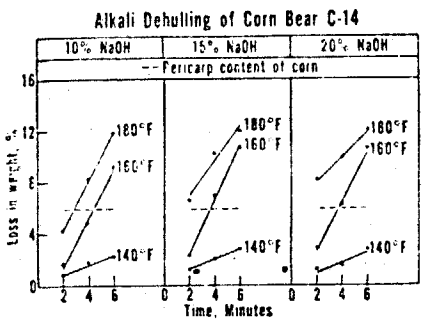
Exploratory studies were conducted with 25-g. samples of sound whole kernels tempered with water at 140°F. for 3 to 4 min. The corn was drained, treated with 50 ml. of aqueous alkali solution for the indicated times and temperatures, and washed immediately with 140°F. water to prevent further action of the alkali. Residual alkali was neutralized by submerging each sample in 50 ml. of 5% glacial acetic acid (v./v.) for 5 min. at 140°F. After rinsing with 140°F. water, the product was drained and air-dried. The corn was stirred continuously during the alkali-treating, neutralizing, and rinsing operations.

Larger samples were prepared similarly. A 1,000-g. sample was tempered at 140°F. for 5 min., drained, and heated with an alkali solution under various conditions. The dehulled corn was drained and washed six to eight times with 140°F. water. Residual alkali in the drained and washed product was neutralized with 2,000 ml. of 5% glacial acetic acid at 140°F. during a 5-min. period. The neutralized product was washed four to six times with 140°F. water. The sample was drained and dried at 113°F. in a rotating dryer to approximately 12% moisture.

The extent of removal or retention of the pericarp and aleurone layer was determined by microscopic examination of kernels stained for 1 to 3 min. with a solution of iodine and potassium iodide. Total niacin, ash, fiber, ether extract, and protein were determined by standard AOAC methods.

RESULTS AND DISCUSSION

Effects of temperature, concentration, and contact time of the various alkali solutions on the amount of material removed from the corn are shown in Figs. 1, 2, and 3. Pericarp content of Bear C-14, Bear X-800, and No. 2 yellow determined by



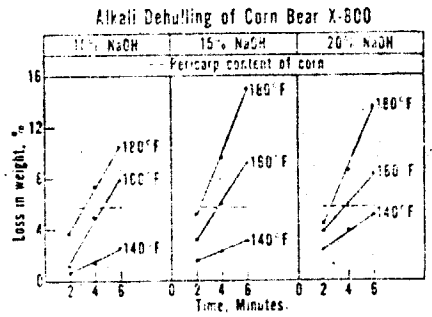


Fig. 1 (left). Effect of time, temperature, and alkali concentration on dehulling of Bear C-14 corn.

Fig. 2 (right). Effect of time, temperature, and alkali concentration on dehulling of Bear X-800 corn.

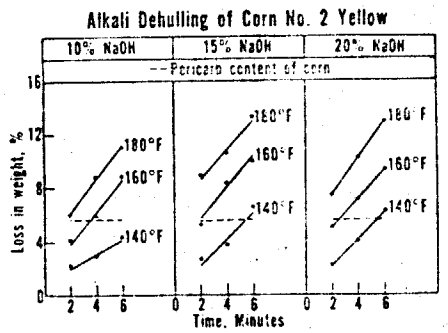


Fig. 3. Effect of time, temperature, and alkali concentration on dehulling of No. 2 yellow corn.

hand-dissection was 5.9, 5.7, and 5.6%, respectively. A loss in weight equivalent to the amount of pericarp in the sample (indicated by the dashed line in the figures) was used as a preliminary guide to select the most satisfactory conditions for dehulling. Alkali dehulling was temperature-dependent. When temperatures were increased from 140° to 160°F., and then to 180°F., larger quantities of material were removed at any specific time or alkali concentration. The slope of the 140°F. line was usually less than that of either the 160° or 180°F. line. This effect was more pronounced with Bear C-14 than with the other two corns. A temperature of 140°F. was not suitable, under the conditions studied, for any of the three corn samples, since the amount of material removed from the kernels was usually less than the weight represented by the pericarp. Apparently a certain temperature was necessary for rapid wetting or penetration of the pericarp. An increase in alkali concentration from 10 to 15% usually was beneficial. However, an increase in concentration from 15 to 20% was not advantageous. On the basis of data obtained with these three corns, the most suitable temperature and alkali concentration for the removal of the pericarp appeared to be 160°F. and 15%, respectively. An

TABLE I. ALKALI DEHULLING OF CORN: COMPARISON OF SAMPLE SIZE

Corn Sample	Time	Loss in Weight[a]		Corn Sample	Time	Loss in Weight[a]	
		Sample, 25 g.	Sample, 1,000 g.			Sample, 25 g.	Sample, 1,000 g.
	min.	%	%		min.	%	%
Bear C-14	2	2.6	5.0	No. 2 Yellow	2	5.7	4.4
	4	6.7	8.1		3	6.9	6.3
	6	10.9	11.3		4	8.1	8.1
					6	10.4	11.9
Bear X-800	2	3.1	3.5				
	4	6.1	7.5				
	6	9.1	11.6				

[a]With 15% NaOH at 160°F.

increase in time, regardless of temperature or alkali concentration, resulted in removal of larger quantities of material.

Larger samples (1,000 g.) were prepared at various time intervals with a 15% sodium hydroxide solution at 160°F. Data obtained with 1,000- and 25-g. samples prepared under the same conditions are compared in Table I. Slightly higher values for some of the 1,000-g. samples are probably due to losses of fines or broken material in the rotary dryer.

Visual inspection of the samples indicated that removal of the pericarp with alkali is essentially due to a solution or dissolving action rather than loosening and then removing by mechanical action. Removal of a portion of the pericarp from the kernel is more noticeable with varieties, such as Bear X-800, which contain a highly pigmented pericarp. Examination under a microscope of alkali-treated whole kernels stained with iodine and potassium iodide provided a means for estimating more quantitatively the degree of removal of pericarp and aleurone layer. Under the conditions of this test the pericarp either does not stain or stains brown; the aleurone layer, yellow; and the endosperm, blue.

Some differences in behavior with respect to alkali dehulling were noted for the three corns (Table II). Treatment of Bear C-14 for 2 min. with 15% NaOH at 160°F. was not effective in removing the pericarp, since 80% of the kernels retained one-half or more of the original quantity of pericarp. Of course, none of the aleurone layer was removed by this treatment. During an intermediate time period, 4 min., the pericarp was almost completely removed, and yet the dehulled sample retained approximately 100% of the aleurone layer. A 6-min. treatment removed both pericarp and aleurone layer.

Data obtained with Bear X-800 and No. 2 yellow were not so clear-cut as with Bear C-14. Treatment of Bear X-800 for 2 min. removed some of the pericarp but none of the aleurone layer; whereas with No. 2 yellow this same treatment removed appreciable quantities of both the pericarp and aleurone layer. A 6-min. treatment removed most of the pericarp and aleurone layer from the kernels of both samples. Of the time intervals examined, 4 min. was selected for Bear X-800 and 3 min. for No. 2 yellow because these time periods removed most of the pericarp and left

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TABLE II. REMOVAL OF PERICARP AND ALEURONE LAYER FROM CORN

Corn Sample[a]	Time	Quantity of Pericarp and Aleurone Layer Remaining after Alkali Treatment									
		Less Than One-Fourth		One-Fourth		One-Half		Three-Fourths		More Than Three-Fourths	
		P[b]	A[b]	P	A	P	A	P	A	P	A
	min.										
Bear C-14	2	10[c]		10		10		15		55	100
	4	90		5		5					100
	6	100	95		5						
Bear X-800	2	15		5		10		55		15	100
	4	70	55	25	25	5	15		5		
	6	100	100								
No. 2 yellow	2	55	20	25	20	10	15	5	5	5	40
	3	95	25	5	35		5		25		10
	4	100	60		30		5		5		
	6	100	75		20		5				

[a]15% NaOH at 160°F.

[b]P, pericarp; A, aleurone layer.

[c]Percentage of total kernels with specified quantity of pericarp and aleurone layer remaining after alkali treatment.

appreciable quantities of the aleurone layer with the endosperm. An average yield of 93% of dehulled material was obtained under these conditions.

Data on the effect of alkali dehulling on composition were obtained with No. 2 yellow corn processed for 2, 3, 4, and 6 min. with 15% alkali at 160°F. (Table III). As previously discussed, a 3-min. processing was considered most satisfactory for this corn. Processing under these conditions had little measurable effect on ash, ether extract, or protein content. Fiber content was reduced from 2.3% in the unprocessed corn to approximately 1% in the 3-min. sample. Further processing did not reduce fiber content. Another effect of alkali dehulling is a reduction in total niacin content. Niacin values reported in Table III represent total niacin (free plus bound), since the AOAC method used for this determination involves hydrolysis.

TABLE III. COMPOSITION OF ALKALI-DEHULLED CORN[a]
(Moisture-free basis)

Time min.	Total Niacin γ/g.	Ash %	Fiber %	Ether Extract %	Protein %
Unprocessed	27.6	1.2	2.3	4.1	9.3
2	14.2	1.3	1.4	3.6	9.7
3	11.4	1.4	1.1	4.1	9.3
4	8.1	1.3	1.0	3.7	9.4
6	4.9	1.3	1.1	3.4	9.1

[a]No. 2 yellow, 15% NaOH, 160°F.

Processing of this particular sample of corn for 3 min. reduced total niacin level to approximately 50% (11.4 γ per g.) of its former value (27.6 γ per g.).

Two areas of research require further investigation. Stress cracks in the endosperm were evident in many of the dry alkali-dehulled kernels. Some of the stress cracks existed in the unprocessed corn, especially the commercial No. 2 yellow. Future studies on alkali dehulling will examine the effect of tempering, washing, and drying temperatures on stress-crack formation. The procedure described here does not remove the tip cap or the highly pigmented hilar layer. The response of alkali-dehulled corn to wet- and dry-milling and the distribution of the tip cap and hilar layer in dry-milled fractions are now under investigation.

Acknowledgment

Two samples of corn were supplied by Bear Hybrid Corn Company, Inc., Decatur, Ill. Miss Irene M. Cull and V. R. Piscitelli assisted in collecting the data reported.

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2,752,248

PREPARATION OF EGG WHITE ADDITIVES

Philip Beck, Chicago, and Ervin W. Hopkins, Hinsdale, Ill., assignors to Armour and Company, Chicago, Ill., a corporation of Illinois

No Drawing. Application May 6, 1952, Serial No. 286,422

7 Claims. (Cl. 99—113)

This invention relates to egg white additives. More particularly, this invention relates to additive compositions for egg whites which improve the foaming properties of the egg whites, such as rate of foam formation, foam volume, and foam stability.

Large quantities of fresh, frozen, and reconstituted egg whites are used to prepare various food products. For most purposes, it is necessary to whip the egg whites into a light, fluffy foam before they are incorporated in the food products. For example, large quantities of whipped egg whites are used in making angelfood cakes, nougat cream, etc. The foaming properties of egg whites, relating both to the rapidity of foam formation and the volume of the foam, are therefore of considerable commercial importance. Also, the stability of the foam is a matter of importance. Egg whites vary considerably as to the time required to whip the egg whites, the resulting volume of the whipped whites, and the stability of the whipped product. Some egg whites can not be made to whip to any appreciable extent within reasonable whipping times. The explanation for this is not understood, nor are the factors or properties of egg whites which tend to produce rapid foam formation, and large volume of foam.

Additives have long been sought which would improve the foaming properties of egg whites, and particularly the properties indicated above, which tend to produce rapid foam formation, large foam volume, and foam stability. To be of practical importance, of course, additives for accomplishing these results must be edible, and also must not unduly interfere with the normal storage and use of egg whites. Heretofore, no satisfactory additives for egg whites conforming to these requirements have been known.

It is therefore a general object of this invention to provide additive compositions for egg whites which are capable of favorably influencing the foaming properties of egg whites, and thereby accomplishing one or more of the objectives set out above; namely, increasing the rate of foam formation, the volume of foam, or the stability of the foam. It is also a primary object of this invention to provide a method for producing additive compositions of the type indicated. Another object of this invention is to provide an improved egg white whipping composition including ordinary egg whites in combination with minor proportions of an additive composition capable of cooperating with the egg whites to improve the properties of the mixture. Further objects and advantages will become apparent as the specification proceeds.

In one of its phases, this invention is based on the discovery that a composition capable of improving the foaming properties of egg whites can be prepared from egg whites themselves by subjecting the whites to a mild alkali treatment. One surprising aspect of this discovery is that the alkali-treated egg whites are of little value when employed by themselves, their unexpected utility arising when they are used as an additive to ordinary, untreated egg whites. Further specific discoveries will subsequently be discussed in detail.

The starting material for preparing the additive compositions of this invention can be designated generally as egg whites. Either fresh, frozen, or reconstituted dried egg whites can be employed. However, fresh or frozen egg whites are preferred, since it is desirable to have the egg whites in liquid form for treatment with the alkaline agent.

In carrying out the method of this invention the egg whites are treated with an alkaline agent capable of bringing about gelation of the egg whites. The alkali metal hydroxides, and particularly sodium and potassium hydroxide, are the preferred treating agents. Excellent results are obtained by using sodium hydroxide. However, other alkaline agents which are well known equivalents of sodium and potassium hydroxide for producing mild alkaline conditions in aqueous solutions can be employed. For example, trisodium phosphate, sodium carbonate, etc., can be used.

Preferably, the alkaline agents are introduced into the egg whites in an aqueous solution, and a sufficient amount of the aqueous solution is added to bring about gelation of the egg whites at ordinary room temperatures (20 to 25° C.). When employing alkali metal hydroxides, such as sodium and potassium hydroxide, it has been found desirable to add at least .5% by weight of the hydroxide to the liquid egg whites. Concentrations of between about 1 to 3% by weight of the alkali metal hydroxide are particularly desirable, and excellent results are obtained when employing concentrations of around 2% by weight. Higher concentrations up to at least 5% by weight may be employed if desired, but there does not appear to be any particular advantage in employing higher concentrations, and in particular concentrations above 5%.

The temperature of the liquid egg whites at the time of the addition of the alkaline agent is not particularly critical. If desired, the addition can be made at ordinary room temperatures (20 to 25° C.). Temperatures from 0 to 60° C. can be employed, but it is desirable to keep the temperatures of the egg whites below about 50° C. to prevent extensive hydrolysis.

No particular technique is required for adding the egg whites other than that usually employed for mixing such additives. For example, the aqueous solution should be added slowly, and preferably with stirring of the egg whites to mix the alkaline agent therewith. A thorough and rapid stirring is necessary to prevent formation of lumps where the local concentration of alkali is high. A gel generally begins to form immediately upon the addition of the alkaline agent, and the egg whites set to a solid, rubbery condition in a few minutes. It is not known whether gelation of the egg whites is essential to accomplish the results of the present process or whether the gelation is merely a physical concomitant of the desired chemical, or physical-chemical modification of the egg whites. However, it is believed that the rapid gelation of the whites on the addition of the alkaline agent tends to inhibit hydrolysis of the protein constituents. Since it is not desired to appreciably hydrolyze the proteins in the egg whites during the alkaline treatment, the production of the gel state is a convenient method of carrying out the treatment. It permits carrying out the treatment at somewhat greater concentrations of the alkaline agents than would otherwise be possible, and also longer contact times can be employed.

The next step in the process is ordinarily the liquefaction of the gel. This may be accomplished either by allowing the egg whites to undergo self-liquefaction or by promoting the liquefaction of the egg whites with

heat. The firmness of stiffness of the gel, and indirectly the relative ease of liquefaction, can be controlled in various ways. For example, additional water can be added to the egg whites to prevent the gel from being too firm, and thereby more difficult to handle. At low concentrations of the alkaline agent, say around .7% by weight, it is necessary to heat the gelled egg whites to bring about liquefaction within any reasonable period of time for a commercial process. At higher concentrations of the alkaline agent, say around 2% by weight, spontaneous liquefaction of the gelled egg whites will occur at ordinary room temperatures. For example, at temperatures of between about 70 to 75° F., gelled egg whites containing 2% sodium hydroxide will spontaneously liquefy in from 24 to 36 hours. However, to speed up the liquefaction process at all concentrations of the alkaline agents, it is highly desirable to heat the gelled egg whites somewhat above room temperature. In general, heating temperatures of between about 30 to 50° C. are preferable. However, temperatures up to 60° C. can be employed, although the results are not as desirable as when the temperature is kept below 50° C. The alkali-treated egg whites are considerably more resistant to heat coagulation than untreated egg whites, and therefore it is possible to employ temperatures somewhat above the normal heat coagulation temperatures of egg whites. However, the use of high temperatures tends to cause the egg whites to develop a red-brown color, which is believed to be due to a reaction between the reducing sugars present in the egg whites and the alkaline agent during the heating period. The reducing sugars can be removed by extensive fermentation of the egg whites with microorganisms, but this additional step is not necessary when the lower temperatures are used as preferred. Furthermore, the liquefaction of the gel is promoted by employing concentrations of sodium or potassium hydroxide within the indicated preferred range of from 1 to 3% by weight.

Upon completion of the liquefaction of the gelled egg whites, it is highly desirable to neutralize the treated whites, or more specifically to restore the treated whites to the normal pH of egg whites. The normal pH of egg whites varies somewhat, but it is usually within the range from about pH 7.5 to 8.5. In some cases, the pH of egg whites may be as high as pH 9.0, but this is rather unusual. pH adjustment of the egg whites should be made with an edible acid, such as hydrochloric, tartaric, citric, etc. An aqueous solution of hydrochloric acid is preferred. The amount of acid to be added to bring the treated egg whites within the desired range can be easily determined by standard titration procedures.

The neutralized, treated egg whites can then be used directly as an additive to improve the whipping properties of untreated egg whites. For example, the alkali-treated whites in liquid form can be added to fresh egg whites or thawed frozen egg whites. The resulting composition can then be used immediately, or preserved by drying or freezing according to the usual procedures. If desired, the liquid alkali-treated egg whites can be dried before addition to untreated egg whites. Any suitable drying procedure can be employed, such as spray drying, pan drying, etc. As indicated above, the tolerance of the alkali-treated egg whites to heat is greater than ordinary egg whites, and therefore the drying temperature are not especially critical.

The amount of the alkali-treated egg whites to be added to untreated egg whites will vary somewhat depending upon the results desired to be accomplished. In general, it is desirable only to employ minor proportions of the alkali-treated egg whites in admixture with major proportions of untreated egg whites. More specifically, a desirable enhancement of the properties of egg whites can be obtained by forming a mixture of alkali-treated egg whites and untreated egg whites containing from about 1 to 10% by weight of the alkali-treated egg whites.

For specific purposes, more limited proportions may be desirable. For example, when the egg whites are to be used to prepare meringue, it has been found desirable to incorporate from 5 to 10% by weight of the alkali-treated egg whites in the mixture. For preparing an egg white composition to be used in making angelfood cake, it has been found that the composition should contain between about 1 to 5% by weight of the alkali-treated whites.

While the above steps result in a highly desirable product which has great utility as an additive to egg whites, it has been found desirable to employ additional steps which still further improve the quality of the product. One of these additional steps involves the treatment of the egg whites to render them substantially free of hydrogen sulfide. The chemical reaction that ensues during the alkaline treatment of the egg whites is detectable by the odor of ammonia gas which is evolved during the course of the reaction. A slight odor of hydrogen sulfide is also detectable, which becomes much more noticeable upon adjusting the pH of the egg whites to their normal pH range upon the completion of the alkaline treatment. Apparently sulfhydryl bonds are broken during the course of the reaction, and some hydrogen sulfide is formed. Upon neutralization of the liquefied egg whites, a considerable amount of hydrogen sulfide is produced, and the odor is readily detectable. Since the presence of hydrogen sulfide in a food product is undesirable, it is preferred to treat the egg whites to either remove or prevent the formation of the hydrogen sulfide so that the finished product is substantially free of hydrogen sulfide. This can be done most conveniently by treating the liquefied egg whites either before or after neutralization with an oxidizing agent capable of destroying hydrogen sulfide, sulfhydryl groups, etc. by oxidation. Hydrogen peroxide is preferred for this purpose, because it decomposes into oxygen and water. However, other edible oxidizing agents can be employed, and in particular peroxide oxidizing agents, such as barium peroxide, sodium peroxide, and organic oxides such as acetyl peroxide and benzoyl peroxide. A sufficient amount of the peroxide treating agent should be employed to prevent the odor of hydrogen sulfide from being noticeable in the neutralized egg whites. When employing hydrogen peroxide, as preferred, concentrations of around .1 to 3% by weight of the hydrogen peroxide in the egg whites are satisfactory, and excellent results are obtained at concentrations between about .1 to .5%. Hydrogen peroxide can be added in the aqueous solution, such as, for example, a 5% commercial aqueous solution of hydrogen peroxide. Although the oxidizing agent can be added either before or after neutralization of the egg whites, it is preferred to add it before neutralization so as to prevent the development of a noticeable hydrogen sulfide odor.

A further step which is desirable in the production of a high quality product is the homogenization of the liquid egg whites to assure that all of the protein constituents are in suspension. Upon addition of the acidifying agent in the neutralization step, following the liquefaction of the gelled egg whites, flock formation is observable as the acid is added, and, even though the mixture is continually stirred during the addition of the acid, upon the completion of the addition, there will generally be some insoluble protein precipitate present. This insoluble precipitate tends to give the egg whites a cloudy appearance, and therefore it is desirable that the precipitate be either resuspended or removed. It can be removed by filtration, but it has been found preferable to merely pass the egg whites through a homogenizer, which results in the breaking up and resuspension of the precipitate. Also, of course, the liquid alkali-treated egg whites can be added to ordinary liquid egg whites, and the whole mixture homogenized.

The chemical nature of the product produced by the process described above and characterized by its utility as an egg white additive has not been completely deter-

mined. However, it is believed to be a composition in which an alkali metal is chemically associated with the egg white proteins. In particular, it is believed that the preferred product can be described as a sodium or potassium egg white proteinate. The egg white additives produced in accordance with this invention are also characterized by being alkali-treated egg whites in which the protein is substantially unhydrolyzed as compared to its original form. For example, analytical tests indicate that the increase in free amino groups during the alkaline treatment is negligible. In other words, severe alkaline hydrolysis of the egg white proteins is not essential to producing the product of the present invention; and, in fact, it is believed to be highly desirable to minimize the increase in free amino groups. More specifically, it is believed that the maximum increase in free amino groups should not exceed 10 mg. amino nitrogen per gm. of egg white solids, and preferably not more than 5 mg. amino nitrogen per gm. egg white solids.

For the purpose of more fully illustrating the present invention it is desired to set out the following examples.

Example I

To 30 g. of defrosted frozen egg white, 0.6 g. of NaOH dissolved in a minimum of water was added with stirring. A stiff gel formed at once, but became liquefied after four days. The alkaline egg white was adjusted to pH 8.3 with HCl, homogenized, and 10 per cent by volume added to fresh egg white. Whipping tests of the egg white under constant conditions gave the following results:

	Height of foam, in.	Drop from 75 g. foam, 1 hr., mm.
Egg white control	[illegible]	[illegible]
Egg white+10% alkali-treated white	[illegible]	[illegible]

A whipping test was done in the same manner with another preparation of egg white treated with NaOH, and then added at the level of 5% to fresh egg white.

	Height of foam, in.	Drop from 75 g. foam, 1 hr., mm.
Egg white control	[illegible]	[illegible]
Egg white+5% alkali-treated white	[illegible]	[illegible]

In either the 5 or 10% addition cases, a greater volume of foam was obtained, and while the foam stability appears to be increased by 10%, 5% of alkali-treated white was not effective.

Example II

The action of egg white prepared in the manner described in Example I was tested in making angel food cakes. Egg white is whipped at a high speed until considerable foaming occurs, cream of tartar, salt and one half of the required sugar are added. Whipping then proceeds until the whites have a dull appearance and the flour and remaining sugar are then folded in gently. It was found that egg white of normal whipping characteristics containing more than 2 or 3% of the alkali-treated whites gave angel food cake batters of large volume, but poor stability. While baking, these batters collapsed to a considerable extent, and cakes with coarse texture were the result. When 2 or 3% of the egg white preparation was contained in the batter, the maximum foam volume developed in shorter than normal time, and cakes with texture of the desired fineness were obtained. A test with egg whites having very poor whipping qualities shows that some cases may require higher percentages of the treated white than 2 to 3%, as follows:

Egg white control—whites whipped for 20 minutes with little or no foaming.

Egg white +5% alkali-treated white—whipping completed in 18 minutes.

In this case a cake of normal texture and volume was made from egg white which would not whip at all within practical limits of whipping time. Even after the alkali-treated white was added, the time required to obtain the required foam valume was about twice that normally experienced, but the addition made it possible to use the egg whites.

Example III

The required quantity of sodium hydroxide to make a 2% concentration in 87.5 lbs. of egg white was dissolved in water, and added gradually with stirring to the egg white. A rubbery gel formed in a few minutes. This was held at a temperature of 70 to 75° F. overnight, and by morning, most of the gel had liquefied. Remaining parts of the gel were broken up mechanically, and when heated to 30 to 35° F. were liquefied after about 3 hours. Hydrochloric acid was added until a pH of 8.5 was reached, and the precipitated protein was resuspended by means of a colloid mill. The egg white was dried in a laboratory spray drier. This preparation when reconstituted to the normal solids content of egg white (10%) and 3% of the solution added to slow-whipping egg white reduced whipping time from 18 to 10 minutes.

Example IV

Baking tests had shown that the addition of 2 or 3% of alkali-treated egg white to either fresh or thawed frozen whites decreased the whipping time of angel food cake batters. An important question was whether the alkali-treated white could be mixed into egg white just before freezing and still retain through a period of storage extending over several months the effect on foaming which was observed in fresh whites.

Well-mixed fresh egg white was divided into two portions, and from one 5 1100 g. amounts were weighed into tin containers. To the other portion, 3% of alkali-treated white was added and mixed in, and this also divided into 5 1100 g. amounts. Four of the containers from the two sets were placed in a freezer, and a pair was removed at intervals up to 4½ mos. for use in making angel food cakes. Storage temperature was 0 to 10° F., the results were as follows:

BATTER WHIPPING TIMES

Time of Storage	Egg white control, min.	Treated egg white[1], min.
None, not frozen	[illegible]	[illegible]
17 days	[illegible]	[illegible]
1 mo.	[illegible]	[illegible]
1½ mos.	[illegible]	[illegible]
4½ mos.	[illegible]	[illegible]

[1] Egg white+3% of alkali-treated white.

Observations on volume and texture of the cakes indicated that these were satisfactory. The results show that the effect on whipping times of the small addition of alkali-treated white at the time of freezing is retained for at least 4½ months.

Example V

In the above examples, the odor of hydrogen sulfide became apparent when the egg white was neutralized after liquefaction of the alkali gel had occurred. At a 2 or 3% dilution of the alkali-treated egg white in normal egg white, there was little or no hydrogen sulfide detectable, but the possibility of even small amounts being present is undesirable. Oxidation of hydrogen sulfide by low concentration of hydrogen peroxide was accomplished as follows:

To 75 ml. of liquefied alkali-treated egg white, 5 ml. of 5% hydrogen peroxide were added. This gave a concentration of 0.31% hydrogen peroxide. After 15 minutes, the egg white was acidified to a pH of 2.5, but no hydrogen sulfide could be detected. Lower concentrations

of hydrogen peroxide, such as 0.21% may also be used, but a longer time is required for complete reaction. In this latter case, residual hydrogen sulfide was apparent on acidifying after 15 minutes, but not after 20 minutes.

Example VI

For the purpose of determining the extent of hydrolysis of the egg white proteins under the conditions employed to prepare the egg white additives of the present invention, the following tests were conducted:

To the 10 g. of fresh egg white, 0.5 ml. of 40% by volume NaOH was added with agitation. This addition gives a 2% concentration of alkali in the egg white. Part of the flasks were incubated at 4–5° C., and part of 37° C. For titration, 90 ml. of distilled water were added. When any lumps of gelled white were present these were broken up, the solution let stand for 1 hour, and the lumps strained out. Dilute acid was added to the phenalphthalein end-point, neutral formaldehyde added, and the solution back-titrated with NaOH.

The titrating solution was 0.238 N. For comparative purposes, it was calculated to 0.2 N basis. A solids content of 12.2% was used in calculating the results to the ml. of 1% solution.

Converting the results to amino-N equivalency (1 ml. 0.2 N alkali=2.8 mg. amino-N), the following table was constructed.

	ml. 0.2 N NaOH per cc.	0.2 N NaOH/g. egg white, ml.	amino nitrogen g. egg white, mg.
Control egg white	0.014	.170	4.76
4–5° C.			
5 days	.0200	.244	6.83
9 days	.0285	.350	7.00
37° C.			
1 hour	.0156	.190	5.32
24 hour	.0174	.212	5.94

Corresponding analytical results on dried egg white preparations were as follows:

	Ml. 0.2 N NaOH per cc.
Lyophilized fresh egg white	.0095
Spray dried egg white	.0107
Alkali-treated white, pan dried	.0048
Alkali-treated white, spray dried	.0050

These figures differ by amounts within the experimental error, and are all somewhat lower than the value found for fresh egg white.

While in the foregoing specification specific embodiments of the present invention have been set out in considerable detail for purpose of illustration, it will be apparent to those skilled in the art that many of the details set forth can be varied widely without departing from the spirit of the invention.

We claim:

1. The process for preparing an egg white additive, which comprises treating liquid egg whites with between about 1 to 3% by weight of sodium hydroxide to bring about the gelation of said egg whites, liquefying the gel thus obtained by heating it at a temperature between 30 to 50° C., and adding a sufficient amount of an edible acid to the liquefied product to restore it to about the normal pH of egg whites.

2. The process for preparing an egg white additive, which comprises treating liquid egg whites with at least .5% by weight of an alkaline agent selected from the group consisting of sodium hydroxide and potassium hydroxide to bring about the gelation of said egg whites, liquefying the gel thus formed by heating said gel, adding an acid to the liquefied product to restore it to about the normal pH of egg whites, and also treating said product with a peroxide oxidizing agent to render it substantially free of hydrogen sulfide.

3. The process steps of claim 2 in which said peroxide oxidizing agent is hydrogen peroxide.

4. The process for preparing an egg white additive, which comprises treating liquid egg whites with between about 1 to 3% by weight of sodium hydroxide to bring about the gelation of said egg whites, liquefying the gel thus obtained by heating said gel at a temperature ranging from 30 to 50° C., treating the liquefied product with hydrogen peroxide to free it of undesirable odors, also adjusting the pH of the liquefied product to about the normal pH of egg whites, and homogenizing the liquefied product.

5. The process for preparing an egg white additive, characterized by the steps of gelling egg whites under alkaline pH conditions without hydrolyzing said egg whites by treating said egg whites with at least .5% by weight of an alkaline agent selected from the group consisting of alkali metal hydroxides and basic alkali metal salts, liquefying the gel thus obtained by holding said gel at a liquefaction-promoting temperature, and restoring the liquefied product to around the normal pH of egg whites.

6. The process for preparing an egg white additive, characterized by the steps of gelling egg whites under alkaline pH conditions without hydrolyzing said egg whites by treating said egg whites with at least .5% by weight of an alkali metal hydroxide, liquefying the gel thus obtained by heating said gel, and acidifying the liquefied product to restore said product to around the normal pH of egg whites.

7. The process steps of claim 6 in which said alkali metal hydroxide is employed in a concentration of between about 1 to 3% by weight.

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An improved method for canning 3-fold concentrated whole milk

By P. W. BOARD
Division of Food Preservation, C.S.I.R.O., Ryde, N.S.W., Australia
and J. T. MULLETT
Hunter Valley Co-operative Dairy Co. Ltd, Hexham, N.S.W., Australia

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Summary. A method is described for canning and sterilizing concentrated whole milk containing 36·5% total solids. The product had acceptable organoleptic properties and did not form sediment or undergo age-thickening during storage. The method involved initially increasing the pH of the raw milk and forewarming. The product was not homogenized nor were polyphosphates added.

Investigations of methods for producing 3-fold concentrated sterile milk have been mainly confined to workers in the U.S.A., who include Leviton, Pallansch & Webb (1962) and Seehafer (1967).

Seehafer (1967) described the commercial process for producing this product. Raw milk is forewarmed for 30 min at about 74 °C and then concentrated under vacuum to about 35% total solids (TS); after chilling, the concentrate is adjusted to 34·2% TS and polyphosphates or sodium carbonate are added to improve the physical stability and flavour of the final product. The concentrate is filtered, heated quickly to 66–71 °C in a heat exchanger and sterilized by heating to 146 °C for 2 s. The sterile product is cooled quickly to 66 °C, homogenized at 41·4 MN/m² (6000 lb/in.²), cooled to 10–16 °C, filtered and sealed into presterilized cans, all operations after that of sterilization being done under aseptic conditions. Milk processed in this way commonly coagulates or forms sediment during processing and storage, and gels or age-thickens after prolonged storage. Fat separation is not a problem unless the product is stored at temperatures above 27 °C, when a cream line forms.

Sediment formation is influenced by such factors as the origin and quality of the raw milk, its acidity and the processing method. Forewarming treatments that reduce whey protein nitrogen to low values decrease the tendency of the product to form sediment whereas direct injection of high pressure steam increases this tendency. Age-thickening may be prevented by the addition of polyphosphates, and sodium carbonate is sometimes added to increase the pH from 6·2–6·3 to 6·5–6·6 to reduce the chalky flavour of the sterilized product. Addition of sodium carbonate improves the heat stability of the product but reduces the antigelling effect of the polyphosphates. For products that are to be stored unrefrigerated for long periods Seehafer (1967) advised against pH adjustment because of the risk of age-thickening.

Some steps in the process described by Seehafer (1967) are known to change the structure of fat and protein particles in milk and may thereby reduce the stability of the final product. For example, Knoop, Wortmann & Knoop (1959) showed that the fat globule membrane was modified by simple agitation as well as by homogenization, and Jones, Hall & Trout (1964) showed that direct steam injection has a homogenizing action on oil globules. Mulder (1957) found that the damaged surface of the fat globule adsorbed serum proteins, and Jackson & Brunner (1960) showed that casein was one of the adsorbed proteins. Again, the procedure outlined by Seehafer does not require pH adjustment to compensate for hydrogen ions produced when the milk is heated and it seems likely that the increased acidity may have an adverse effect on the stability of the product. High-temperature, short-time (UHT) processes may sometimes result in incomplete destruction of enzymes which on reactivation could destabilize the product.

Burton (1969) reviewed the many problems encountered in processing milk concentrates by UHT methods. There is clearly a need to develop alternative processing methods that avoid as much as possible the use of elaborate equipment and treatments which induce instability; the present paper gives the results of an investigation of such methods.

EXPERIMENTAL

The experimental work comprised 2 parts; the first involved a canning trial of 2×2 factorial design to determine the effects of pH adjustment and of homogenization on the stability of 36·5% TS sterile whole milk. The second experiment was a study of the storage stability of canned 36·5% TS sterile whole milk prepared by the best of the 4 treatments as determined in the first part of the investigation.

Development of the process

Raw milk from the Hunter Valley of New South Wales was standardized to 3·25% fat, and 8·56% solids-not-fat and divided into 2 lots. To 1 lot was added 8·8 ml N-NaOH/kg(4 ml/lb) and nothing was added to the other. NaOH was used to adjust the pH because unpublished work carried out in 1955 at the Hunter Valley Co-operative Dairy Co. Ltd, based on the findings of Sommer & Hart (1926), showed that NaOH was superior to phosphates or citrates for stabilizing otherwise unstable concentrated spring milk intended for spray drying. Exploratory experiments in this present investigation also showed that NaOH had a better stabilizing effect than sodium hexametaphosphate with 28% TS and 36% TS sterile concentrates. Maximum stability for processing was obtained when the pH values of the sterilized concentrates were 6·85 (28% TS) and 6·65 (36% TS) and these pH values were obtained by adding 8·8 ml N-NaOH/kg (4 ml/lb) raw milk. This addition gave an average pH of 7·37 which varied by less than $\pm 0{\cdot}05$ in 8 samples of raw milk treated during the 4 seasons of the year in the period 1965–9.

After addition of the NaOH to one lot of milk, both lots were forewarmed to 97 °C in 20 min, cooled and each divided into 2 sublots, one of which was homogenized at 71 °C at 13·8 MN/m^2 (2000 lb/in.^{2}), while the other was not homogenized. The treatments were designated 1–4, as follows.

Treatment 1: NaOH added, not homogenized.
Treatment 2: NaOH added, homogenized.
Treatment 3: NaOH not added, not homogenized.
Treatment 4: NaOH not added, homogenized.

The samples from the 4 treatments were then vacuum-concentrated and standardized to 36·5 % TS, and stored at 3 °C overnight. Polyphosphates were not added at any stage. The concentrates were then quickly preheated to about 80 °C, filled into plain 301 × 214 (8 oz) cans which were closed and processed in steam at 128 °C for 3½ min while spinning at 150 rev/min with their cylindrical axis horizontal. The cans were then spin-cooled in water. This process causes greater inactivation of enzymes than does the UHT process which has the same sterilizing efficiency towards sporing organisms.

Storage investigation

For the second part of the investigation, raw Hunter Valley milk was processed using treatment 1. The canned concentrate was stored at 5, 20 and 30 °C and examined at intervals up to 210 days.

Examination of processed milk

The pH and apparent viscosity of the samples were measured at 21 °C at each stage during processing and at each examination during storage. A Radiometer pH Meter Type TTT 1 C was used for the pH measurements and the relative viscosities of the samples were observed from the time they took to drain from a 20-ml pipette; the time taken by water to drain from this pipette was 14 s. The reflectance of the concentrated products from the storage experiment was measured in the visible region against a magnesium carbonate standard using a Beckman Model B spectrophotometer, and the concentrations of tin and iron in the concentrates were determined by colorimetric methods after wet oxidation (Board & Elbourne, 1964).

The pH, apparent viscosity and solubility index of the reconstituted single-strength samples were also determined. The solubility index was determined by the method given in an Australian standard based on the method described in Bulletin 916 of the American Dry Milk Institute (Standards Association of Australia, 1965). The organoleptic qualities of the reconstituted samples were compared with those of fresh pasteurized milk from the Sydney domestic supply.

RESULTS

Development of the process

Table 1 gives the results of pH, viscosity and solubility index determinations on the 4 samples in the factorial experiment at different stages during preparation, and at 2 days after processing. These data show that addition of NaOH maintained the pH at least 0·4 units above that of the equivalent unadjusted product through all stages of the process, and that the pH of the final canned product containing added alkali was similar to that of the untreated raw milk.

The viscosity of the concentrated milk in treatment 1 decreased with each preparative step, but increased with each step in the other 3 treatments to such an extent that the canned concentrates in treatments 3 and 4 were soft solids. All the canned concentrates dispersed in water with gentle stirring to give reconstituted milks having

viscosities of about 15s: the samples from treatments 2, 3 and 4 contained large quantities of sediment as shown by the data for solubility index in Table 1. The sediment from treatment 2 appeared fine and chalky, and that from treatments 3 and 4 consisted of large irregular grains. When reconstituted milk from the 4 treatments was held for 1 h at 21 °C in cylinders measuring 9 in. in height and 1½ in. in diameter, the following amounts of sedimentation occurred: treatment 1, none; treatment 2, 0·1 in., treatment 3, 4·7 in. and treatment 4, 5·2 in.

Table 1. *pH, Viscosity and solubility index of samples in the 2 × 2 factorial experiment*

	Treatments			
	NaOH added		NaOH not added	
Stage in process	Not homogenized 1	Homogenized 2	Not homogenized 3	Homogenized 4
	pH			
Before forewarming	7·37	7·37	6·77	6·77
After concentration to 36·5% total solids (TS)	6·90	6·91	6·37	6·37
Preheated before canning	6·91	6·90	6·35	6·35
2 Days after canning	6·67	6·67	6·23	6·22
	Viscosity of concentrated milk, s			
After concentration to 36·5% TS	110	270	41	43
Preheated before canning	78	231	55	57
2 Days after canning	69	427	Solidified	Solidified
	Viscosity of reconstituted milk, s			
Preheated before canning	16	16·4	15·2	15·2
2 Days after canning	15·6	15·6	15·0	15·0
	Solubility index of reconstituted milk			
Preheated before canning	0·05	0·1	0·1	0·1
2 Days after canning	0·1	10·5	13·5	11·5

All the concentrates had a slight caramel colour which was hardly noticeable after they had been reconstituted to single strength. The concentrates also had a caramel flavour which was described as 'pleasant but slightly cooked' in the single-strength milks. Concentrates from treatments 2 to 4 were unacceptable because they contained sediment.

Storage investigation

The results of pH measurements on canned concentrated milk prepared by treatment 1 and stored for periods up to 210 days at 5, 20 and 30 °C are shown in Fig. 1. The pH of the single-strength milk obtained by adding distilled water to the concentrate was 0·2–0·3 units higher than that of the corresponding concentrated sample.

The viscosity of the concentrated milk held at 5 °C increased steadily during 210 days storage, from 70 to 128 s (Table 2). Concentrate stored at 20 and 30 °C showed little or no change in viscosity up to 160 days, but at 210 days all samples showed an increase in viscosity of about 10 s and the first signs of gel formation were seen in the samples stored at 30 °C. Some serum separation occurred in concentrates

stored at 20 and 30 °C but the serum recombined with the product on stirring. There was no evidence of separation of cream or free fat. Despite the changes in viscosity of the concentrates, all the reconstituted samples had viscosities of 15–16 s and there was little or no evidence of sediment or physical instability. For instance, solubility index determinations on reconstituted samples at the 85-day examination gave values of 0·05 for all samples; fresh raw milk gave a value of 0·02.

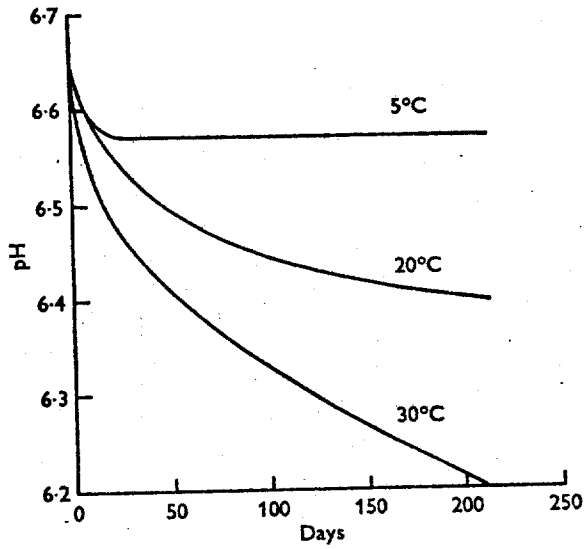

Fig. 1. Change of pH of canned concentrated milk during storage at 5, 20 and 30 °C.

Table 2. *Relative viscosity and reflectance at* 450 *nm of milk concentrate stored at* 5, 20 *and* 30 °C

	Relative viscosity, s			Reflectance, %		
Storage temperature, °C	5	20	30	5	20	30
Storage time, days:						
0	70	70	70	—	—	—
7	85	70	61	30	30	29
85	118	74	65	31	29	23
160	108	72	65	30	30	22
210	128	83	76	31	27	22

The reflectance spectrum of freshly prepared concentrate showed minimum readings near 450 nm rising through an inflection at about 500 nm to a broad maximum near 700 nm. As the samples darkened during storage at the higher temperatures the reflectance decreased over the whole spectrum, the decrease being greater at shorter wavelengths. Reflectance values at 450 nm are recorded in Table 2.

Caramelization was most rapid at 30 °C, slower at 20 °C and apparently absent at 5 °C. Milk reconstituted from concentrate stored at 30 °C for 85 days showed marked caramelization and was probably unacceptable; after storage for 160 days the concentrate gave a single-strength milk with unacceptable colour and flavour. Milk made from concentrates stored at 5 and 20 °C had acceptable quality at all examinations;

the samples had a slight cooked flavour, a full-bodied texture with no dry chalky taste, and a faint caramel colour. In contrast, milk made from commercial samples of canned evaporated milk (29% TS, pH 6·2) had a watery taste.

Corrosion of the unlacquered electrolytic tinplate cans was by slow detinning and was negligible except in cans stored at 30 °C for 210 days. Many small detinned areas were found on the bodies of these cans, and the side-seam region that had been modified by the heat of soldering during manufacture was also detinned.

DISCUSSION

Evaluation of the 4 procedures used for preparing concentrated whole milk of 36·5% TS showed that an acceptable product which did not form sediment, undergo age-thickening, or show cream separation during storage for up to 210 days, may be obtained by initially increasing the pH of the raw milk to counteract acid formed during subsequent heat treatments, and by forewarming to denature 90% of the whey protein. The process involving addition of 8·8 ml N-NaOH/kg raw milk (4 ml/lb) (treatment 1) gave stable canned concentrates on 8 occasions during the 4 seasons of the year. Variations of ±0·05 pH unit from the average of 7·37 for the NaOH-treated raw milks had no observable effect on the stability or quality of the 8 lots of canned concentrate. The repeatability of the process probably depends in part on the fact that seasonal variations in the properties of Hunter Valley milk are minimized by the current practice of calving throughout the year. It is possible that milk from other areas may have different pH and buffering capacities and may require different amounts of NaOH to give stable concentrates. It seems likely, however, that the procedure of increasing pH of raw milk to about 7·37 to improve heat stability may have general application. Morrissey (1969) showed that there are two pH regions in which milk is highly heat stable, one at about pH 6·7 and another at pH > 7·1. Exploratory experiments in the present investigation covered pH values up to 7·8 and maximum stability was found to be at about pH 7·4. At pH values above 7·4 increased browning and peptization of casein caused quality deterioration. It is interesting that Jenness & Patton (1959) suggested that sodium ions have a specific stabilizing effect on milk.

Homogenization, which is widely used commercially, gave products that formed sediment and thickened with age. It appears that improved stability could be imparted to the current commercial product if the pH of the raw milk was increased and if treatments such as high velocity pumping, steam injection, flash cooling, and homogenization itself were eliminated or minimized. Under such circumstances it may not be necessary to add polyphosphates to improve physical stability and the chalky taste may not then be a problem.

Although the rate of browning during processing increases with increase in pH, the use of a rapid in-can heating process reduced this quality defect to the extent that it was not commercially significant.

The importance of storing concentrated milk at low temperatures to retain quality was shown by the results of the storage investigation, which were consistent with those reported by Patton (1952) and by Loney, Bassette & Claydon (1968). Although the stored concentrate was physically stable for long periods at elevated

temperatures, the colour and flavour deteriorated rapidly. These adverse changes are commonly found in concentrated foods and are generally classified as browning reactions. There is no method known, other than cold storage, that will prevent such changes in products of this type.

The changes in fine structure that the protein bodies and fat globules undergo during the preparation and processing of canned concentrated milk are complex and are described in a paper by Board, Bain, Gove & Mullett (1970).

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With 3 plates

Structural changes in whole milk during the production of sterile concentrates: an electron microscope study

By P. W. BOARD, JOAN M. BAIN and D. W. GOVE
Division of Food Preservation, C.S.I.R.O., Ryde, N.S.W., Australia
and J. T. MULLETT
Hunter Valley Co-operative Dairy Co. Ltd, Heyham, N.S.W. Australia

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Summary. Electron microscopy was used to study the effect of addition of NaOH, forewarming, homogenization, concentration, and heat sterilization on the structure of whole milk during the production of 4 heat-sterilized concentrates that showed different degrees of physical stability. The samples were prepared for electron microscopy by adding fixative either to liquid or freeze-dried material, and then embedding for sectioning.

Noticeable structural changes occurred during forewarming of samples containing added NaOH, during homogenization, and during heat sterilization. Addition of NaOH and forewarming decreased the closeness of packing of the subunits in the casein micelles. Homogenization reduced the average size of the fat globules, and protein became attached to their surfaces. Heat sterilization caused coalescence of protein. In non-homogenized sterilized concentrate with added NaOH, protein bodies of about 100 times the volume of the original casein micelles were formed; these were free-floating and the concentrate was stable. In non-homogenized sterilized concentrate with no added NaOH the protein bodies were about 15 times the size of the casein micelles and bridged to each other, thereby forming a sediment consisting of large irregular particles. Very large protein bodies containing fat globules formed during heat sterilization of the homogenized samples, both in the presence and in the absence of NaOH, and were responsible for the formation of sediment in these 2 products.

Needle crystals observed in most samples were identifed as $CaCO_3 . H_2O$ by selected area diffraction; KCl crystals were also detected.

Board & Mullett (1970) prepared heat-sterilized whole-milk concentrate (36·5% total solids) using 4 different processes (see Table 1). All 4 products were stable after they were concentrated, but sediment formed in 3 samples during the final heat sterilization process. These differences in stability depended on whether or not the pH was increased at the initial stage of processing and on whether or not the milk was homogenized before concentration. A physically stable product was obtained when the initial pH was increased and the milk was not homogenized; a chalky sediment formed when the pH was increased and the milk was homogenized; sediment composed of

large irregular grains formed in non-homogenized and homogenized milk when the pH was not increased.

The present electron microscope study was carried out to examine the structure of raw milk and to observe the changes brought about by increased pH, forewarming, homogenization, concentration, and heat sterilization.

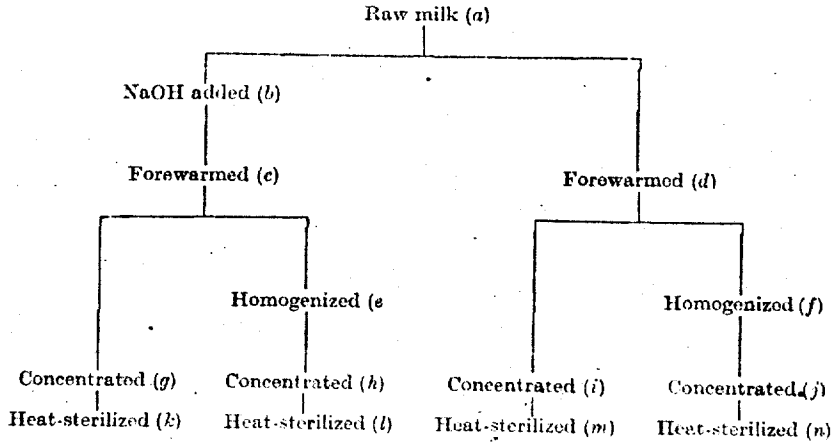
Several previous workers have investigated the fine structure of milk, examining either the structure of fat globules (Knoop, Wortmann & Knoop, 1958) or of casein micelles in fat-free material (e.g. Shimmin & Hill, 1964, 1965; Calapaj, 1968; Carroll, Thompson & Nutting, 1968; Schmidt, 1968; Schmidt & Buchheim, 1968). Although Schmidt (1968) claimed that fat globules seriously interfered in electron microscope studies of casein particles, we examined whole milk because both fat and protein could be important in determining the physical stability of whole-milk concentrates. Eggmann (1969) has investigated the structure of whole milk using the freeze-etching technique.

METHODS

Processing

The processing treatments applied to the milk are outlined in Table 1 and were described in detail by Board & Mullett (1970). Samples taken from each treatment during processing are designated by letters (*a*)–(*n*).

Table 1. *Processing treatments applied to the milks*



Preparation for electron microscopy

Attempts were made to examine the samples directly in the microscope. Material diluted 1 in 50 with distilled water was applied to the grid as a drop and allowed to dry. It was examined after no further treatment and after fixing in osmium vapour for 30 min. Diluted material was also fixed in glutaraldehyde, but osmium fixation gave the best results. Although the overall structure of the milk examined in this way was consistent with the structure shown later in sectioned material and with that observed by optical microscopy after staining with crystal violet, the method of examination was not satisfactory because of the damage caused by the electron beam (Plate 1*a*)

and because of the considerable contamination of the microscope. Carbon, evaporated on to the sample, gave some protection of the fat globules from beam damage, but focusing was difficult at magnifications greater than 5000. Shadowing of the samples with platinum–palladium masked the structure of the casein micelles and other protein bodies.

Henceforth, material was taken at each stage in processing and fixed either in the liquid or in the freeze-dried state.

Liquid samples. One ml of milk was fixed in 9 ml of 1 % OsO_4 in cacodylate buffer (pH 6·8) for 2½ h. The black pellet that formed during fixation was washed with buffer solution and dehydrated in an alcohol series, the sample being centrifuged at each change of solution; staining with uranyl acetate (1 % in 70 % alcohol) was carried out for 1 h during dehydration. Pieces of the pellet were then embedded in Araldite, and sectioned using a Porter–Blum MT 2 ultramicrotome. The sections were stained with lead citrate on the grid (Reynolds, 1963) and examined in a Siemens Elmiskop 1 electron microscope.

Freeze-dried samples. Samples of milk, each of 5 ml, were frozen on dry ice or in liquid nitrogen and then freeze-dried. Pieces of dried material (*c.* 1 mm^3) were fixed in 1 % buffered OsO_4 for 2½ h and then treated as above, except that the material was not centrifuged.

Both methods gave satisfactory results, but because fat tended to separate during centrifugation of material prepared from liquid samples, the method based on freeze-dried material was preferred and was generally used throughout the investigation.

RESULTS

Structure of raw milk

Electron micrographs of raw milk showed fat globules and casein micelles (up to 0·2 μm diam.) and diffuse material which probably was serum protein; needle crystals (*c.* 0·1 μm long) were frequently associated with the protein fraction (Plate 1*b*). The fat globules were surrounded by a double membrane (Prentice, 1969) and the casein micelles appeared to be granular with a rough surface (Shimmin & Hill, 1965; Rose, 1969); some casein micelles appeared to be associated with the fat globules (Plate 1*c*).

Effect of increased pH

The addition of 8·8 ml N-NaOH/kg milk (4 ml/lb) as recommended by Board & Mullett (1970) had no effect on the apparent closeness of packing of the subunits in casein micelles. Larger additions of NaOH appeared to reduce the density of the individual micelles. With additions of 17 ml or more, micelles disintegrated and the opacity of the milk decreased.

Effect of forewarming

Forewarming causes interaction of casein and serum proteins (Sawyer, 1969), so from this stage in the processing, the large micelle structures are referred to as protein bodies rather than as casein micelles.

Forewarming of the samples with 8·8 ml NaOH added (sample *c*) reduced the apparent closeness of packing of the subunits in the protein bodies and may have

caused partial disintegration of some former casein micelles; the amount of electron-dense material in the serum appeared to have increased. Forewarming of the milk containing no added NaOH (sample (*d*)) resulted in the formation of diffuse particles of about 0·02 μm diam. in the serum; many of these particles were attached to the surface of the dense protein bodies. The double membrane around the fat globule was no longer obvious after forewarming.

Effect of homogenization

Homogenization of forewarmed milk increased the number of fat globules, reduced their average size and increased their total surface area. It appeared that serum proteins and occasionally protein bodies were attached to the fat globules. Eggmann (1969) also observed protein associated with the fat globules in whole milk powder from homogenized milk.

Effect of concentration

The structures of the 4 samples at this stage in processing are shown in Plate 2. The protein bodies in all the samples at this magnification appeared to be similar in size and structure to the casein micelles of raw milk. The homogenized samples (*h*) and (*j*) contained more diffuse material in the serum and the average size of the fat globules was smaller than in the non-homogenized samples (*g*) and (*i*). At higher magnification, the subunits of the protein bodies of the samples with added NaOH (samples (*g*) and (*h*)) appeared to be less closely packed than in casein micelles of raw milk or in the protein bodies of samples (*i*) and (*j*).

Effect of heat sterilization

Heat sterilization caused the most marked changes in the structure of the milk concentrates. These changes involved fusion of the protein bodies, and sometimes fat globules and protein bodies, to form entirely new structures. In sample (*k*) (Plate 3*a*) these structures were physically stable, but in sample (*l*) (Plate 3*b*) the new structures formed a chalky sediment and in samples (*m*) and (*n*) (Plate 3*c*, *d*) the structures formed large, soft, irregularly shaped particles of sediment. The areas shown in Plate 3 (*b*–*d*) are only a small part of the individual sediment particles in samples (*l*), (*m*) and (*n*), and as such do not adequately represent the gross morphology of the samples.

Sample (*k*) (*Plate* 3*a*). The protein bodies in this non-homogenized concentrate containing NaOH increased more than 100-fold in volume during heat sterilization. The protein bodies (diam. 1·0 μm) were now approximately 5 times the diameter of the casein micelles in raw milk. Most of the protein bodies were free-floating. Fat globules were either free or attached to single protein bodies. Diffuse background material was present as in raw milk. The absence of sediment in this sample was associated with the free-floating nature of the protein bodies, fat globules, and protein–fat complexes.

Sample (*l*) (*Plate* 3*b*). The particles in this fine chalky sediment were made up of enlarged irregularly shaped protein bodies which were several μm across and which contained numerous fat globules. The addition of NaOH was again responsible for enlargement of the protein bodies in the milk and homogenization caused the fat

globules to be embedded in them. The diffuse background material was present as in raw milk.

Sample (*m*) (*Plate* 3*c*). The sediment particles in this non-homogenized sample with no NaOH added were much larger and softer than those in sample (*l*). The protein bodies were about 15 times greater in volume than the casein micelles in raw milk, but were smaller (0·5 μm diam.) than any other protein bodies in the concentrated and heat-sterilized samples. Their surface was covered with strands of protein which appeared to bridge with neighbouring protein bodies to form the sediment particles in the product. Fat globules usually had one or more protein bodies attached to their surface, but the globules were not deeply embedded as in the homogenized product (samples (*l*) and (*n*)).

Sample (*n*) (*Plate* 3*d*). The effect of homogenization in this sample was similar to that in sample (*l*) (Plate 3*b*), but there was not so much fusion of the protein bodies nor embedding of the fat globules in the absence of NaOH. At higher magnification, there was evidence that the protein bodies were joined to each other by protein bridges as in sample (*m*), which would have helped stabilize the sediment particles.

Crystalline deposits

Many samples of raw milk and processed milk contained needle crystals about 0·1 μm long, which were usually associated with the protein fraction (e.g. Plates 1*b*, 2*a*, 3*a*). Electron diffraction measurements made on these preparations indicated interference by the fixative or staining materials, so selected area diffraction measurements were made on unfixed and unstained samples applied directly to the grid. The electron diffraction patterns for the needle crystals corresponded to those published for $CaCO_3.H_2O$; an additional pattern, obtained from the serum region, corresponded to that of KCl, though no crystals were visible (Table 2).

Table 2. *Electron diffraction parameters for $CaCO_3.H_2O$ and KCl, compared with those for crystalline components of milk preparations*

Values for needle crystals	$CaCO_3.H_2O$*	Values from serum region	KCl*
2·37	2·37	3·14	3·15
2·17	2·16	2·22	2·22
1·93	1·93	1·81	1·82
1·76	1·76	1·58	1·57
1·34	1·37	1·42	1·41
		1·28	1·28

* X-ray powder data file index of American Society for Testing and Materials (1962)

DISCUSSION

The electron micrographs of milk at various stages in the production of the unsterilized concentrates showed that addition of NaOH and/or homogenization caused changes in the structure of the protein bodies and fat globules, and these changes influenced the way in which the concentrates reacted during heat sterilization.

The decrease in apparent packing density of the subunits in the casein micelles on addition of NaOH and forewarming was probably caused by the breaking of apatite

bridges (Rose, 1969). It may, therefore, be significant that samples containing added NaOH generally contained more needle crystals having a diffraction pattern corresponding to that of the $CaCO_3.H_2O$ than did samples which contained no added NaOH. It seems likely that the open-structured casein micelles and free subunits reacted with whey proteins during forewarming (Burton, 1969) and that during heat sterilization about 100 of the resultant protein bodies coalesced to form each of the large free-floating spherical protein bodies found in sample (*k*). A similar sequence of reactions probably led to coalescence of protein in sample (*l*), but the form of the final protein bodies was also influenced by earlier homogenization. Coalescence of protein bodies also took place in the heat-sterilized concentrate with no NaOH added (samples (*m*) and (*n*)), but to a lesser extent. The difference in the amount of protein coalescence, in samples with and without added NaOH, may indicate differences in the distribution of whey proteins in the surface of the protein bodies in the concentrates immediately before heat sterilization.

Two consecutive steps seem to be involved in the formation of sediment in the heat-sterilized samples of homogenized milk (samples (*l*) and (*n*)). The first step occurred during homogenization and involved deposition of serum proteins and occasionally protein bodies on the newly formed fat–serum interfaces. The deposited proteins effectively embedded most of the fat globules even though up to the stage before sterilization (samples (*h*) and (*j*)) there was no evidence of sediment. The second step in sediment formation involved coalescence of neighbouring protein bodies as occurred in the non-homogenized samples (*k*) and (*m*) during heat sterilization. Fusion of these bodies gave the irregularly shaped particles of sediment containing embedded fat.

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EXPLANATION OF PLATES

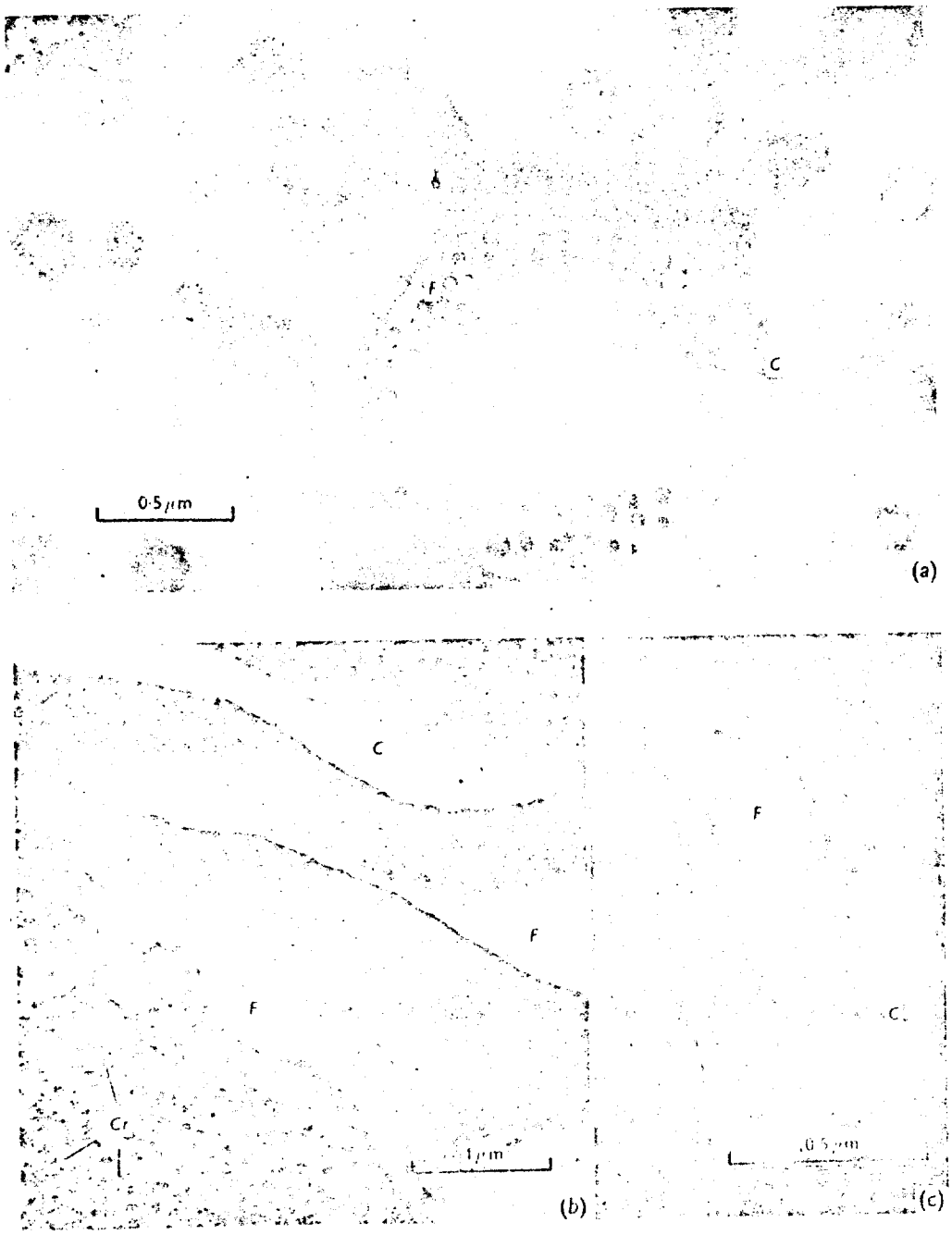
All plates, except Plate 1 (*a*), are electron micrographs of sections of freeze-dried milk which was fixed in 1% OsO_4 in cacodylate buffer and stained with uranyl acetate during dehydration. Sections were stained with lead citrate on the grid.

PLATE 1

RAW WHOLE MILK

(*a*) Electron micrograph of material fixed on the grid by exposure to osmium vapour for 30 min. Casein micelles (*C*) are in close contact with a fat globule (*F*) which has suffered damage in the electron beam. ×40000.

Journal of Dairy Research, Vol. 37, No. 3 Plate 1



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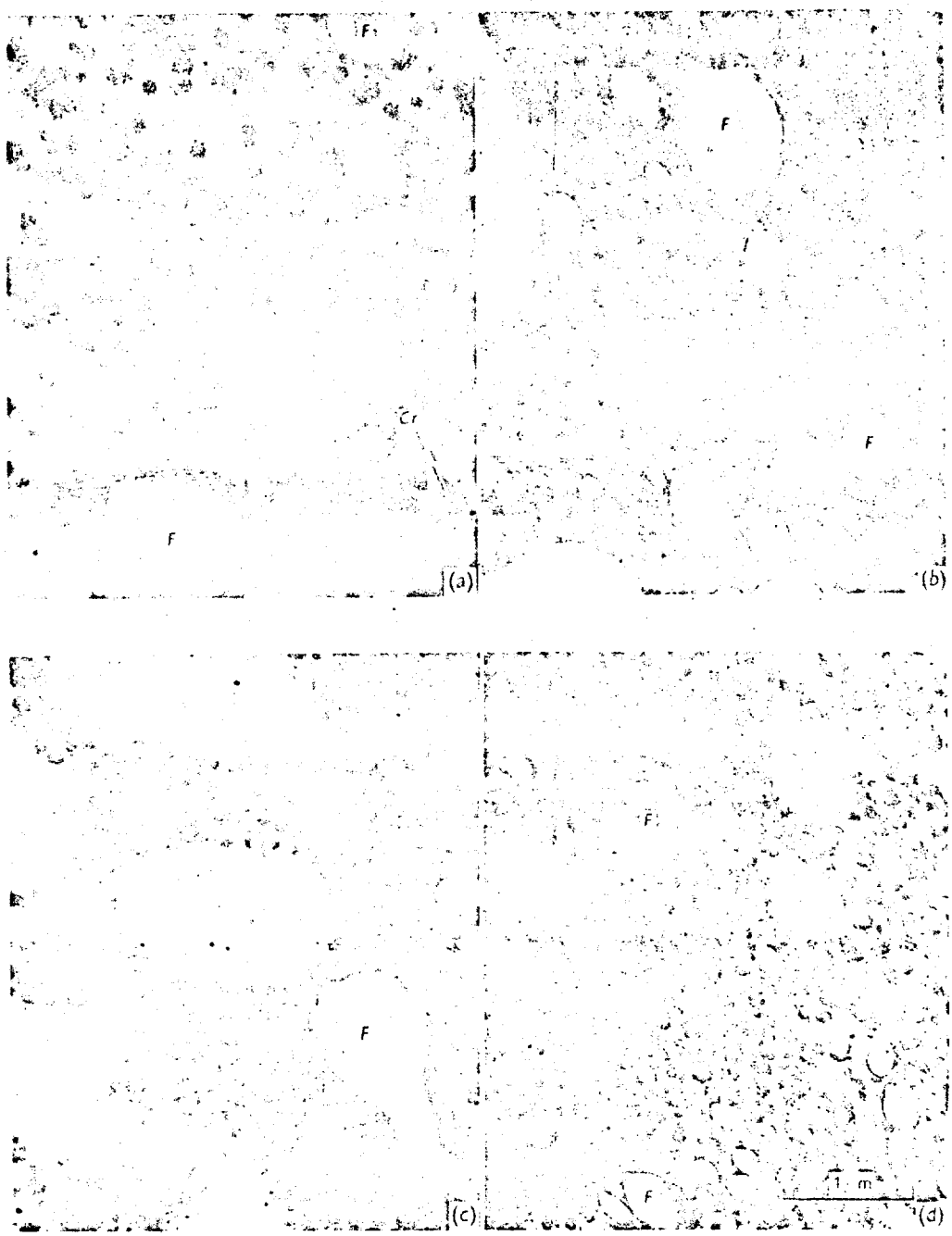

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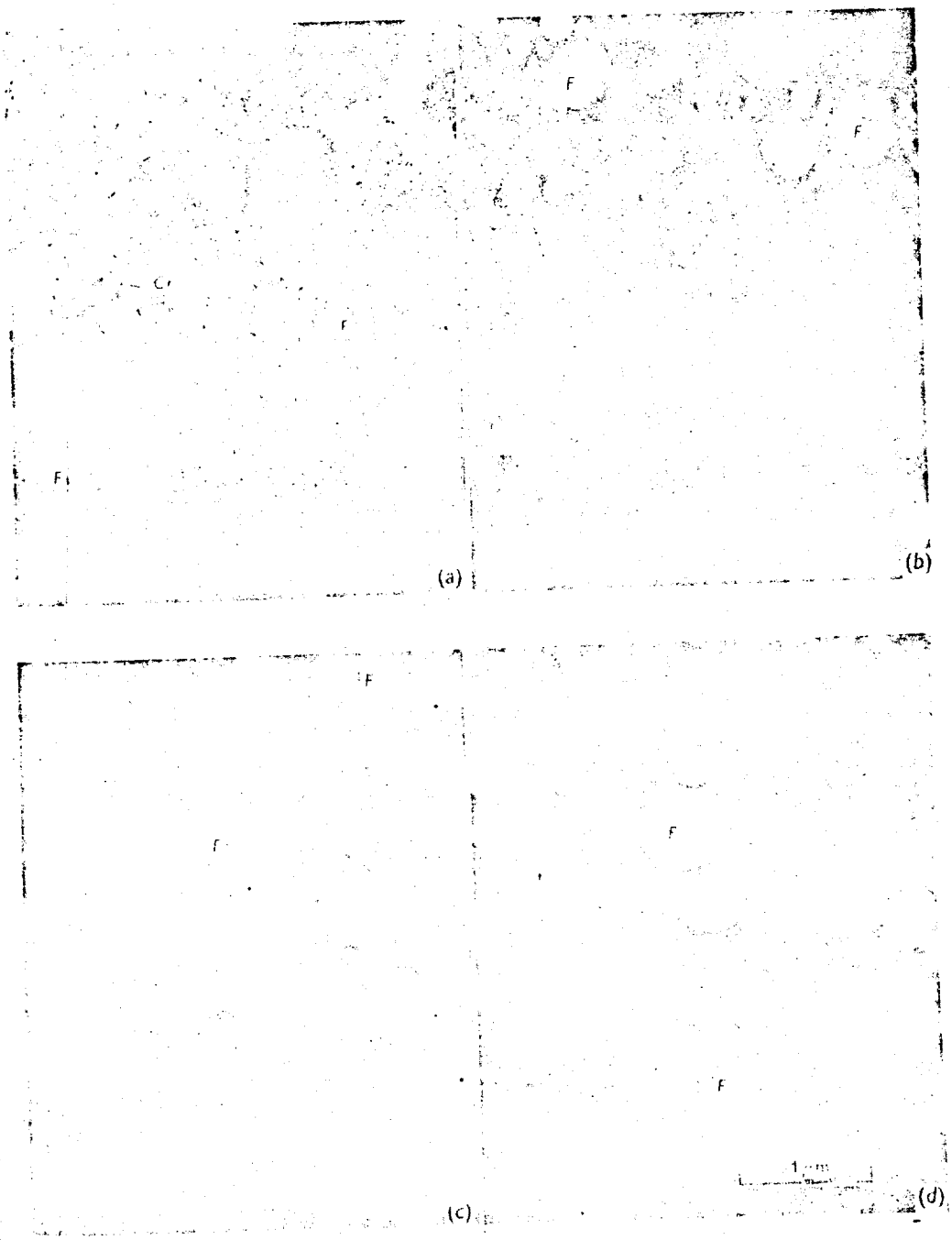

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(*b*) Distribution of casein micelles (*C*) and fat globules (*F*). Needle-like crystals (*Cr*) are indicated. ×20000. (*c*) Detail of casein micelles (*C*) and of a fat globule (*F*) shown surrounded by a double membrane. ×60000.

PLATE 2

CONCENTRATED WHOLE MILK

Protein bodies in all 4 samples appear similar to those in raw milk (Plate 1*b*). Fat globules (*F*) are smaller in the homogenized samples. Needle crystals are indicated by (*Cr*). ×20000. (*a*) Sample (*g*). NaOH added, forewarmed, not homogenized. (*b*) Sample (*h*). NaOH added, forewarmed, homogenized. (*c*) Sample (*i*). NaOH not added, forewarmed, not homogenized. (*d*) Sample (*j*). NaOH not added, forewarmed, homogenized.

PLATE 3

HEAT-STERILIZED CONCENTRATED WHOLE MILK

(*a*) Sample (*k*). NaOH added, forewarmed, not homogenized. Showing emerged, roughly spherical protein bodies. Fat globules (*F*) are found in contact with a single protein body. Needle crystals (*Cr*) are associated with the protein. ×20000. (*b*) Sample (*l*). NaOH added, forewarmed, homogenized. Numerous small fat globules (*F*) are embedded in large areas of protein. ×20000. (*c*) Sample (*m*). No NaOH added, forewarmed, not homogenized. Protein bodies have coalesced, but to a lesser extent than in sample (*k*). They appear packed to one another by protein strands. Some protein bodies are associated with the fat globules (*F*). ×20000. (*d*) Sample (*n*). No NaOH added, forewarmed, homogenized. Numerous small fat globules (*F*) are embedded in protein, as in sample (*l*), but the protein bodies have not coalesced to the same extent. ×20000.

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PAK 37

COMPARATIVE CARCINOGENIC POTENCY OF COMMON AGENTS

By Emil Bogen, M.D.
AND
Russell N. Loomis, M.S.
Olive View

THAT malignant tumors often develop at the site of repeated or continuous irritation of tissues has been observed by clinicians and investigators for a long time, both in animals and in man.[1] Not every form of chronic irritation, however, is capable of producing cancerous changes. Most workers who have used physical and chemical nonspecific irritants have failed to observe true malignant changes.[2]

TABLE 1.—*Comparative Carcinogenic Potency of Common Agents*

Substance	Mice Surviving Six Months	Tumors Developed		Average Incubation Months
		Benign	Malignant	
1-2-5-6 Dibenzanthracene	33	1	16	11
Tar A	34	3	17	10
Coal Tar	6	0	6	8
Tar B	9	2	2	15
Asphalt	8	0	2	30
Tobacco tar	27	2	0	—
Pine tar	6	0	0	—
Nicotine	11	0	0	—
Lard oil	5	0	0	—
Sour cream	5	0	0	—
Nitric acid	5	0	0	—
Hydrochloric acid	5	0	0	—
Sodium hydroxid	7	1	0	—
Pinching	6	1	0	—
Cutting	7	0	0	—
Burning	6	2	0	—
Pressure	3	0	0	—

NATURE OF THE EXPERIMENTS

At the hospital in which we work, during the past few years white mice, with a known low incidence of spontaneous tumors but which were known to yield a high incidence of true tar tumors, were subjected to various nonspecific types of irritants. . . .

Although some warty growths appeared in a few of these animals, in no case did a true cancer develop, and most of the warts regressed spontaneously in spite of the continuance of the irritant. In one of six mice treated with 10 per cent sodium hydroxid, as directed by Narat,[5] a tumor developed within four months, but its exact nature was not made out. Recognizing that with larger numbers of animals we might have observed more tumors, and even malignancy, we cannot but be impressed by the extreme infrequency with which this may occur.

Known active carcinogenic agents, on the other hand, produce a much more positive effect.[4] After several applications of an active gas-house tar to the back of white mice, for example, there is usually an epilation, or local loss of hair, sometimes associated with transient generalized loss of hair, which, however, usually soon returns. The local area, however, becomes permanently bald, and the skin becomes dry, thickened and rough. After a number of months, however, papillary warty growths appear in the tarred region, slow growing, well confined, and tending to recede and disappear if the tarring is then stopped. Some of them, however, after a varying time, suddenly take on an accelerated growth rate, invasive character, and general malignant features.

CHARACTERISTICS OF THE INDUCED CANCERS

Such induced cancers are typical epithelial neoplasms, showing histological criteria of malignancy, such as frequent mitotic figures, atypical cell size and shape, irregular nuclear patterns, and irregular conformation and invasion of normal tissues and blood vessels. There are usually clinical evidences of malignancy also, such as invasion, resulting in death of the animal if the tumor is not removed; metastases, usually late, however, recurrence after incomplete removal; and in a number of instances growth on transplantation through a series of related mice.

RELATIVE ACTIVITY OF CERTAIN SUBSTANCES

Extensive experiments have shown that not all such tars are equally effective in producing tumors in white mice. It was our purpose to determine the relative activity of a number of substances in the production of such tumors. Kennaway and Cook, in their epoch-making researches in the past few years, discovered that the pure crystalline substance 1-2-5-6 dibenzanthracene is a powerful carcinogenic agent.[6] Accordingly, we procured some of this substance and have used it as a control.[6]

When applied to the skin of white mice in 0.3 per cent solution in benzene, 1-2-5-6 dibenzanthracene does not manifest the marked local irritant effects of most crude tars. The epilation, thickening and roughening of the skin, are usually much less prominent. At about six months after the treatment has started, however, the warty papillomata develop, just as with the tars, and within a few months more malignant change is almost always evident.

Altogether, more than five hundred mice have been used in this investigation, divided into numerous cages, usually with but five mice each. Treatments have been administered usually twice a week, but occasionally more or less often for special purposes. For all of these experiments young

mice of from three to six months of age were taken from a homogeneous stock of white mice with a very low incidence of spontaneous tumors. The few mice still surviving in this experiment are all well past the minimal time for the appearance of tumors, none of them now being less than a year old.

The long time required for the tumors to appear results in a comparatively high mortality rate from other conditions during the period of observation. In addition, many of the substances used for the study are themselves somewhat toxic, and kill off the mice at an earlier age than would otherwise have been attained. The majority of the mice developing cancers died of the tumor itself, or of the attempt at operative removal of it. On the other hand, some of them continued to carry warty growths for many months, and in some instances the dry warty growths dropped off spontaneously, or were destroyed by ulceration, or were absorbed. Some tumors, histologically malignant, were surgically removed with recovery of the mice, which continued to live for as much as a year more without showing any further signs of tumor growth.

FACTORS IN TUMOR FORMATION

The most important factor determining whether a tumor will form is the chemical composition of the material applied.[7] The 1-2-5-6 dibenzanthracene produced tumors in about half of all of the mice surviving six months of treatment, and nearly all of these exposed for a full year. A preparation of gas-house tar (called Tar A), obtained through the courtesy of the Crocker Institute, and a pharmaceutical pure coal tar gave an even higher score. On the other hand, only a third of the mice treated with another gas-house tar (called Tar B), and a fourth of those treated with a California roofing asphalt, developed tumors. Vegetable tars, such as pine tar and tobacco tar,[8] nicotin, lard oil, and sour cream, as well as the chemical and physical nonspecific measures attempted, were completely ineffective.

Other factors affecting the frequency in the development of tumors, as shown in this study, are:

1. *Concentration of the Agent.*—Small differences, as Woglom[4] showed, are unimportant, and 50 per cent suspensions are more easily handled and less toxic than pure tars, with equal results. On the other hand, greater dilutions lead to less frequent tumors.

2. *Frequency of Application.*—The tar may remain for some time after application, but tends to be less effective, and the more often it is applied, up to once daily, the earlier are the tumors. Previous epilation of the treated area, removal of dried material from previous applications, heating the tar before applying it, or injuring the site of application, may all affect the efficacy of a preparation, but the differences are slight.

3. *The Medium, in which the Tar Is Applied is of Some Importance.*—Glycerin, which Woglom and Herly[9] used with Tar A, is a poor solvent for most tars. Benzene, which Kennaway advises with 1-2-5-6 dibenzanthracene, is irritating and toxic in itself, so that the mice must be kept in the open when it is applied. We have used chloroform and ether for some purposes, with good results. Lard oil seemed to lessen the frequency of the tumors formed.

4. *Treatment of the Tar Has Been Reported to Change Its Properties.*—Passing a current of steam through Tar A to remove phenols and other toxic impurities does not seem to affect its carcinogenic properties.

5. *The Strain of Mice Used.*—Strains of mice, with both high and low incidence of spontaneous tumors, have been used with no observable difference in results; but other workers report significant differences between various strains.

COMMENT

It appears that cutaneous cancer in mice is not a general reaction of the tissues to any type of prolonged irritation, but that it is more specifically induced by certain special kinds of complex unsaturated organic compounds. These may arise in the external environment, and come in contact with or enter the body; or they may arise themselves as a result of biochemical reactions within the tissues.

The prolonged exposure to gas-house tars and other industrial agents, as well as the application of crude coal tar in medicaments, have resulted in the development of cancers in man. The prolonged incubation period of occupational cancers and the shifting industrial population retards recognition of such instances. It has not yet been demonstrated to what extent the widespread use of asphalt, which we have found carcinogenic in our experience, may be responsible for the development of pulmonary carcinoma.

The use of white mice enables us to detect carcinogenic properties in substances within a small fraction of the time required for the development of malignant neoplasms in man. That there are many forms of cancer for which the responsible agent cannot now be named certainly should not deter us from attempting to minimize, so far as possible, the exposure of human beings to those agents whose carcinogenic properties may be demonstrated in so simple and conclusive a manner. Further work should be undertaken with other substances which may play a part in the development of cancers in man, and measures should be taken to prevent such occurrences.

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* (Peeling method, in particular for potatoes.)
Verfahren zum Schalen von Fruchten, insbesondere Kartoffeln.
Bottner, K.-H.; Weidenmuller, E.; Geissler, B.; Weiss, J. (Bottner KG)
West German Patent Application 1 582 546 (1970) (De)

Potatoes etc. are immersed in a 20% NaOH solution at 60 C and then conveyed to a lye-peeling machine. Neutralization is in a 0.4-0.6% acetic acid bath, and impurities are removed by an abrasive device. The product is sterilized in a 0.8% sodium sulphide solution. All units are arranged in a continuous operation line. W&Co

* Unable to obtain copy from our patent office.

Epidemiology of Lye Poisoning in the United States

H. W. BROWN, M.D., F.A.P.H.A., AND
GLENN KISER, M.D.

School of Public Health, University of North Carolina, Chapel Hill, N. C.; and Duke University, School of Medicine, Durham, N. C.

IN 1927, culminating the years of continuous struggle of Dr. Chevalier Jackson, a national law was enacted requiring the label "poison" to be placed on all commercial products containing lye and other caustic chemicals. In spite of similar laws enacted by the various states before and after this date, burns due to lye ingestion with resultant esophageal strictures continue to be a serious public health problem. Thus Vinson (1927) reported that 102 of the 186 esophageal strictures seen at the Mayo Clinic were due to the ingestion of lye. Taylor reports 53 lye poisoning cases in Pennsylvania for the year 1934. He also points out that many new caustic preparations have been placed on the market since the Federal Caustic Act was enacted—such household helps as drain pipe cleaners, paint brush cleaner, and paint remover. However, his data indicate that in spite of these new preparations, and increased advertising, lye cases have been reduced somewhat in number.

Martin and Arena (1939) reported a study of 48 cases of esophageal strictures seen at Duke University Hospital in the preceding eight years. It is evident from their report that lye ingestion continues to be an important source of esophageal strictures. Legislation has failed to eliminate this problem. A part of the difficulty arises from the fact that the Federal Caustic Poison Act (1927) in referring to lye defines dangerous caustic or corrosive substances as any containing 10 per cent or more of sodium or potassium hydroxide. Products used for household purposes containing such amounts of lye must be labeled poison. Products containing less than 10 per cent lye do not come under the Act.

Lye poisoning is not one of the diseases which is required by law to be reported. A number of reports of lye poisoning are scattered throughout the literature, but fail to give an adequate picture of the extent of the problem in the United States. The present study has attempted to gather these data in order to form a basis for rational preventive procedures.

METHODS

An attempt was first made to ascertain lye ingestion morbidity and mortality from records of the country's 48 state health departments. Practically no detailed information was secured from this source in view of the fact that, although lye poisoning is a reportable entity as a cause of death, in state summaries it is included under the general heading "acute accidental poisoning by solids or liquids." Deaths due to lye ingestion are usually not reported as such but grouped as "accidental

poisoning" or merely "stricture of the esophagus." Frequently no mention of causative agent is given. Lye poisoning morbidity is not required by law to be reported to the health authorities.

Inasmuch as state vital statistics records provided such a paucity of data, it was decided to send questionnaires to a number of hospitals in each state. Letters were written to four or five or more of the larger and more prominent hospitals in each state. They were requested to send data on the total number of lye cases treated each year, together with the total hospital admissions. Data were also requested upon the race, sex, age, and financial status of the patient while in the hospital. The larger and more prominent general hospitals only were questioned, because it was felt they would furnish more data, their records would be more available, and it appeared possible that lye stricture cases would gravitate to them. It is recognized that the hospital samples and data are not entirely representative inasmuch as small hospitals, due to the inadequacy of their records, were not included in the study. It was thought that case duplication might be a complicating factor if numerous small hospitals were also included in the study. The hospitals responded admirably, and some data were received from most of them. Our data are admittedly incomplete both in numbers and desired detail. To our knowledge, however, they represent the first attempt to study the problem on a nation-wide scale.

RESULTS

Distribution of Lye Poisoning in the United States

A total of 1,334 cases of lye poisoning were reported in a total of 9,184,055 hospital admissions by the 181 hospitals during 1914 to 1939, inclusive. Therefore, for the 181 hospitals as a whole over the period studied, lye poisoning accounted for 14.5 per 100,000 of the hospital admissions. The year in which the lye poisoning occurred was given in 1,031 of the 1,334 cases reported. They are tabulated in Table 1. A casual inspection of this table suggests a gradual increase in cases. Whether or not this is real cannot be stated, as the yearly admission figures for the hospitals reporting these cases are not available. Part of the increase in reported cases is no doubt due to improvement in hospital records. It may also be due in part to increased bed capacity of some of the hospitals and to the trend toward hospitalization in the United States. It is apparent, however, that the Federal Statute enacted in 1927 did not eliminate lye poisoning. The average yearly number of cases reported by the hospitals in the study group from 1920 to 1927 was 22, while from 1928 to 1939 it was 67. There were 303 lye cases reported without information on the year of their occurrence. Whether or not these cases had a peculiar time distribution which would affect the trend in Table 1 is unknown.

TABLE 1.

Cases of Lye Poisoning Reported by the 181 Hospitals Coöperating in the Study

1914	1	1927	27
1915	1	1928	29
1916	10	1929	36
1917	12	1930	42
1918	14	1931	59
1919	12	1932	55
1920	18	1933	61
1921	19	1934	83
1922	19	1935	81
1923	18	1936	107
1924	23	1937	103
1925	29	1938	85
1926	24	1939	63

During the period 1914–1939 the 181 hospitals included in this study admitted 1,334 cases of lye poisoning among a total of 9,184,055 hospital admissions. In the year 1939 there were 9,879,244

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hospital admissions in the United States. If the proportion of lye poisoning cases to the hospital admissions in 1939 remained the same as it was during 1914 to 1939 it follows that in 1939 there were at least 1,430 new cases of lye poisoning in the United States. However, since our study included data only from general hospitals, whereas the total hospital admission for 1939 includes hospitals for the insane and tuberculous, it is very likely that the total cases of lye poisoning would be less than 1,430. These figures represent only those seeking hospital care. There are no doubt many cases which did not come to the hospital but were treated by physicians in their offices.

The geographic distribution of the cases reported by the 181 hospitals included in this study is shown in Table 2. On the basis of experience in these institutions, hospital admission rate for lye poisoning cases per 100,000 patients is given for each state. The State of Utah had a rate of 411.8 cases of lye poisoning per 100,000 hospital admissions. The data from this state were too few, however, to be of any significance. Study of Table 2 shows that, except for the District of Columbia with a rate of 111.7, the southern states Tennessee, North Carolina, Florida, South Carolina, and Georgia have the highest rates (61.4 to 85.9 per 100,000 hospital patients). On the other hand, Mississippi with a rate of 8.0, and Louisiana with 13.7, are typically southern states with low rates.

The hospitals queried in 7 states—Rhode Island, Vermont New Hampshire, North and South Dakota, Nevada, and New Mexico—did not report a single case of lye poisoning in a total of 449,886 hospital admissions (Map I).

Pennsylvania reported the greatest number of lye cases, 283. As they were distributed among 1,681,711 hospital patients, the lye poisoning rate was only 16.0 per 100,000. It is quite possible that Pennsylvania's rate as well as the rates of other states, with physi-

MAP I. LYE POISONING PER 100,000 HOSPITAL ADMISSONS

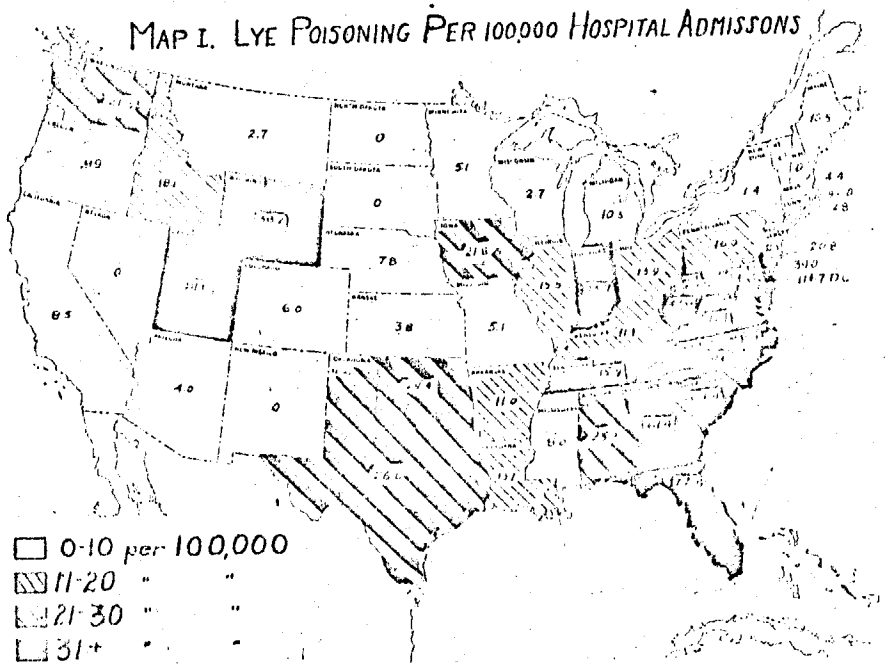

TABLE 2

Number of Lye Poisoned Cases Reported by the 181 Questionnaired Hospitals in 48 States and the District of Columbia from 1914 to 1939

State	Number of Lye Cases in Hospitals	Total Number Hospital Admissions	Lye Cases per 100,000 Hospital Admissions	Adjusted Lye Poisoning Rate
Utah	3	679	411.8	144.1
District of Columbia	95	85,950	111.7	111.7
Tennessee	35	40,782	85.9	20.6
North Carolina	60	73,491	81.6	20.4
Florida	67	85,961	77.9	21.8
South Carolina	54	82,347	64.4	14.1
Georgia	43	70,058	61.4	15.4
Indiana	14	31,910	43.7	12.7
West Virginia	3	7,149	42.9	13.3
Virginia	32	76,084	42.1	12.2
Delaware	16	41,283	39.0	15.6
Wyoming	20	52,319	38.2	15.7
Oklahoma	64	218,397	29.4	5.9
Texas	33	124,081	26.6	6.9
Alabama	12	48,458	25.0	5.0
Iowa	82	377,033	21.8	7.2
Washington	1	4,465	21.3	9.3
Maryland	18	86,610	20.8	8.3
Idaho	1	5,500	18.1	7.4
Pennsylvania	283	1,681,711	16.0	5.6
Illinois	75	488,066	15.3	5.7
Ohio	71	511,498	13.9	4.2
Louisiana	9	65,275	13.7	5.6
Kentucky	21	189,364	11.1	2.3
Arkansas	2	17,185	11.0	1.7
Maine	6	56,733	10.5	3.6
Michigan	78	755,308	10.3	3.6
California	28	330,450	8.5	3.5
Mississippi	2	25,075	8.0	1.7
Nebraska	4	50,812	7.8	2.1
Colorado	9	147,750	6.0	2.9
Missouri	14	273,724	5.1	1.6
Minnesota	18	336,251	5.1	2.4
Massachusetts	28	640,969	4.4	1.9
Arizona	1	25,100	4.0	1.7
Kansas	3	79,700	3.8	1.2
Connecticut	3	107,731	2.8	1.2
Wisconsin	10	365,616	2.7	1.1
Montana	2	72,825	2.7	1.4
New Jersey	2	77,931	2.5	0.9
New York	11	799,452	1.4	0.6
Oregon	1	103,286	0.99	0.4
Rhode Island	0	57,051	0.0	0.0
Vermont	0	129,208	0.0	0.0
New Hampshire	0	58,923	0.0	0.0
South Dakota	0	27,934	0.0	0.0
North Dakota	0	127,315	0.0	0.0
Nevada	0	22,983	0.0	0.0
New Mexico	0	26,472	0.0	0.0

cians and hospitals noted for the care of esophageal obstructions, is unduly great due to influx of patients to these centers. The data furnished by the hospitals unfortunately were not sufficiently detailed to permit us to allocate patients to place of origin. Hence it is impossible to estimate the effect of this factor on the lye poisoning rates of the various states.

It is perhaps somewhat illogical to calculate lye poisoning rates upon hospital admission rates. The hospitalization rate varies greatly from state to state. For example, in Washington, D. C., the yearly hospitalization rate is 19 per cent of its total population, in Georgia it is 5 per cent. It is evident that such great variations in hospital rates in part, at least, indicate that there is a tendency in certain areas to go to the hospital for many illnesses

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rather than to remain at home. For certain serious conditions such as operations and lye strictures, it is quite likely that hospitalization rates are rather uniform throughout the country. Variations in the general hospitalization rate influence greatly the ratio of lye poisoning admissions to total admissions. Thus, if the southern states have a low per capita general hospitalization rate, yet if all the lye poisoning cases go to the hospital the lye poisoning admission rate would be relatively high. On the other hand, a state with a high general hospitalization rate would have a correspondingly lower ration of lye poisoning admissions to general admissions.

A correction was therefore made for the variation in general hospitalization rates in the several states. This was done by multiplying the hospital admission rate* of the state by the lye poisoning admissions rate. Thus states which had a high lye poisoning admissions rate due to the fact that the general hospital admission rate was low, showed a reduction in the final lye poisoning rate. For example, in North Carolina the lye poisoning cases admitted to the hospitals queried represented 51.6 per 100,000 of hospital admissions. Now North Carolina has a relatively low general hospitalization rate which in turn results in relatively higher lye poisoning rates. The adjusted lye poisoning rate for North Carolina becomes 20.4. The adjusted rates for the various states are shown in Table 2. This correction in general resulted in a reduction in the lye poisoning rate in southern states with a low general hospital admissions rate. Even with this correction the southern states still showed the highest lye poisoning rates.

Grouping of the lye poisoning into the generally used nine divisions of the United States further emphasizes the more common prevalence of this condition in the South (Table 3).

TABLE 3

Hospital Admission Ratio for Lye Poisoning per 100,000 General Hospital Admissions in Nine Areas

	Cases
New England States	2.4
Pacific States	6.8
West North Central States	9.3
Mountain States	10.1
East North Central States	11.1
Mid Atlantic States	11.6
East South Central States	23.1
West South Central States	25.4
South Atlantic States	34.9

RACE

There were only 349 cases upon which data were available as to age, race, and sex (Table 4). A total of 249 cases were white (71.6 per cent), and 100 (28.4 per cent) were colored. Inasmuch as the Negro race constitutes approximately 12 per cent of the total population in the United States, and yet appears to account for 28.4 per cent of the lye poisoning cases, it is apparent that this condition is more common among them than among the whites. This is especially apparent if one compares the ratio of lye poisoning reported among white and Negro children under 5 years of age (Table 4). In this particular group, 61 per cent of the cases occurred in white children, and 39 per cent in Negro children. In other words, it was 4 times as common in Negro children as in white children when the racial populations are taken into consideration. Ignorance, crowded living conditions, and carelessness are no doubt factors in the high Negro rate. Another factor responsible for the high Negro rate is their rural distribution.

* The hospital admission rate of the District of Columbia was 0.19. Since this hospital admission rate was the highest encountered, it was given the value of 1.0 for computation of the adjusted lye poisoning rate, and the other states' calculations were made accordingly.

AGE

Lye poisoning is 2 to 3 times as prevalent among rural persons as urban inhabitants. This is largely due to the habits of house cleaning and soap making of rural southern inhabitants.

Our figures indicate that the greatest percentage of the lye poisoning in both races occurs in the group under 5 years of age. Sixty-five per cent of Negro cases occurred in children under 5 years of age. In the whites, on the contrary, only 41 per cent occurred in children under 5 years of age (Table 4). Two possible explanations are suggested both of which may play a rôle in this age distribution of lye poisoning. The amount of lye swallowed by Negro children may be greater than that usually swallowed by white children and therefore medical and hospital care are sought at once. Among both the whites and Negroes there may be instances where the amount of lye swallowed is so small that hospitalization is not immediately necessary. Hospitalization may therefore come a number of years later due to a slowly progressive stricture. In such instances the case might be classified under the age of onset rather than the age at which the lye was ingested. In fact a number of our records indicate the lye stricture for which the patient was hospitalized dates to lye ingestion some years previously. A second possibility accounting for the higher lye poisoning incidence in whites in later life is the more common use of this material for suicidal purposes by the white race.

An analysis of the age incidence of lye poisoning in the 0–4 age group demonstrates that children under 1 year of age seldom are affected. One and 2 year olds are the ones most commonly poisoned, although all ages through the 5–9 year group furnish numerous cases. The frequency of attack then falls off until the 20–24 year age group is reached. Beginning at this age and extending through the next 40 years there is a continuous moderate amount of lye poisoning, especially among the whites. This increase in lye poisoning in adults may in part be due to late manifestations of lye poisoning incurred in childhood. Our data, however, indicate that a considerable amount originates in adults and to a large extent as a result of attempted suicides.

There were 60 persons over 12 years of age whose history gave the method of poisoning. In only 3 instances, 5 per cent was "accidental" definitely given as the cause of lye ingestion. There were 5 cases which were called "recent" and possibly all of these 5 could have been accidental—though this is not probable. Should one consider, however, all of these "recent" cases due to accidental cause, even then lye accidents by adults, in this case over 12 years of age, would amount to

TABLE 4

Distribution of Lye Poisoning by Age, Sex, and Race

Age		Under 1	1	2	3	4	0–4	5–9	10–14	15–19	20–24	25–34	35–44	45–54	55–64	65+	Adult	Infant	Under 4	Total
White	Male	0	16	13	10	11	50	6	3	2	7	10	14	11	4	2	7	0	4	120
	Female	2	18	20	4	3	47	7	2	15	14	14	9	7	7	2	4	1	0	125
	Total	2	34	33	14	14	97	13	5	17	21	24	23	18	11	4	11	1	4	249
Negro	Male	1	1	12	7	5	29	7	4	1	1	3	2	1	0	0	3	0	1	53
	Female	0	14	11	7	1	33	1	1	1	2	3	3	2	0	0	0	0	1	47
	Total	1	18	23	14	6	62	8	5	2	3	7	4	3	0	0	3	0	2	100
Total		3	52	56	28	20	159	21	10	19	24	31	28	21	11	4	14	1	6	349

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only 13.3 per cent—quite different from the 100 per cent accidental ratio found in the study of lye cases in children. To sum up the adult lye poisoning cases we find 66.7 per cent of them were due to suicide, 20 per cent were poisoned as children, and possibly 13.3 per cent were accidentally poisoned as adults (Table 5). In the study of adult cases (over 12 years of age) at Duke Hospital, where a more careful investigation could be made of the method of poisoning (but with the disadvantage of having far fewer cases), it was found that 18.2 per cent of adults had taken lye by accident as an adult, 45.4 per cent had taken lye by accident as a child, and 36.4 per cent used lye with suicidal intent (Table 6).

TABLE 5

Summary of Accidental and Suicidal Lye Poisonings in Persons Over Twelve Years of Age

Reason for Poisoning or Time When Poisoned	*No. of Cases*	*Per cent of Total*	
Suicide	40	66.7	
Poisoned "when child"	9	15.0	20.0% as children
"Old" cases	3	5.0	
"Recent" cases	5	8.3	13.3% probably accidental
Poisoned by "accident"	3	5.0	

TABLE 6

Analysis of Eleven Lye Cases at Duke University Hospital in Persons Over Twelve Years of Age

Reason for Poisoning or Time When Poisoned	*No. of Cases*	*Per cent of Total*
Poisoned "when child"	5	45.5
Suicide	4	36.4
Poisoned by "accident"	2	18.2

A total of 40 instances of suicide by lye ingestion were reported. Thirty-eight (95.0 per cent) of the 40 suicide cases were white, 2 (5.0 per cent) colored. White females were responsible for 22 (55.0 per cent) of the suicides, white males for 16 (45.0 per cent), colored females and males each responsible for 1 (2.5 per cent). Since lye is a substance used more often by the poorer classes, it is not surprising that 70 per cent of these suicidal cases entered the hospitals as charity patients, while only 14.6 per cent were private patients. The financial status of 15.4 per cent of the patients was not given.

SEX

Lye poisoning as reported was slightly more common among white females than males and more common in Negro males than among Negro females. Our data show that among the whites, 48.2 per cent of the cases were males and 51.8 per cent females, among the Negroes 53.0 per cent were males and 47.0 females. It is impossible to state why the rate for white males should be less than that for the females while among the Negroes the males had a greater lye poisoning rate than the females. The difference in lye poisoning rates of the sexes is very small, however, and more data are needed before any significance can be attributed to these apparent differences. One might have expected a higher rate among the females than the males inasmuch as the former spend more time in the home and theoretically should have a greater exposure to accidental ingestion. This may be balanced by the fact the females usually prepare the lye for use, are familiar with it, and hence avoid its accidental ingestion. Study of the incidence of lye poisoning among children under 5 (Table 4) also indicates no appreciable sex difference.

LYE ACCIDENTS

All the cases of lye poisoning in our series can be placed into two groups: accidental and intentional. The former group includes persons of all ages who

by accident ingested lye. The latter group, taking lye intentionally, were attempting suicide. This latter group is composed almost exclusively of adults.

Accidental lye poisoning was frequently due to ingestion of the powdered lye mistaken for salt or sugar. Even more common was the drinking of lye solutions which were mistaken for milk or sugar water. Classification of 53 cases upon which data concerning the type of material ingested were available indicates that 45.3 per cent of the patients had swallowed lye solution; 24.5 per cent had ingested powdered lye; 30.2 per cent were merely stated to have "swallowed lye."

The following excerpts from lye poisoning case histories indicate how the accident took place.

Powdered Lye:

"Lid of lye can put in mouth."
"Spoon containing lye put in mouth."
"Pellets of lye swallowed."
"Picked up lye can and started eating contents."

Lye Solution:

"Solution mistaken for drinking water."
"On wash day."
"Drank 'sugar water' from glass which once contained lye."
"Solution of lye and water put on potato and eaten."

Our data and a review of the literature show that lye, or its preparations, have been mistaken for the following: cough mixture, drinking water, sugar, medicine, milk, epsom salts, wine, and coffee.

Histories of the majority of accidental lye poisoning in children indicate that the lye was being used in solution for washing of clothes and floors or making soap. These solutions or lye pellets were carelessly left within reach of children.

LYE POISONING—AN ECONOMIC PROBLEM

Lye poisoning, both accidental and intentional, is to a large extent a problem of families in the low income group. A total of 239 of 284 cases (85 per cent) upon which the financial status of the patient was given were charity patients. Those taking lye for suicidal purposes were charity patients in 70 per cent of the instances. The cost of hospitalization and treatment were assumed by the hospitals, local charity, or local governments. That this expense may be rather great is indicated by the findings of Martin and Arena. They reported that 68 per cent of the lye poisoning cases studied by them required an average of 48 days hospitalization. Sixteen per cent of the patients were hospitalized 1 to 6 days, and the remaining 16 per cent were followed in the dispensary. In addition to the immediate hospital treatment, the patients frequently must return over a period of months and even years for dilatation of esophageal strictures. Thus the total expense of an accidental lye poisoning may be a matter of several hundred dollars.

PREVENTION

Our data indicate that the prevention of accidental lye poisoning is largely a matter of eliminating this substance as an accidental item of diet or beverage of children. Children under 5 years of age are especially involved in this problem. Adult and child education would appear to be the first logical step in the prevention of lye poisoning. Education of the adults is important because they are responsible for using the lye preparation and carelessly leaving the material lying about. They should be informed of the usual household chemicals that contain lye or caustic materials and also the grave dangers and consequences of ingesting these materials. In addition to stressing prevention of poisoning, it should also be emphasized that if poisoning does take place, whether the

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Lye-Peeling of Green Papaya (*Carica papaya* L.)

L. E. Cancel, I. Hernández, and E. Rodríguez-Sosa[1]

INTRODUCTION

Great efforts are being made presently to bring the papaya fruit of Puerto Rico into industrialization. Many papaya products can be processed into regular consumer items such as our traditional sliced papaya in extra heavy syrup (Ca 62° Brix), papaya canned in light, medium or heavy syrup, and glazed papaya slices. Suitable techniques for removing the peel from green papayas obviously are necessary for such processing. Canners now have the peel removed from the fruit by hand or by immersing the fruit in hot lye solutions.

This study was made to evaluate some of the economic factors in the lye-peeling process such as the most effective concentrations of lye, the length of time required for adequate peeling at different lye concentrations, and also to determine residual alkalinity and possible textural changes in the resulting papaya pulp. The results of this study are reported below.

REVIEW OF LITERATURE

Woodroof (*1*)[2] stated that the lye peeling method is suitable for peeling all sizes, shapes and varieties of most fruits, vegetables, and root crops, but that the appropiate relation of lye concentration, temperature of the solution, and time of immersion should be established for each product.

Mazzola (*2*) peeled potatoes in 10 seconds by dipping them in a 53-percent lye solution at 300° F. with only 7-percent waste. Greig and Manchester (*3*) recommended a method which requires an 8-minute immersion in a 15-percent lye solution at 155° F. Dunlop (*4*) reported that he had peeled potatoes satisfactorily by dipping them for 2 minutes in a boiling 14-percent lye solution. This shows the need for exact knowledge of the appropriate relationships between the concentration and temperature of the solution and immersion time.

Lye peeling of many fruits and vegetables has been studied by a number of investigators. Woodroof and Atkinson (*5*) peeled sweetpotatoes by dipping the tubers in a boiling 10-percent lye solution for five minutes. Beets (*6*) can be lye-peeled by immersing the roots for three minutes in a boiling 7-percent lye solution. Stateler (*7*) obtained well-peeled carrots by

[1] Food Technologist, Assistant Chemical Engineer, and Research Assistant, respectively, Food Technology Laboratory, Agricultural Experiment Station, Mayagüez Campus, University of Puerto Rico, Río Piedras, P.R.

[2] Italic numbers in parentheses refer to Literature Cited, p. 27.

dipping them for 70 seconds in an 18-percent lye solution at 218° F. It has been reported (*6*) that carrots are well peeled by immersing them for 2½ minutes in a boiling 3-percent lye solution. No information occurs in the literature, apparently, concerning lye-peeling of green papayas.

MATERIALS AND METHODS

PAPAYAS

Commercially grown varieties of green papayas (*Carica papaya* L.) were used in all the experiments. It must be noted, however, that commercial varieties of papaya grown in Puerto Rico are not of a fixed type. They vary in size, shape, color, and thickness of pulp. The fruits studied were obtained from an experimental plot at the Gurabo Substation and from commercial plantations in the area of Isabela. The fruits for the lye-peeling studies were harvested completely green. The average weight of the fruit used in these experiments varied from 3 to 5 pounds.

LYE SOLUTION

Lye solutions were prepared from 76-percent pure sodium hydroxide flakes. The sodium hydroxide concentrations in these solutions varied from 92 to 104 g. per liter for the 10-percent solution, 152 to 180 g. per liter for the 15-percent solution, and 212 to 220 g. per liter for the 20-percent solution. The concentration of the solutions was readjusted periodically during the experiments. Twenty-five ml. of the lye solutions were titrated with 4 *N* hydrochloric acid. Flakes were added until the titration value indicated the concentration was within an acceptable range in the desired percent by weight concentration.

Boiling temperatures for the several lye solutions were 216°, 221°, and 226° F., for the 10-, 15-, and 20-percent by weight concentrations, respectively.

In this study, 125 pounds of solution were prepared for each batch because the capacity of the lye-peeler was limited to 14 to 17 gallons of lye solution.

SHEAR-PRESS DETERMINATION

After the papaya was peeled and washed, it was diced in a hand-operated mechanical dicer equipped with a 1-inch die. The resulting papaya cubes were then stacked in the sample holder of a Lee-Kramer shear-press. For these determinations the instrument was adjusted to the 2,000-pound range and to a velocity of 1.45 mm./second. The sample holder in this instrument takes 8 ounces of 1-inch diced cubes.

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LYE-PEELING

The lye peeler used consisted of a rectangular steel vessel divided into two chambers. Each chamber had a perforated basket which provided for a rapid separation of the fruit from the lye solution and quick immersion in fresh water. The lye solution was heated by a steam coil of high heating capacity.

The lye solution in this study was heated to the boiling point, then the papayas were immersed in the boiling solution for the predetermined period of time. After the specific time elapsed, the papayas were dumped into fresh cold water for fast cooling. The hydrolyzed tissue around the fruit was removed with water sprays until all softened tissue disappeared and the reaction of the surface of the peeled papaya was no longer alkaline to a phenolphthalein indicator.

RESULTS AND DISCUSSION

RELATION BETWEEN TIME OF IMMERSION AND THE LYE SOLUTION CONCENTRATION ON THE LYE-PEELING OPERATION

A series of experiments was conducted using lye solutions of 10-, 15-, and 20-percent lye by weight. The time of immersion of the papaya fruit varied from 2 to 7 minutes, 2 to 6 minutes, and 2 to 5 minutes, respectively, for each lye concentration. The average values and ranges for three replications are shown in table 1. The data presented reveals that a good peeling operation may be obtained from any of the three following combined boiling lye solution treatments: 6 minutes in a 10-percent solution, 4 minutes in a 15-percent solution, and 3 minutes in a 20-percent solution.

The results of the experiments to determine possible undesirable effects on the papaya pulp with respect to acidity and hardness are shown in table 2. The average values for hardness and acidity of the hand-peeled papaya pulp, compared with the values of lye-peeled papaya pulp, show them to be quite similar. Average values for these two properties for hand-peeled papaya pulp were 0.42 percent for total acidity and 6.62 square inches for hardness. The titrable acidity values for the lye-peeled papaya pulp expressed as citric acid, which range from 0.030 to 0.059 percent, do not show any specific trend as compared with 0.42 percent found for hand-peeled pulp. The average hardness value of 6.62 square inches found for hand-peeled papaya pulp is within the range obtained for the lye-peeled papaya pulp, which varied from 5.7 to 6.8 square inches.

PEELING CAPACITY OF THE BOILING LYE SOLUTIONS

A series of experiments was performed to determine the peeling capacity of the boiling lye solutions under study. The conditions of the experiments

TABLE 1.—*Relation between time of immersion and lye solution concentration on the effectiveness of the lye-peeling operation of green papayas*

Lye solution concentration (percent/weight)	Time of immersion in the lye solution	Visual evaluation of the peeling action	Loss in weight of the whole fruit in lye peeling	
			Average	Range
	Minutes		*Percent*	*Percent*
10	2	Questionable[1]	4.4	3.0–5.7
	4	do.	7.2	6.2–8.06
	5	do.	10.5	9.86–11.4
	6	Good[2]	13.2	11.8–14.52
	7	Very good[3]	11.0	9.84–12.12
15	2	Questionable	6.5	6.1–7.0
	4	Good	10.5	10.3–11.1
	5	Very good	12.0	11.6–12.3
	6	do.	15.5	14.3–16.7
20	2	Questionable	5.5	1.82–9.10
	3	Good	12.1	12.0–12.2
	4	Very good	15.6	14.3–17.02
	5	do.	14.4	12.0–15.9

[1] Questionable—large unpeeled spots or many small unpeeled spots remained.
[2] Good—one or two small unpeeled spots remained which could be easily removed in a trimming operation.
[3] Very good—completely peeled.

TABLE 2.—*Effect on hardness and titrable acidity of the papaya pulp when lye-peeled under one of the recommended conditions*

Lye solution concentration (percent/weight)	Time of immersion in the lye solution	Weight lost by the whole fruit on lye-peeling	Shear-press determination		Titrable acidity (calculated as citric acid)
			Maximum inflection on curve	Area under the curve	
	Minutes	*Percent*	*Units*	*Square inches*	*Percent*
10	6	15.1	1700	6.8	0.057
10	6	14.5	1770	6.5	.050
10	6	12.9	1670	6.0	.059
15	4	11.1	1820	6.7	.055
15	4	12.2	1740	6.6	.030
15	4	11.6	1580	5.9	.039
15	4	13.2	1500	5.7	.038
20	3	9.8	1660	6.0	.034
20	3	11.6	1680	6.5	.060

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were: A 6-minute immersion of the fruit in a 10-percent boiling lye solution, a 4-minute immersion in a 15-percent boiling lye solution, and a 3-minute immersion in a 20-percent boiling lye solution. The solution in the lye peeler was maintained at the 16-gallon level for all experiments. Table 3 shows the data obtained.

The data shows that a batch lye-peeling operation, in which a pound of papaya per gallon of lye solution relationship is maintained, results in a peeled fruit that can at least be evaluated as "good". The data also shows that the surface area of fruits being so peeled has an effect on the quality of the operation. When peeling 20 pounds of papaya, represented by a large number of fruits in 16 gallons of lye solution, the treatment results normally in peeled papayas which are evaluated as "questionable". However, when the 20 pounds of papaya are represented by a small number of fruits, the peeling operation results in one that may be classified as "good". The results presented in table 3 tend to show that the relation between the volume of boiling lye solution to weight of papaya is dramatically affected by the relation of the volume of lye solution to the surface area of the papayas being peeled.

HAND-PEELING OF PAPAYA FRUIT

A series of experiments were performed in which hand-peeled papayas were used. The results are shown in table 4. Hand peeling of fruits and vegetables obviously is a high labor-consuming operation; also, the quality of hand peeled papaya fruit and the quantity of waste are uncertain.

Table 4 shows that the loss as peel varied from 13.2 to 19.8 percent, or an average of 16.1 percent. The total combined loss as stems, seeds and peel fluctuated from 19.8 to 30.7 percent, or an average of 24.5 percent.

Table 5 shows the results of a series of pilot plant runs when papayas were lye-peeled using a 15-percent lye solution concentration and a 5-minute immersion. When the results obtained from a hand-peeling operation (table 4) are compared with those from a pilot plant lye-peeling operation (table 5) the difference in total percent lost in weight is very small with the added advantage of a low labor cost such as the lye-peeling method provides.

SUMMARY

A good lye-peeling operation may be obtained when whole green papayas are peeled under any of the following three combined treatments: 6 minutes in a 10-percent boiling lye solution, 4 minutes in a 15-percent boiling lye solution, and 3 minutes in a 20-percent boiling lye solution. The average weight lost as peel for each of the above treatments was 14.2, 11.6, and 11.5 percent, respectively. Changes in the titrable acidity and hardness of the pulp in lye-peeled papayas fell within the values obtained in hand-peeled

TABLE 3.—*Determination of ratio of papaya fruit to lye solution for proper peeling action*

Lye solution concentration (percent/weight)	Time of immersion in lye solution	Boiling temperature of lye solution	Weight of papaya fruit used		Number of fruits used in trial	Weight lost by whole fruit in lye-peeling	Visual evaluation of peeling action
	Minutes		*Pounds*	*Ounces*	*Units*	*Percent*	
10	6	216° F.	5	1	2	14.9	Very good
			5	2	2	17.1	Do.
			8	2	3	12.3	Do.
			8	2	3	15.4	Do.
			11	3	4	15.1	Do.
			11	4	3	13.9	Do.
			14	3	4	14.1	Do.
			14	4	4	15.4	Do.
			16	14	4	13.0	Do.
			17	3	5	13.8	Do.
			20	0	6	15.6	Questionable
			20	4	7	13.8	Do.
			23	0	7	12.2	Do.
15	4	221° F.	5	0	2	10.0	Good
			5	1	2	12.4	Do.
			8	3	3	14.5	Very good
			8	4	4	9.1	Questionable
			11	2	3	16.9	Very good
			11	4	4	12.2	Do.
			14	1	6	13.0	Good
			14	3	5	13.2	Very good
			16	14	6	13.7	Good
			17	2	10	10.8	Questionable
			20	2	5	11.5	Good
			20	8	8	11.6	Questionable
20	3	226° F.	5	4	2	16.7	Very good
			5	4	2	8.3	Good
			8	1	3	17.8	Very good
			8	3	3	14.5	Good
			11	1	3	14.1	Very good
			11	2	4	12.9	Good
			13	14	5	13.9	Very good
			14	2	4	15.5	Do.
			17	0	6	15.4	Good
			17	2	7	13.2	Questionable
			19	15	7	16.0	Good
			20	1	6	16.2	Do.

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papayas. In the lye peeling of whole green papayas, best results were obtained when the papayas were used at the rate of 1 pound for each gallon of lye solution used. It also was found that the average loss in weight of papayas as stems and seeds was 9 percent.

TABLE 4.—*Results from experiments with hand-peeled papayas*

Experiment number	Papayas	Weight of the papayas	Peeled pulp obtained	Stems and seeds	Peels	Total loss as stems, seeds, and peels	Average weight per papaya
	Number	*Pounds*	*Percent*	*Percent*	*Percent*	*Percent*	*Pounds*
1	6	20½	73.5	8.5	18.0	26.5	4.9
2	5	20½	74.2	9.8	16.0	25.8	4.1
3	5	20¾	73.5	10.8	15.7	26.5	4.2
4	7	25¼	69.3	10.9	19.8	30.7	3.6
5	6	24	71.8	9.4	18.8	28.2	4.0
6	7	28¾	77.4	5.2	17.4	22.6	4.1
7	6	26¼	75.3	7.6	17.1	24.7	4.4
8	6	25½	76.5	7.8	15.7	23.5	4.3
9	7	24¾	76.7	8.1	15.2	23.3	3.5
10	8	28	77.7	8.0	14.3	22.3	3.5
11	8	27½	76.3	8.2	15.5	23.7	3.4
12	8	27¼	78.0	7.3	14.7	22.0	3.4
13	7	30½	80.2	6.6	13.2	19.8	5.0
14	7	26¾	77.6	8.4	14.0	22.4	3.8
15	7	27½	75.6	8.2	16.4	24.6	3.9
16	8	30	75.0	9.2	15.8	25.0	3.8
Average values			75.5	8.4	16.1	24.5	3.9

TABLE 5.—*Pilot plant lye-peeling operation of papayas using a 15-percent lye solution and a 5-minute immersion time*

Experiment No.	Weight of the papayas	Peeled pulp obtained	Stems and seeds	Peel lost in lye-peeling	Total lost in lye-peeling plus stems and seeds
	Pounds	*Percent*	*Percent*	*Percent*	*Percent*
1	288	73.0	11.9	15.1	27.0
2	118½	73.4	9.3	17.3	26.6
3	161½	73.1	8.5	18.4	26.9
4	158½	74.5	8.8	16.7	25.5
5	137¼	75.4	8.4	16.2	24.6
6	91¾	73.0	9.3	17.7	27.0
Average values	955½	73.8	9.4	16.8	26.2

RESUMEN

Al revisar la literatura no se encontró información alguna sobre las condiciones más adecuadas para pelar la papaya usando soluciones de soda cáustica. Los experimentos realizados con otras frutas y vegetales sugirieron la conveniencia de investigar este aspecto del problema para determinar las relaciones correctas entre la concentración de la soda caústica y el tiempo de inmersión requerido para pelar la fruta.

En este trabajo se usó un tanque rectangular de acero, dividido en el medio para formar dos cámaras. Una de las cámaras contenía la solución de soda cáustica y la otra, el agua para el lavado de la fruta tratada con soda. El tanque tenía una cabida máxima funcional para 17 galones de solución.

Para el lavado de la papaya, la fruta se vació en un canasto de acero perforado, el canasto se sumergió luego en la cámara que contenía la solución de soda en ebullición y al terminar el tiempo prescrito para la inmersión, el canasto se sacó y se sumergió inmediatamente en la cámara con agua fría, donde fue que se removió la cáscara digerida por la soda.

La papaya (*Carica papaya* L.) que se usó en estos experimentos se obtuvo de un predio experimental sembrado en la Subestación de Gurabo y de plantaciones comerciales en el área de Isabela. Es bueno señalar que las variedades comerciales que se siembran actualmente en Puerto Rico no son de un tipo fijo, pues varían en tamaño, forma, color y espesor de la pulpa. El peso promedio de las frutas que se usaron fluctuó entre 3 y 5 libras.

Los resultados obtenidos de estos experimentos indican que cuando se pelan las papayas verdes y enteras es una solución de soda caústica, debe aplicarse uno de los tres tratamientos siguientes: Inmersión durante 6 minutos en una solución en ebullición de soda cáustica al 10 por ciento; inmersión durante 4 minutos en una solución en ebullición de soda cáustica al 15 por ciento; o inmersión por 3 minutos en una solución en ebullición de soda cáustica al 20 por ciento, por peso. La pérdida que se atribuye a la cáscara en cada uno de los tres tratamientos recomendados fue de 14.2, 11.6 y 11.5 por ciento, por peso, respectivamente. Los cambios en la acidez que pueden evaluarse y en la dureza de la pulpa de la papaya pelada con una solución de legía coinciden con los valores obtenidos para la pulpa de papaya pelada a mano. Existe una relación óptima entre el volúmen de la solución de legía y el peso de la papaya a pelarse. Los mejores resultados se obtuvieron cuando se trató la fruta a razón de 1 libra por galón de solución de soda cáustica. Las pruebas que se llevaron a cabo demuestran que la pérdida en el peso de la fruta que puede atribuirse a los tallos y semillas representa aproximadamente un 9 por ciento.

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Lower Esophageal Sphincter Response to Gastric Alkalinization

A New Mechanism for Treatment of Heartburn with Antacids

Cdr. DONALD O. CASTELL, MC, USN, F.A.C.P., and Lt. Cdr. STEPHEN M. LEVINE, MC, USNR,

Philadelphia, Pennsylvania

Recent studies have shown that the hormone gastrin has a profound effect on lower esophageal sphincter pressure. The present study was performed to evaluate the effect of different gastric alkalinizing agents. Sphincter pressure was monitored continuously in a group of normal subjects, using fluid-filled catheters. Instillation of 0.1 N hydrochloric acid into the stomachs of these subjects resulted in consistent decreases in lower esophageal sphincter pressure. The subsequent instillation of 0.1 N sodium bicarbonate resulted in striking increases in sphincter pressure to values much greater than basal levels. Similar results were obtained when 0.1 N sodium hydroxide or a commercial antacid preparation was placed into the stomach. Alkalinization of the stomach by various agents results in marked increases in lower esophageal sphincter pressure. The proposed mechanism of action is through release of endogenous gastrin from the antrum. Treatment of heartburn with alkali appears to have a dual effect—neutralization of gastric acid and an increase in the lower esophageal sphincter pressure barrier.

THE PHYSIOLOGIC sphincter at the distal end of the esophagus is readily identified as a zone of increased pressure between the stomach and the esophagus. It is generally accepted that the primary function of the lower esophageal sphincter is to provide a barrier against the reflux of acid gastric contents into the esophagus. In recent months the hormone gastrin has been shown to have a profound effect on this sphincter, with marked increases in sphincter pressure resulting from injection of pentagastrin or after various maneuvers to cause release of endogenous gastrin from the antrum (1, 2). Thus, it appears that antral gastrin causes physiologic increases in gastric acidity and also a simultaneous increase in the antireflux function of the lower esophageal sphincter.

Deacidification, or alkalinization, of the antrum is a known mechanism for producing endogenous gastrin release (3). Since alkali in various forms is widely used in the treatment of heartburn, the present investigation was undertaken to evaluate the effects of these agents on lower esophageal sphincter pressure.

Methods

PATIENTS STUDIED

Studies were performed in normal subjects and in patients referred to the gastrointestinal unit of this hospital for evaluation of sphincter competence. A total of 34 patients were studied, including 28 patients with normal pressure at the lower esophageal sphincter and 6 with abnormally low pressure (incompetent sphincter) (4).

INTRALUMINAL PRESSURE MEASUREMENT

In all subjects, after an overnight fast, a specially designed four-lumen tube was passed into the stomach. Three water-filled polyvinyl tubes 1.4 mm in internal diameter transmitted intraluminal pressure to external transducers (Sanborn 267A). These pressures were graphed on a multichannel, direct-writing recorder. The tubes were arranged so that intraluminal pressures were recorded at three points 5 cm apart through lateral orifices 1.2 mm in diameter. Gastric secretion was collected by an additional tube with multiple perforations over a 7-cm segment. The three tubes used to record intraluminal pressure and the tube used to collect gastric secretions were combined into a single four-lumen assembly with a total outside diameter of 5 mm. The midpoint of the area of multiple perforations used to collect gastric secretions was 15 cm distal to the point

► From the Gastroenterology Branch, Medical Service, U. S. Naval Hospital, Philadelphia, Pa.

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from which lower esophageal sphincter pressures were recorded.

Studies were done while the patients were in the supine position. Belt pneumographs around the chest and over the larynx allowed respirations and swallows, respectively, to be monitored. The tube assembly was slowly withdrawn from the stomach with manometric monitoring until the middle recording orifice was positioned in the lower esophageal high pressure zone (sphincter). The tube assembly was then anchored. Intraluminal pressures were thus recorded simultaneously from the distal esophagus, lower esophageal sphincter, and fundus of the stomach. The tube used to record pressures was infused with distilled water at a rate of 0.4 ml/min. Pressures were recorded in millimeters of mercury with mean gastric fundic pressure being used as the zero reference. Pressure in the lower esophageal sphincter was expressed as the mean (midway between inspiration and expiration) pressure level greater than fundic pressure.

Gastric secretions were also collected continuously and then divided into 5-min samples. Intermittent suction by hand syringe was used as necessary. The pH of each specimen was measured directly using a Beckman glass electrode and the hydrogen iron concentration calculated using the method of Moore and Scarlata (5). The pH electrode was calibrated before each study using the National Bureau of Standards K-tetroxalate dihydrate buffer (pH 1.679 at 25 C) recommended by Moore (6).

GASTRIC ACIDIFICATION AND ALKALINIZATION

After a 15-min basal period, during which intraluminal pressures and gastric secretion were monitored as described above, 0.1 N hydrochloric acid was instilled into the stomach through the lumen opening into the gastric fundus. The acid was instilled at a rate of 6 to 10 ml/min over a 20-min period, with a resulting fall in intragastric pH to less than 1.5 in all subjects. After

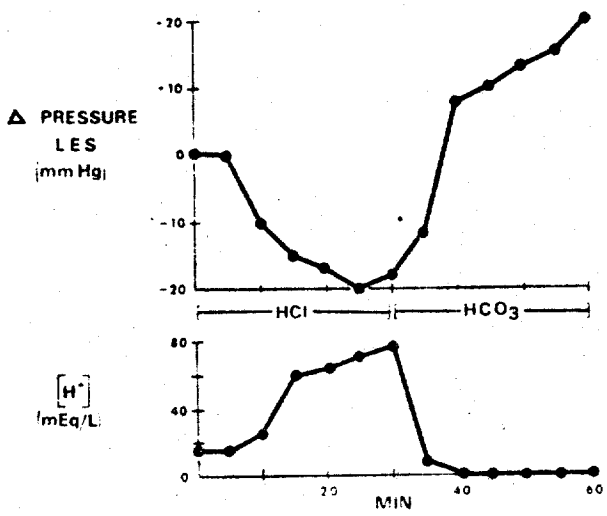

Figure 1. Change in lower esophageal sphincter (LES) pressure from the basal level and intragastric hydrogen ion concentration at 5-min intervals in one normal subject during instillation of 0.1 N hydrochloric acid and 0.1 N sodium bicarbonate into the stomach.

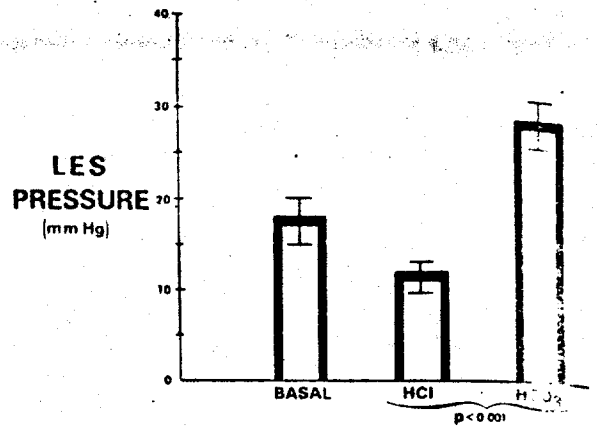

Figure 2. Mean pressure in the lower esophageal sphincter (LES) ± 1 SE in 14 subjects during the basal state and with subsequent gastric acidification (hydrochloric acid) and alkalinization (bicarbonate).

gastric acidification the stomach was emptied through the collecting lumen of the tubing, and alkali was instilled into the stomach. Either 0.1 N sodium bicarbonate or 0.1 N sodium hydroxide was administered also at a rate of 6 to 10 ml/min through the lumen opening into the gastric fundus, and lower esophageal sphincter pressure was monitored for an additional 20 to 30 min. In some studies a commercial antacid preparation containing magnesium hydroxide and aluminum hydroxide was used. For the antacid studies the solution was administered as a single bolus of 60 ml of antacid after the removal of the hydrochloric acid from the stomach.

ALKALINIZATION WITHOUT PRIOR ACIDIFICATION

Six subjects were studied without prior acidification of the stomach in order to observe the effect of the antacid preparation on sphincter pressure with the patient in a basal state. During these studies basal pressure and intragastric acidity were recorded for 15 min before the instillation of 60 ml of the antacid. After alkalinization the pressures and intragastric acidity were monitored for an additional 30 min.

Statistical significance between sphincter pressures obtained before and after gastric alkalinization in each group of subjects was evaluated using the Student's *t* test for paired samples.

Results

In Figure 1 are shown the results of the initial studies in one normal subject. Changes in lower esophageal sphincter pressure and intragastric hydrogen ion concentration for each 5-min period demonstrate that as the gastric acidity increases, there is a striking decrease in sphincter pressure. In this subject the lower esophageal sphincter pressure decreased by 20 mm Hg from an initial basal level of 25 to a low point of only 5 mm Hg. The effect of the subsequent alkalinization of the stomach with sodium bicarbonate is equally dramatic. With alkalinization of the stomach a marked rise in sphincter

pressure occurred again within the first few minutes. Pressure increased to a peak of 45 mm Hg, 20 mm Hg greater than the initial basal pressure.

Similar studies were performed in 14 subjects, using sodium bicarbonate as the alkalinizing agent. Figure 2 illustrates a summary of the results obtained. In this and subsequent figures the bars represent mean pressure in the lower esophageal sphincter for the group of subjects, and the vertical lines indicate ± 1 SE of the mean. The mean basal pressure was 17.6 ± 2.6 mm Hg. During gastric acidification pressure fell to 11.3 ± 1.8 mm Hg. With subsequent alkalinization lower esophageal sphincter pressure rose to a level of 28.0 ± 2.7 mm Hg. The overall change in sphincter pressure between that occurring with the stomach acidified and after gastric alkalinization was 17.0 ± 2.8 mm Hg. The difference between lower esophageal sphincter pressures obtained during gastric acidification and with subsequent alkalinization with 0.1 N sodium bicarbonate was highly significant ($P < 0.001$). The sphincter pressures after gastric alkalinization were also significantly greater than basal pressure levels ($P < 0.001$).

Figure 3 shows the results obtained with similar studies in five subjects, using 0.1 N sodium hydroxide as the alkalinizing agent. Mean basal lower esophageal sphincter pressure was 20.0 ± 4.1 mm Hg. With acidification of the stomach the pressure fell to a mean level of 10.0 ± 2.0, and with subsequent alkalinization with sodium hydroxide pressure rose to 35.4 ± 4.0 mm Hg. The overall change in sphincter pressure from the acidified to the alkalinized stomach was 25.6 ± 4.9 mm Hg. The difference between the mean sphincter pressures during gastric acidification and after subsequent alkalinization with sodium hydroxide was again highly significant ($P < 0.01$). The pressure levels after sodium hydroxide were also significantly greater than basal pressures ($P < 0.05$).

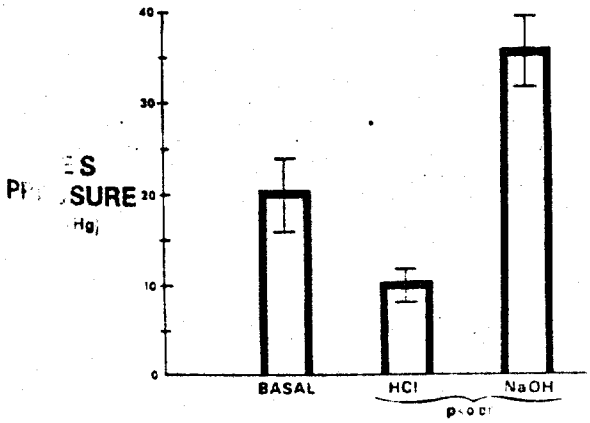

Figure 3. Mean pressure in the lower esophageal sphincter [illegible] 1 SE in five subjects, during the basal state and with [illegible] gastric acidification (hydrochloric acid) and [illegible] (sodium hydroxide).

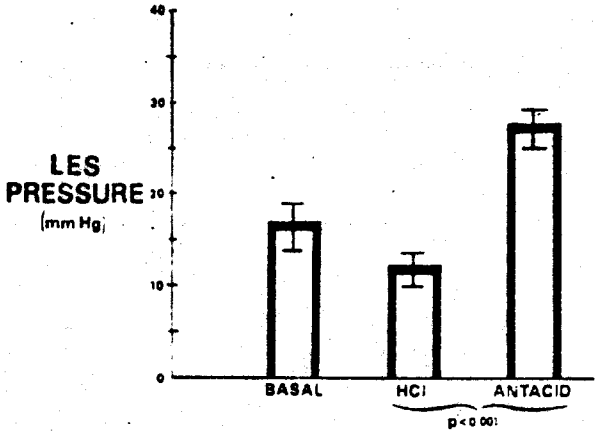

Figure 4. Mean pressure in the lower esophageal sphincter (LES) ± 1 SE in nine subjects during the basal state and with subsequent gastric acidification (hydrochloric acid) and alkalinization (antacid).

The results of similar studies using 60 ml of a standard antacid preparation in nine subjects are shown in Figure 4. The mean basal lower esophageal sphincter pressure was 16.4 ± 2.6 mm Hg. With gastric acidification pressure fell to 11.7 ± 1.7 mm Hg and with subsequent alkalinization with antacid pressure rose to 30.1 ± 2.4 mm Hg. The overall change in sphincter pressure was 14.1 ± 1.4 mm Hg. Again the difference between the mean values for lower esophageal sphincter pressure during gastric acidification and with subsequent gastric alkalinization with antacids was highly significant ($P < 0.001$). Pressure levels after antacid administration were also significantly greater than basal sphincter pressures ($P < 0.001$).

The results of gastric alkalinization with a commercial antacid preparation without preceding acidification are shown in Figure 5. Here the mean basal pressure for six subjects was 16.0 ± 2.1 mm Hg. With subsequent alkalinization with 60 ml of commercial antacid the pressure rose to 30.1 ± 2.4 mm Hg, the change in pressure being 14.1 ± 1.4 mm Hg. The difference between mean basal pressure and mean pressures after gastric alkalinization was highly significant ($P < 0.001$).

RESPONSE IN PATIENTS HAVING AN INCOMPETENT SPHINCTER

Results of lower esophageal sphincter pressures before and after gastric alkalinization in six patients with abnormally low sphincter pressures are shown in Table 1. Mean basal pressure for these six subjects was [illegible] mm Hg. With subsequent alkaliniza-

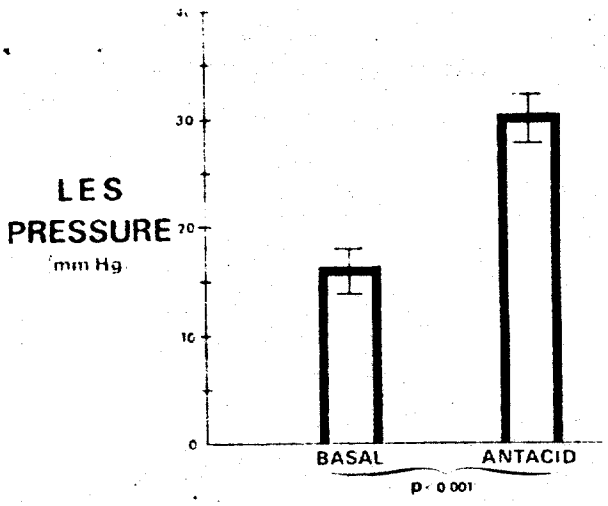

Figure 5. Mean pressure in the lower esophageal sphincter (LES) ± 1 SE in six subjects during the basal state and with subsequent gastric alkalinization with a commercial antacid.

tion with a commercial antacid sphincter pressure rose to a mean level of 19.2 ± 0.98 mm Hg. The difference between these mean values is highly significant ($P < 0.001$).

Discussion

Alkalinizing agents in various forms have been the mainstay of the treatment of heartburn for many years. This therapy has been given primarily to neutralize any acid being refluxed from the stomach. Our recent studies on the effect of the hormone gastrin on lower esophageal sphincter pressure have suggested the possibility of another mechanism in the treatment of reflux with alkali (1). Various maneuvers, including alkalinization of the stomach, known to cause release of endogenous gastrin from the antrum have been shown to result in increases in lower esophageal sphincter pressure and thus potentially to increase the antireflux mechanism of this region.

The present studies have shown that after gastric acidification the addition of sodium bicarbonate to the stomach results in significant increases in lower esophageal sphincter pressure. These increases in sphincter pressure were noted to occur within the first 10 min after gastric alkalinization and would appear to be consistent with the early measurable release of gastrin into the serum, as shown by McGuigan, Jaffe, and Newton (7).

The subsequent studies using 0.1 N sodium hydroxide as the alkalinizing agent were performed to ascertain that the effect observed with sodium bicarbonate was not a specific effect for this agent but was more likely caused by alkalinization per se. Again highly significant increases in sphincter pressure were observed in all subjects after sodium hydroxide was instilled into the acidified stomach. These striking increases in lower esophageal sphincter pressure occurring after either sodium bicarbonate or sodium hydroxide instillation would indicate that neutralization of gastric contents has a profound effect on the sphincter pressure barrier.

In clinical practice, however, gastric alkalinization is more commonly accomplished with commercial antacid preparations. Therefore, the effect of an antacid preparation on lower esophageal sphincter pressure was studied. As in the studies using other alkalis, highly significant increases in sphincter pressure were observed in all subjects when 60 ml of the antacid was instilled into the previously acidified stomach.

The effect of the commercial antacid preparation on lower esophageal sphincter pressure without prior acidification of the stomach was also studied. Again highly significant increases in sphincter pressure were noted after instillation of 60 ml of antacid into the stomach. These results confirm the effect of gastric alkalinization in promoting marked improvement in the antireflux barrier provided by the lower esophageal sphincter under usual clinical conditions.

The real clinical importance of treatment of heartburn with antacids obviously involves those patients who have incompetent lower esophageal sphincters. Therefore, the data from the six subjects included in our studies who had lower esophageal sphincter pressures in the range (less than 10 mm Hg) that had previously been shown in patients with sphincter incompetence are of particular interest. Mean sphincter pressure for these patients rose from a basal level of 5.5 mm Hg to 19.2 mm Hg after antacid administration. It is important to note that during the basal state these subjects all demonstrated lower esophageal sphincter pressures in the range previously

Table 1. Response of Lower Esophageal Sphincter Pressure to Gastric Alkalinization in Patients Having an Incompetent Sphincter

Patient	Basal Sphincter Pressure		Sphincter Pressure After Alkalinization
		mm Hg	
D.B.	4		20
W.S.	4		18
E.S.	3		20
M.R.	8		22
H.B.	9		15
D.R.	5		20
Mean	5.5		19.2
SE	1.0		0.98
Student's *t* test		8.4	
P		<0.001	

shown to be found in patients with clinical incompetence—in other words, patients with definite symptoms of reflux (4). After alkalinization the pressures in the total group were all within the range of pressures found in the resting state in normal subjects—in other words, in those subjects without symptoms of reflux.

The results of these studies indicate that alkalinization of the stomach results in marked increases in lower esophageal sphincter pressure. This effect is apparently produced through release of endogenous gastrin from the gastric antrum. This effect has been shown to occur after administration of various alkalinizing agents, including commercial antacids. It would appear that alkali has a dual effect in the treatment of heartburn, not only neutralizing the gastric acid but also resulting in significant increases in the lower esophageal sphincter pressure barrier.

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► Requests for reprints should be addressed to Donald O. Castell, M.D., U.S. Naval Hospital, Philadelphia, Pa. 19145

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A study of various chemical treatments to remove lignin from coarse roughages and increase their digestibility

BY SURESH CHANDRA* AND M. G. JACKSON
College of Agriculture, U.P. Agricultural University, Pantnagar, District Nainital, U.P., India

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SUMMARY

Several delignifying agents, used in various paper-making processes, have been compared for their ability to remove lignin and increase the digestibility of several roughages in laboratory experiments using a spray method of treatment recently suggested by Wilson & Pigden (1964). The chemicals were dissolved in small quantities of water which were then used to moisten the roughage. No washing was done after treatment. Sodium hydroxide was the most effective chemical; at a rate of 10 g per 100 g of roughage, the lignin content of ground maize cobs was reduced by 26 % and rumen dry-matter digestibility increased by more than 100 %. Bleaching powder was as effective as sodium hydroxide in removing lignin, but residual chlorine apparently inhibited rumen microbial digestion.

Maize cobs, wheat and paddy straws, sorghum stover and sugar cane tops, though of widely differing initial digestibility, were brought to an equal level of rumen digestibility if treated with 4 % or more of sodium hydroxide. Drying the treated roughage at 100 degrees instead of at room temperature increased digestibility only in the case of maize cobs. The alkali spray treatment was as effective in increasing the digestibility of chaffed as of ground wheat straw. Data are presented which show the amounts of residual alkali in treated roughage as a function of time after treatment and of concentration of alkali used in the treatment. Further, data are presented which show the loss of nutrients from treated roughage if washed as in the traditional method.

INTRODUCTION

A sodium hydroxide treatment to break down the lignin and thus improve the feeding value of roughages was developed many years ago by Beckmann (1921) in Germany and its effectiveness subsequently confirmed by Ferguson (1942) in England and Sen, Ray & Talapatra (1942) in India. The procedure involves soaking the roughage in vats of sodium hydroxide solution and then washing it. Processing costs are, however, high and for this reason alkali-treated roughage is an economical feed only when the prices of better quality feeds are very high. In most countries of the world, even including India with its relatively high feed prices, alkali-treated roughage is not an economical feed at present. Costs could be reduced considerably by the alkali spray method of treatment reported by Wilson & Pigden (1964). In this, the roughage is treated with half its own weight of sodium hydroxide solution. The moist roughage can then immediately be fed to stock or dried and fed later, but in any case it is not washed. The heavy loss of dry matter by leaching (20–30 %) in Beckmann's method, which increases the cost of the treated roughage so sharply, is thus avoided.

As a first step in a programme of research designed to determine the true utility of this spray method of chemical treatment for producing economical treated roughage, two series of laboratory experiments were conducted. They are described in this paper. In the first series several different chemical reagents, all known to react with lignin, were compared for their effectiveness in removing lignin and in increasing digestibility. From these experiments sodium hydroxide was found to be the most effective reagent and, in view of its relative cheapness, its effect was studied in more detail in a second series of experiments.

EXPERIMENTAL

The roughages used in these experiments (Table 1) were initially ground in a hammer mill through a

* Present address: Department of Physiology and Bacteriology, S.P. Medical College, Bikaner, Rajasthan, India.

Table 1. *Chemical composition of roughages* (% of dry matter)

Roughage	Ash	Silica ash	Crude protein	Crude fibre	Ether extract	Lignin: Pepsin method*	Lignin: Russian method†	Cellulose‡
Maize cobs	2·2	0·4	1·9	31·0	1·4	19·4	21·9	34·2
Wheat straw	8·7	3·5	3·8	40·3	1·9	31·6	34·7	45·9
Paddy straw	12·0	4·9	3·7	34·9	1·6	33·2	38·6	42·6
Sorghum stover	8·1	3·7	5·6	39·9	1·7	33·6	39·8	38·0
Sugar cane tops	7·7	2·9	4·5	29·8	1·0	33·8	38·6	28·7

* Thacker (1954), † Pearl (1967), ‡ Crampton and Maynard (1938).

3 mm (5 mm for maize cobs) sieve and then reground in a laboratory mill through a 2 mm sieve. The chemical treatments given are described below.

Series I. Ground maize cobs was selected as a reference roughage for this series of experiments. The reagents selected were those used in the various processes of paper making (for a review see Pearl, 1967). They are shown below, along with the concentrations tested. All the chemicals used were of analytical reagent grade.

Reagent	Concentration (g reagent/100 g roughage)
1. Sodium sulphide	0, 1, 2, 3, 5, 7, 10, 12, 15
2. Sodium sulphite	0, 1, 2, 3, 5, 7, 9, 12, 15
3. Sodium hydroxide	0, 1, 2, 3, 4, 6, 8, 10, 12, 15
4. Sodium carbonate	0, 1, 2, 3, 4, 6, 8, 10, 12, 15
5. Equal parts of sodium hydroxide and sodium carbonate	0, 2, 4, 6, 8, 12, 15
6. Bleaching powder	0, 1, 2, 4, 6, 8, 10
7. Hydrogen peroxide	0, 1, 2, 3, 4, 5, 6

In addition to these, several organic reagents that are used in laboratory studies of lignin chemistry, e.g. alkaline nitrobenzene, methanol and phenol, were tried in preliminary trials. The results (Chandra and Jackson, unpublished data) showed that they had to be used in large amounts in relation to the amount of roughage taken to give even a small increase in digestibility. The use of large amounts of expensive chemicals would not be commercially feasible. Further, unreacted residues of these chemicals would be likely to be toxic to animals.

The treatment of the maize cobs was done as follows: Ten g of dry ground cobs was taken in a 500 ml beaker. To this, reagents 1–5 were added in appropriate quantities, dissolved in 10 ml of water. The mixture was stirred, allowed to stand for 1 h and then oven-dried at 100 degrees. (Sodium sulphide-treated material was dried at 50 degrees because it charred if dried at 100 degrees). For the bleaching powder treatment, 10 g of material was taken in a 500 ml conical flask with a rubber stopper. Ten ml of the bleaching powder solution was added and mixed, followed by 10 ml of a sulphuric acid solution the concentration of which was half that of the bleaching powder solution used. The flasks were tightly stoppered and shaken periodically for 1 h. The treated material was dried at 50 degrees. The same procedure was used for the treatment with hydrogen peroxide, except that 10 ml of a 0·001 N sulphuric acid solution was used uniformly for all concentrations of hydrogen peroxide. With both the bleaching powder and the hydrogen peroxide, drying was done at 50 degrees to avoid charring of the treated material.

Series II. The following experiments were done:

1. The following roughages were treated with sodium hydroxide at 0–10% concentrations and the comparative effect on rumen dry-matter digestibility was determined.

(*a*) maize cobs,
(*b*) wheat straw,
(*c*) paddy straw,
(*d*) sorghum stover,
(*e*) sugar cane tops.

2. The effect of drying temperature upon the extent of increase in digestibility was studied. Each of the first four roughages listed above, after treatment with 0–15% sodium hydroxide, was dried either at room temperature or at 100 degrees.

3. The amount of residual sodium hydroxide in treated maize cobs and wheat straw as a function of concentration of sodium hydroxide and of time after treatment was determined by washing a sample of treated roughage until the washings were free from alkali. The collected washings were then titrated with 0·5 N sulphuric acid, using phenol-red indicator.

4. The relative losses of various nutrients from sodium hydroxide treated maize cobs washed as in the method of Beckmann were determined by analysis of oven-dried washings.

5. The comparative effect of sodium hydroxide treatment of ground and chaffed wheat straw on dry-matter digestibility was determined.

Determination of lignin content and dry-matter digestibility. In series I experiments on maize cobs, lignin was determined by the Russian standard method, Gost 1856, for the determination of lignin in wood (Pearl, 1967). The protein content of the cobs was so low that a protein digestion pre-treatment, as in the method of Thacker (1954), was not thought to be necessary. The standard method was modified only at the filtration stage, where a hydrochloric acid treated Whatman No. 40 filter paper was used instead of a G-2 Schotta crucible. The granular precipitated lignin was washed with hot water and dried to constant weight. Dry-matter digestibility was estimated by the nylon bag, rumen digestion technique of Kearen & Heineman (1962). While this technique does not necessarily give values equal to conventional *in vivo* digestibility values, it is considered a valid technique to determine the relative digestibilities of feeds. All the treatments in a given experiment formed one lot for the determination of dry-matter digestibility. Duplicate lots were placed simultaneously in the rumens of two fistulated Sahiwal steers and kept for 72 h. Each digestibility value is thus a mean of two independent determinations. Dacron cloth having 55–58 threads per cm was used. One gramme of dried material was placed in each bag. The steers, weighing 250–300 kg, were given a low-bulk ration to facilitate the insertion and removal of the samples. The ration consisted of 5 kg fresh lucerne herbage and 3 kg of a concentrate mixture containing salt and trace minerals. This ration was fed throughout the course of all these experiments, and beginning 1 month before the experiments started. The animals were watered twice daily at fixed times.

RESULTS AND DISCUSSION

Series I. The effect of various reagents, at increasing concentrations, on lignin content and rumen dry-matter digestibility is shown in Fig. 1. All the reagents acted on lignin. The resulting increases in rumen dry-matter digestibility were roughly proportional to the degree of lignin breakdown, with the exception of the bleaching powder treatment. Sodium hydroxide was clearly the most effective reagent. The shape of the curve for rumen dry-matter digestibility for sodium hydroxide is similar to that obtained in an *in vitro* experiment (Wilson & Pigden, 1964) with wheat straw similarly treated, a steady linear increase in digestibility up to the 10% level and a levelling off after that. It must be remembered here that these increases in digestibility value are really potential increases and may not be realized in actual feeding trials with the same feeds because unreacted alkali at the higher levels of treatment (see results Exp. 3, series II of this paper, p. 15) and/or high sodium intakes might upset the rumen fermentation and thus prevent the potential digestibility of the feed from being realized. The results of the feeding trials conducted by Singh & Jackson (1971) do, in fact, suggest this. They fed animals sodium hydroxide spray-treated, straw-based rations made up of straw treated with 0, 3·3, 6·7 and 10% alkali. Digestibility increased only up to the 3·3% level and levelled off thereafter. Similarly the Canadian workers (Pigden, private communication) found a levelling off of digestibility above 6% NaOH in feeding trials with sheep.

The bleaching powder was as effective as sodium hydroxide, over an equivalent range of concentration, in removing lignin. When it came to digestibility, however, the residual chlorine in the treated material may have been toxic to rumen micro-organisms and thus digestion inhibited beyond the 2% level of treatment. Dry chlorine or chlorine dioxide gases were both found to be effective de-lignifying agents when passed through ground roughages (Sullivan & Hershberger, 1959). The advantage of the dry-gas treatment over the wet treatment with bleaching powder tried here was that, after aeration, the residual gas was removed and digestibility was not inhibited. With wheat straw they were able to reduce the lignin content by 75% and increase *in vitro* digestibility by 140%. The results of further studies by these authors suggest that dry treatment with chlorine or chlorine dioxide gas might be feasible on a commercial scale if suitable large, air-tight, rotating mixers could be designed and the gas obtained in big tanks at bulk rates (Sullivan, personal communication).

Series II. The comparative effect of sodium hydroxide on the rumen dry-matter digestibility of five important roughages is given in Table 2. The data show that although the digestibility of the untreated roughages varied considerably the differences were eliminated from 3 to 4% of sodium hydroxide upwards. The rate of improvement in digestibility from 0 to 4% was thus more rapid with the poorer roughages. The results suggest that these roughages would have equivalent feeding value, provided the level of sodium hydroxide treatment was 3–4% or more.

The effect of temperature on the action of sodium hydroxide in increasing the rumen digestibility of four roughages is presented in Fig. 2. There was an improvement in rumen digestibility by drying at 100 degrees as compared to air-drying at room temperature with maize cobs only and not with other roughages. Hot-air-drying would thus probably not be an economical adjunct to the alkali treatment, and the treated roughage would as well be

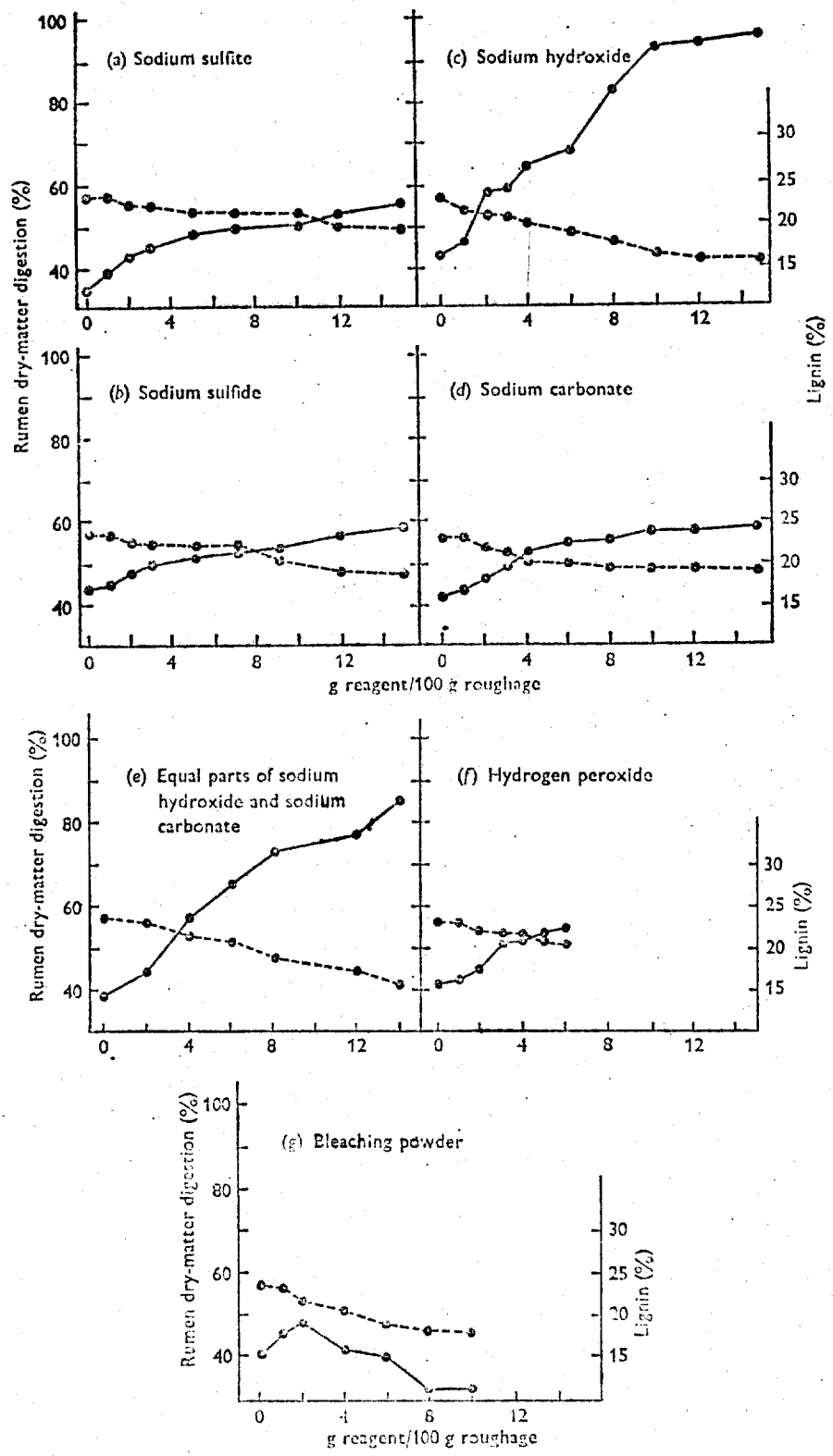

Fig. 1. The effect of chemical treatments on the lignin content (○----○) and rumen dry-matter [illegible] of ground maize cobs: (a) sodium sulphite; (b) sodium sulphide; (c) sodium [illegible] (d) equal parts of sodium hydroxide and sodium carbonate; (e) hydro[illegible]

fed without drying at all, except if it was desired subsequently to pellet it.

The amount of residual alkali in ground maize cobs and wheat straw as a function of concentration of alkali used and of the time after treatment is presented in Tables 3 and 4, respectively. The residual alkali increased rapidly to high levels with increasing concentration. These might well interfere with microbial fermentation or even prove hazardous to livestock health if high concentrations of alkali were used for treating the roughage. The extent of nutrient losses if the treated roughage is thoroughly washed, however, is shown in Table 5. As was reported earlier by several workers, these are serious and would increase the cost of the finished material considerably. Against the possible liability of residual alkali in the unwashed roughage, must be set this increased cost.

Table 4 shows that a slight reduction (15–20 %) in the amount of residual alkali occurs with time. Stated in other terms, if the reaction of the alkali with the straw is considered completed at 18 h, then the reaction was 93–95 % completed at 5 min and 97–98 % at 1 h. While the reaction may not have been completed at 18 h, the shape of the reaction curve when the data of Table 4 is plotted suggests that very long periods, i.e. periods of several days, would have been required to obtain further significant reaction. While it might be worthwhile for a small farmer preparing small batches of alkali-

Table 2. *Comparative rumen dry-matter digestibility of roughages* (%)

Roughages	g NaOH/100 g roughage 0	3·3	6·7	10
Maize cobs	46	66	78	93
Wheat straw	51	67	80	89
Paddy straw	57	69	82	89
Sorghum stover	58	68	83	87
Sugar cane tops	56	65	78	86

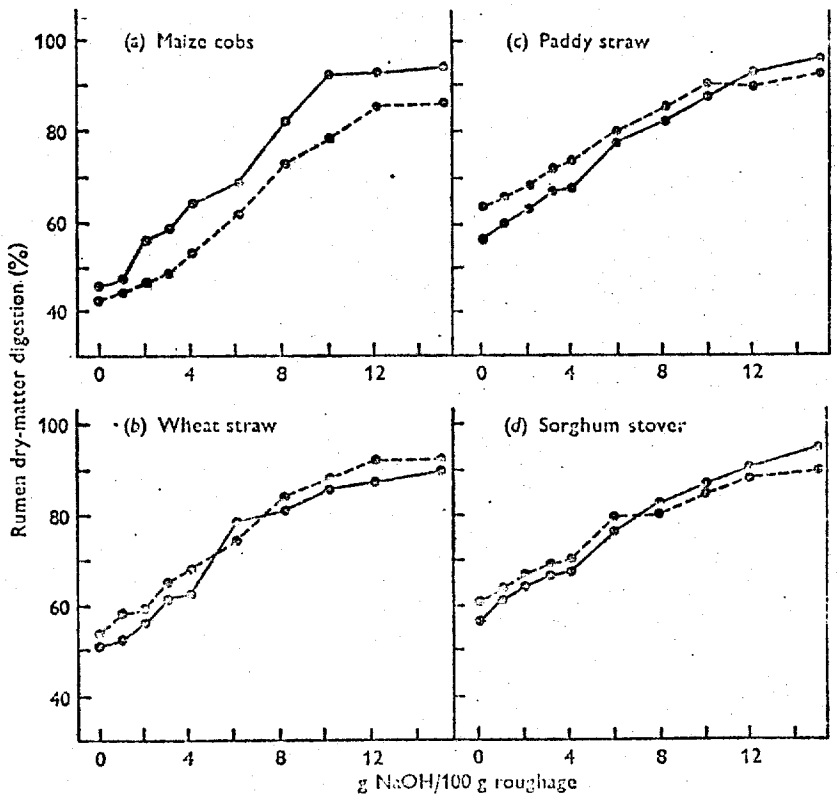

Fig. 2. Effect of drying temperature on the action of sodium hydroxide in increasing rumen dry-matter digestibility. ○——●, Drying at 100 degrees; ○- - - -●, drying at room temperature. (*a*) Maize cobs; (*b*) wheat straw; (*c*) paddy straw; (*d*) sorghum stover.

treated straw rations by hand to allow the treated straw to lie about for several hours or days, in any mechanized treating set up, the provision of more than 10–15 min 'curing' time has been found, in the experience of the authors, to be difficult and expensive. The benefit from longer curing times is certainly marginal, and probably would not be economical.

One group of workers has neutralized unreacted alkali with acetic acid (Donefer, Adeleye & Jones, 1969), but the necessity for or benefit from doing this has not been established experimentally. From the data in Table 3, it is obvious that there is no unreacted alkali to neutralize unless more than 2–3 % of alkali is used in the treatment. At higher levels of treatment such as used by Donefer *et al.* (1969) (8 %), the neutralization of the unreacted alkali might be desirable. Acetic acid is, however, quite expensive and its use would considerably raise the cost of treated roughage. Since acetic acid also supplies energy, this extra cost would be partly offset. Carefully planned, long-term feeding trials are needed to determine whether neutralization is necessary or beneficial and at what minimum cost it could be done.

The data presented in Table 6 indicate that sodium hydroxide treatment is as effective in increasing the digestibility of chaffed wheat straw as of ground straw. The large difference between the digestibility of chaffed and ground roughage would probably not obtain when the two were fed to animals and the chaffed material ruminated.

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Table 3. *Residual sodium hydroxide as a function of sodium hydroxide concentration, % of total sodium hydroxide added*

Treatment, g NaOH/100 g roughage	Maize cobs	Wheat straw
0	0	0
1	0	0
2	2	2
3	3	8
4	4	12
6	12	24
8	21	36
10	24	34
12	32	40
15	32	40

Table 4. *Residual sodium hydroxide in 6 % treated roughage as a function of time after treatment, % of total sodium hydroxide added*

Time	Maize cobs	Wheat straw
5 min	23	32
30 min	22	30
1 h	20	29
4 h	19	29
18 h	19	27

Table 5. *Proportion of original nutrients lost by washing sodium hydroxide treated maize cobs as a function of concentration of alkali used (%)*

Treatment, g NaOH/100 g maize cobs	Dry matter	Organic matter	Ash	Crude protein	'Carbohydrates'*
0	9	8	32	1	9
3	13	11	54	21	11
6	19	14	76	32	14
10	29	22	85	44	21
12	32	30	88	50	29
15	35	32	90	59	31

* 'Carbohydrates' were estimated by subtracting crude protein from organic matter. It thus includes, in addition to true carbohydrates, traces of fat, lignin and lignin breakdown products.

Table 6. *Rumen dry-matter digestibility of sodium-hydroxide treated chaffed and ground wheat straw as a function of alkali concentration (%)*

Physical condition	g NaOH/100 g wheat straw									
	0	1	2	3	4	6	8	10	12	15
Chaffed	39	40	43	45	48	59	63	68	73	77
Ground	52	52	58	62	64*	78	81	89	88	91

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583. THE EFFECT OF STEAM, HYPOCHLORITE AND CAUSTIC SODA USED FOR TREATING DIRECT-TO-CAN MILKING EQUIPMENT, ON THE BACTERIOLOGICAL QUALITY AND FLORA OF MILK

By D. F. C. CARREIRA,* L. F. L. CLEGG, P. A. CLOUGH and C. C. THIEL

with technical assistance from

D. N. Akam and Marie Gruber

National Institute for Research in Dairying, University of Reading

and Elizabeth Hirons

National Agricultural Advisory Service, Aylesbury, Bucks

(With 1 Figure)

The rubber surfaces of a milking machine that come into contact with milk (i.e. the teat cup liners and long milk tubes) are the most difficult parts to clean and sterilize. The reason for this is that fat from milk and the surface of the udder promotes oxidation (1) which so affects the rubber surface that it can only be cleaned adequately by materials which penetrate. Heat penetrates and sterilizes but does not clean rubber. Detergents and disinfectants which act at the surface are effective only with prolonged contact. This led Johns (2) to recommend wet-storage of teat cup liners in 0·5% caustic soda. This method, or an alternative, using 2% alkaline dairy detergents plus 125 p.p.m. sodium hypochlorite, is now recommended in Advisory Leaflet 422 (3).

The use of 5% caustic soda at room temperature proved to be satisfactory in defatting rubber (4, 1). The solution is also strongly bactericidal (5).

A preliminary trial at this Institute (1953) of direct-to-can milking with the complete milking equipment (with stainless steel metal parts) cleaned by immersion in 5% cold caustic soda between milkings, gave excellent bacteriological results. A trial on eight commercial farms was made during 1951, and a detailed report of the process now known as immersion cleaning is described by Thiel, Clough & Clegg (6).

As this method of cleaning and sterilizing yielded milk of excellent keeping quality in a small trial, it was decided to find out whether the bacterial flora of the milk was different from that produced by equipment cleaned by brushing in a hot detergent solution and sterilized by steam or sodium hypochlorite, and also to examine the bacteriological quality of the milk. These two aspects of this work are reported in separate sections.

BACTERIOLOGICAL QUALITY OF MILK

Experimental methods

Technique of immersion cleaning

Immersion cleaning is a method of cleaning the milking equipment associated with direct-to-can milking. All the milk-contact surfaces except the can are either of stainless steel or rubber, and are completely immersed in caustic soda between milkings. The only articles which are in contact with milk during milking are the teat cup cluster with stainless steel shells and claw piece, the long milk tube and the can fitted with a specially designed stainless steel lid and rubber gasket. In this series of experiments these utensils, together with the stall tubes, were placed in a mild steel basket fitted with two handles to lower it into a mild steel container filled with 5% caustic soda. Before milking the basket was removed from the caustic soda container, allowed to drain, and transferred to a plain water container to rinse off the greater part of the caustic soda. The milking parts were then removed by hand and immersed in water plus chlorine (60 p.p.m.) to complete the rinsing without contamination. After milking the clusters were rinsed in the same chlorinated water, the rubbers demounted inside the shells, to allow subsequent rinsing free from caustic soda, and together with the lids were fitted into the basket and again immersed in caustic soda until the next milking. (For a more detailed description of the process see pp. 158–159 of this number of the *J. Dairy Res.*)

Both the caustic soda solution and the water in the preliminary rinse tank were renewed at monthly intervals.

Farm methods

Two farms were selected for the experiment, which covered the period February–September 1954. Farm A was a small farm with an outside bail where the utensils were treated with sodium hypochlorite. Farm B was a larger farm with a milking parlour where the utensils were sterilized by steam. On both farms the experimental treatment alternated with the control treatments for successive periods of 2 months. While the experimental treatment was in use at one farm a control treatment was used at the other. The rubberware coming in contact with milk was renewed at the beginning of each period of 2 months on both farms. The following are the methods of utensil treatment at the farms.

Farm A. After the morning milking the equipment was rinsed in cold water and brushed in a hot detergent chlorine wash. After the evening milking the equipment received only a plain water rinse followed by a cold rinse in chlorinated water, as recommended in *Advisory Leaflet*, no. 422.

Farm B. After each milking the equipment was rinsed in cold water and washed in hot detergent solution. The clusters were steamed on jets and the rest of the equipment in a steam chest.

At both farms sodium hypochlorite was used in the udder washing water, but in the first part of the work no special care was taken to see that udder washing was well done.

The treatment with caustic soda was used first on farm B and continued for 8 weeks. At the same time sodium hypochlorite was used as a control on Farm A. At the end of 8 weeks the treatments were changed, with farm B using steam sterilization as control.

Bacteriological tests

So that the results were not influenced by the bacteriological condition of cans returned from the creamery, a can which had been steamed for 30 min. was supplied to each farm on the day of sampling. Once every week a milk sample was taken during the morning milking from each farm, iced and tested within 3–4 hr.

At farm A the first can contained the milk from only 3–4 cows, whereas at farm B it contained the milk from 6–7 cows.

* British Council Scholar of Estaçao de Lactinios, Tapada da Ajuda, Lisboa, Portugal.

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At the same time samples of the 5% caustic soda solution and the liquid in the preliminary rinse tank were taken for bacteriological examination and to check the amount of caustic in the solutions. Periodically, clusters were rinsed 5 times with 500 ml. of ¼ strength Ringer's solution.

The milk samples were divided into two parts; one was tested on the day of sampling, and the other was aged at 22° C. for 24 hr. in a water-bath. Caustic soda and water samples were neutralized before testing (see below).

The following tests were begun between 9.30 and 10.30 a.m.:

Keeping quality. This was determined by incubating the milk at 22° C. and after 24 hr. examining every 3 hr. for the clot-on-boiling end-point.

Colony count. Plates were poured in triplicate with Yeastrel milk agar (Y.M.A.) and incubated at 30° C. for 3 days.

Total spore counts. Approximately 10 ml. of milk, caustic soda or rinse solution (previously neutralized) were heated at 80° C. for 10 min., cooled, and 1 ml. quantities plated with Y.M.A. and incubated at 30° C. for 3 days.

Resistant spore count. Quantities of 50, 5 and 0·5 ml. were heated for 30 min. at 100° C., cooled and incubated for 14 days at 30° C. before examination to determine the most probable number. The smallest quantity was diluted in 10 ml. autoclaved litmus milk. Those tubes which showed no change were tested by the alcohol test for sterilized milk (80% alcohol containing 0·02% neutral red).

Coli-aerogenes test. Quantities of 10, 1 and 10^{-1} ml. of milk were inoculated into MacConkey's broth and incubated at 30° C. for 3 days. For the aged samples 10^{-2}, 10^{-3} and 10^{-4} ml. dilutions were used. When using 10 ml. quantities, double-strength MacConkey's broth was used.

Results

The results from the two farms are given separately for comparison. Fig. 1 shows that the differences between the colony counts during the different periods are small. Differences between treatments were expected to be more apparent with aged samples, but no appreciable differences were found. During the fourth period on both farms, i.e. with both caustic and steam treatments, there was an increase in the number of samples with high initial counts. This difference may have been caused by some factor outside the experimental technique, probably the season. While in both initial and aged samples there are only small differences between control and caustic treatments, these differences are in favour of the caustic treatment on all but one occasion.

During the first three 8-week periods plates for colony counts were also incubated at 22° C. for 5 days with the unaged samples, but as the results at 22 and 30° C. were similar the former test was discontinued. Similar results were also observed with colony counts incubated at 22 and 30° C. with aged samples.

Table 1 shows the variation in numbers of coli-aerogenes throughout the four periods. The counts generally were low, indicating good cowshed technique. The initial count bears no relation to that of the aged milk. While the absence of coli-aerogenes/100 ml. in the fresh milk may mean a similar result in the aged milk, there were instances where initially these organisms were absent in 100 ml. but the aged milk gave high counts.

As it seemed possible that variations in udder washing or the cleaning of the clusters were responsible for variation in results, a rigid technique of udder washing was used for

the fourth period at both farms. Individual samples were taken from those cows whose milk was sampled from the first can; samples were also taken from the udder washing water and from cluster rinses.

The udder washing technique consisted of washing the udders with a solution of 1 oz. sodium hypochlorite to 2 gal. water, wringing out the cloth outside the bucket, washing the udder a second time, followed by wringing out the cloth again outside the bucket, and, finally, wiping the udder dry. Where the udders were visibly dirty they were cleaned with water before using the hypochlorite solution.

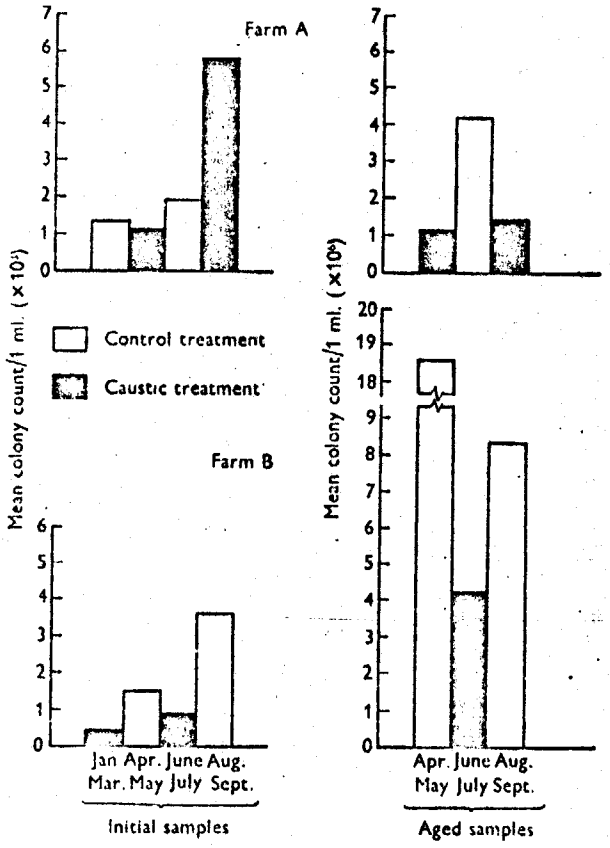

Fig. 1. Mean colony counts at 30° C. of initial and aged milk samples on two farms with control and caustic treatments.

As the bacteriological rinsing of clusters might have influenced the normal cleaning the rinses were not taken from the clusters used to produce the milk which was sampled.

Table 1 shows that this udder washing technique during the last period brought about no apparent reduction in the number of coli-aerogenes organisms in the milk. No coli-aerogenes organisms were found in the udder washing solutions which were neutralized immediately on sampling. Neither were coli-aerogenes organisms found in any of the cluster rinses. In approximately 80 individual cow samples, coli-aerogenes organisms were only determined in three samples, and then only in 10 ml. quantities.

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Table 1. *Individual numbers of coli-aerogenes with fresh and aged milk samples from different treatments*

Most probable number of coli-aerogenes/100 ml., 3 days at 30° C.

	Farm A			Farm B		
Week	Treatment	Initial	Aged	Treatment	Initial	Aged
2	Hypochlorite	3	—	Caustic soda	7·3	—
3		3·6	—		0	—
4		28	—		0	—
5		23	—		0	—
6		0	—		0	—
7		0	—		0	—
8		0	—		0	—
9	Caustic soda	39	—	Steam	23	—
10		0	—		0	—
11		3·6	—		150	—
12		75	—		0	—
13		0	—		0	—
14		0	0		1,100 +	11,000,000
15		0	0		0	930,000
16		0	0		9·1	150,000
17	Hypochlorite	0	0	Caustic soda	3·6	1,100 +
18		28	11,000,000		3·6	46,000
19		0	0		43	110,000 +
20		15	45,000,000		0	1,100,000 +
21		0	0		0	0
22		3·6	150,000		3·6	110,000 +
23		3	0		43	110,000 +
24		9·1	230,000		7·2	11,000,000 +
25	Caustic soda	3·6	0	Steam	19	53
26		0	0		0	0
27		240	11,000,000		0	0
28		23	36,000		0	0
29		0	0		1,100	11,000,000 +
30		6·2	21,000		33	1,000,000 +
31		0	0		3	1,000,000 +
32		0	0		0	110,000 +

Table 2. *Keeping quality results (to C.O.B. end-point at 22° C.) with different treatments*

Distribution of keeping quality results

	Farm A				Farm B			
Hr. to C.O.B.	Hypochlorite	Caustic soda	Hypochlorite	Caustic soda	Caustic soda	Steam	Caustic soda	Steam
33	0	0	0	0	1	0	0	0
36	0	0	0	0	0	0	0	0
39	0	0	0	0	0	1	1	2
42	2	0	1	1	3	1	2	1
45	0	1	0	0	1	1	0	0
48	1	1	0	0	0	1	0	0
51	3	4	3	2	1	4	2	4
54	1	2	3	2	1	0	2	0
57	0	0	1	3	0	0	1	1
Mean values/period (hr.)	48·4	50·6	51·5	52·8	44·1	47·2	48·7	47·8
Mean values/treatment/farm (hr.)		49·9	51·7			46·4	47·5	
Average A.S.T./period (° F.)	44·3	52·6	63·8	62·4	44·3	52·6	63·8	62·4

The distribution of the keeping quality results of the milk from both fa[illegible]s given in Table 2, together with the mean values for each period and for each treatment. With farm A the caustic soda treatment gave better average keeping quality than the hypochlorite control in both periods, but the means of both periods yield a difference of only 1·8 hr. in favour of the caustic soda. The results from farm B favour the steam control, although the mean difference was only 1·1 hr.

Table 3 shows a remarkable uniformity of total spore counts between samples. Further, no differences can be attributed to differences in treatment. Ageing of the milk samples for 24 hr. has had no effect on numbers except in two cases.

Table 3. *Number of total spores in individual samples in different treatments*

Colony count/ml. 3 days at 30° C.

	Farm A			Farm B		
Week	Treatment	Initial	Aged	Treatment	Initial	Aged
2	Hypochlorite	4	—	Caustic soda	20	—
3		10	—		13	—
4		46	—		27	—
5		13	—		6	—
6		20	—		12	—
7		8	—		11	—
8		8	—		8	—
9	Caustic soda	12	—	Steam	1	—
10		18	—		1	—
11		<1	—		1	—
12		1	—		76	—
13		3	—		6	—
14		<1	24		4	28
15		6	11		13	30
16		9	11		14	11
17	Hypochlorite	6	7	Caustic soda	2	6
18		15	14		2	14
19		4	15		5	8
20		3	10		8	16
21		15	12		61	64
22		3	3		4	14
23		2	13		2	5
24		0	4		0	0
25	Caustic soda	0	1	Steam	1	11
26		2	4		1	5
27		*	*		*	*
28		12	7		*	*
29		1	*		2	9
30		1	*		3	30
31		1	2		65	*
32		7	*		*	*

* Results masked by micrococci.

The numbers of resistant spores (Table 4) show a similar picture to that of total spores, except that the incidence of resistant spores is much lower. Here there are no apparent differences between treatments, and ageing of the milk for 24 hr. brought about no change.

Tests on the caustic soda solution and the first rinse water were made each week and total colony count and total number of spores determined. In no case was the total colony count of the caustic solution higher than 100/ml., or of the water higher than 500/ml. The spore counts in the caustic soda solution did not exceed 10/ml. and were usually 1 or 2/ml.,

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and in the water were not higher than 50/ml. and usually about 5/ml. There was no relationship between the bacteriological results and the gradual fall in concentration of caustic soda from 5 to 3·5% during the month before the solutions were changed.

Table 4. *Number of resistant spores in individual samples in different treatments*

	Most probable number/100 ml. 14 days at 30° C.					
	Farm A			Farm B		
Week	Treatment	Initial	Aged	Treatment	Initial	Aged
2	Hypochlorite	0	—	Caustic soda	0·36	—
3		2·3	—		0	—
4		0·91	—		0·91	—
5		2·3	—		2·3	—
6		0	—		0·36	—
7		0	—		0·36	—
8		0·36	—		0	—
9	Caustic soda	0	—	Steam	0	—
10		0	—		0	—
11		0·36	—		0	—
12		0	—		0	—
13		0·36	—		0	—
14		0	0		0	24
15		0	0		0	0
16		0	0		0	0·6
17	Hypochlorite	0	0	Caustic soda	0	0
18		0	4·6		0	4·6
19		0	0		0	0
20		0	0·72		0·72	0·72
21		0·72	5·8		0·6	4·6
22		0	1·88		0	3·2
23		48	5·8		0	0
24		4·6	4·6		0	4·6
25	Caustic soda	48	3	Steam	1·46	0
26		3·0	0		0	0
27		8·6	4·6		0	0
28		0	0		0	0
29		0	3·1		1·82	120
30		0	0		0	0
31		49	19·6		49	49
32		0	0		0	0

DETERMINATION OF MAIN BACTERIAL TYPES

The main types of micro-organisms occurring in all the milk samples in this investigation were examined to find if there were differences between the caustic soda and control treatments. The observations included the flora of the milk when fresh and when aged for 24 hr. at 22° C. This ageing was chosen as representing the worst condition under which milk could be received at the creamery.

Methods

From each farm thirty-two milk samples were examined before and after ageing. The following method was used to distinguish the main types of micro-organisms.

From Y.M.A. plates incubated for 3 days at 30° C. showing 150–200 colonies, 10 colonies were picked (using the statistical method of Harrison (5)), inoculated into plain litmus milk and incubated at 30° C. for 3–4 days. Y.M.A. plates were streaked from the litmus milk culture and incubated at 30° C. for 24–36 hr. Where necessary a single purification of the culture was made. This was sufficient for a broad grouping of micro-organisms. A Y.M.A.

slope was prepared from each colony, incubated at 30° C. for 24 hr., and kept in the cold store for further reference. The scheme in Table 5 shows the criteria used for the differentiation of the main types.

Table 5. *Methods of differentiating main groups of micro-organisms*

Colony from streaked Y.M.A. plate

- Catalase +
  - Gram +
    - Cocci: Micrococci
    - Rods: Corynebacteria, Bacilli
  - Gram −
    - Rods: Coli-aerogenes, Achromobacter, Chromobacter, Pseudomonas, Miscellaneous*
- Catalase −
  - Gram +
    - Cocci: Streptococci, Leuconostoc L[1]
    - Rods: Lactobacilli

* Gram-negative or doubtful.

Results

During the first 13 weeks only the initial milk flora was studied and 20 colonies were picked from one plate of the milk from each farm. After this the scheme was modified to include the milk flora of milk aged for 24 hr. at 22° C. The number of colonies was limited to ten from each plate, i.e. four sets of ten colonies were picked weekly.

Table 6. *Percentage distribution of main groups of micro-organisms in fresh milk*

	Farm A				Farm B			
	Hypochlorite	NaOH	Hypochlorite	NaOH	NaOH	Steam	NaOH	Steam
Micrococci	90·0	97·2	72·8	75·3	69·1	37·7	78·5	31·8
Streptococci	2·1	0	0	11·1	5·8	48·8	7·6	43·9
Bacilli	3·2	0	1·0	1·2	21·6	3·8	3·2	0
Achromobacter	2·1	1·1	12·2	3·7	0·7	3·3	6·5	17·1
Pseudomonas	0	0	1·0	1·2	0	2·7	1·0	4·8
Corynebacteria	1·3	0	1·0	0	0·7	1·6	0	1·2
Coli-aerogenes	0	0	6·0	0	0	0·5	0	0
Miscellaneous	0	0	1·0	0	0	0·5	0	0
No growth	1·3	1·7	5·0	7·5	2·1	1·1	3·2	1·2

Table 7. *Percentage distribution of main groups of micro-organisms in aged milk*

	Farm A				Farm B			
	Hypochlorite	NaOH	Hypochlorite	NaOH	NaOH	Steam	NaOH	Steam
Micrococci	—	96·8	49·0	70·5	—	41·8	70·5	38·3
Streptococci	—	0	0·9	12·6	—	24·1	0	5·4
Bacilli	—	0	4·1	0	—	6·1	4·2	0
Achromobacter	—	0	19·9	2·4	—	20·8	11·5	31·5
Pseudomonas	—	0	12·7	3·7	—	0	4·2	23·2
Corynebacteria	—	0	0	0	—	0	0	0
Coli-aerogenes	—	0	6·2	4·8	—	7·4	9·4	0
Miscellaneous	—	0	2·0	1·2	—	0	0	0
No growth	—	3·2	5·2	4·8	—	0	0	1·3

Tables 6 and 7 show the distribution of the main groups of micro-organisms during the different periods for the fresh and the aged samples, expressed as a percentage of the total cultures isolated during each period.

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In the fresh samples of milk micrococci predominated with both caustic and hypochlorite treatments at farm A. This was also true of the caustic treatment in farm B, but not with the steam treatment. We can offer no explanation for this. The types of micro-organisms were more restricted with the caustic treatment.

With the aged samples the flora isolated during the caustic treatments shows little change with ageing. With both control treatments there is a general small increase in most of the less dominant groups.

SOURCE OF MICRO-ORGANISMS

An attempt was made to determine the main sources of micro-organisms by taking individual milk samples from all cows contributing to the bulk milk, and by taking rinse samples from the clusters and sampling the udder washing water. The milking units were the only articles coming into contact with the milk, apart from the milk cans which were virtually sterile. The caustic and water rinse solutions had been tested consistently throughout the work and were known to contribute little to the final count of the milk.

The udder washing solutions, the chlorine in which was neutralized immediately on sampling (100 ml. sample neutralized with 1·8 g. sodium thiosulphate sterilized in the sampling bottle) never gave counts in excess of 50/ml., and sometimes gave 0/ml.; tests for coli-aerogenes organisms were always negative in 1 ml. The cluster rinse results show that there were insufficient organisms on the cluster to alter the bulk milk count, being on an average 62 × 10^3/cluster at farm A and 77 and 37 × 10^3 respectively on two clusters at farm B, equivalent to 1–2 organisms/ml. if distributed in 10 gal. of milk.

Table 8. *Comparison of colony counts from individual cow samples and the corresponding bulk milk*

	Farm A (weekly samples)							
Individual cow samples	970	1,840	630	2,400	3,680	460	1,665	200
	9,080	11,310	11,600	9,500	1,870	680	620	5,000
	540	50	3,500	625	—	—	—	60
	8,010	10,160	13,560	1,600	—	—	—	—
Mean	4,650	5,847	7,322	3,825	2,058	570	1,142	1,420
Bulk milk	1,390	2,930	9,330	5,900	2,860	5,850	11,300	7,400
	Farm B (weekly samples)							
Individual cow samples	420	1,576	570	460	510	2,860	420	1,960
	220	680	1,000	250	780	130	1,230	8,700
	1,890	1,570	750	2,500	1,600	680	620	430
	950	1,860	6,550	2,140	2,090	870	110	40
	17,400	3,160	1,090	570	3,660	1,490	110	1,730
	270	240	2,800	6,000	2,400	1,950	350	730
	232	920	—	—	—	800	190	150
Mean	3,054	1,429	2,126	1,987	1,840	1,254	432	1,962
Bulk milk	2,620	9,930	6,530	1,400	3,890	1,930	1,620	990

The counts from the individual milk samples are given in Table 8, together with the result from the corresponding bulk milk sample. It can be seen in most cases that the arithmetic mean of counts of milk from individual cows corresponds fairly closely to the bulk milk count. Therefore, in the majority of cases the numbers of organisms in the individual milk samples could account for the numbers in the bulk milk. In 25% of the cases, however, the bulk milk counts were considerably higher, no doubt as a result of dirt being drawn into the machine during milking.

On one occasion the predominant types of micro-organisms were determined from individual samples of these cows whose milk gave counts in excess of 1000/ml. The individual cow samples from farm A were predominantly micrococci. Nearly 85% of the flora of all the bulk milk samples from this farm were micrococci. From farm B the types predominant in the individual milk samples were much more diverse. This was in keeping with the types isolated from the bulk milk.

GENERAL DISCUSSION

It is well established that correct use of steam or sodium hypochlorite, following adequate cleaning of dairy utensils, results in milk of a low bacterial count. The bacteriological results obtained during the control periods on each farm were used to ensure that the control treatments were effective. The fact that there is no difference in bacteriological results between the caustic soda and the control treatments indicates that the caustic treatment gave results similar to the best that can be expected under commercial conditions from steam and hypochlorite. If this work had been done on farms where the control treatments were carried out less effectively, and a higher proportion of the bacterial flora had been contributed by the utensils then considerable differences in bacterial flora and quality of the milk would have been noted.

Perhaps the most important conclusion from this work is that the satisfactory bacteriological results of milk samples have been achieved without brushing the milk bearing surfaces, which is necessary in both control treatments.

Generally, differences in prevailing atmospheric temperature alters the bacterial flora of milk. The fact that this was not observed in this work suggests that such changes in bacterial flora, if they occur, may be associated with imperfect cleaning, though it must not be overlooked that mean atmospheric shade temperatures of 67° F. were only rarely experienced and were only once sustained for as long as three days.

At the outset of this work it was expected that the utensils would contribute a significant proportion of the micro-organisms found in the milk. The results show that the majority of micro-organisms in the bulk milk came from the udder. This was borne out by the similarity of the predominant types in individual cow and bulk milk samples. However, as most bacteria from the normal udder have little effect on keeping quality, those bacteria which influence keeping quality must either come from the utensils or in the course of milking. Although the numbers of bacteria from the utensils were small it is possible that these might have had some effect on keeping quality.

As the utensils contribute so few micro-organisms to the milk either from the control or caustic treatments, we are unable to explain whether the change in the majority micro-flora at farm B was a result of the change of treatment or not. On both farms the micro-flora of the milk from the caustic treatment was simpler than that from steam or hypochlorite, and on ageing of the milk for 24 hr. at 22° C. it was observed that with steam and hypochlorite treatments the less dominant members of the flora generally increased in numbers. This was not the case with the caustic treatment.

Because of the effectiveness of the cleaning methods used it was necessary to test relatively large quantities of milk so that determinations of coli-aerogenes organisms could be expressed as numbers/100 ml. The results indicate that these organisms are probably never absent from raw milk (apart from small aseptic samples) though it may be necessa

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Experiments on Lye Treatments in Ripe Olive Pickling*

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By W. V. CRUESS
Food Technology Division, University of California

OVER the past twenty-five years commercial ripe olive pickling technique in California has greatly improved in respect to the attainment of a black to dark brown color. No doubt the discussions at the annual olive processors' conferences during that period have contributed much to this improvement in the appearance of canned ripe olives. The color of ripe olives at time of purchase by the consumer depends, first, on the effectiveness of processing (pickling) to attain good color and secondly, on retention of the color in the can or glass jar. The present brief report deals with several factors studied in connection with development of color during processing (pickling) and does not touch upon retention of color in the final package.

It seemed desirable to undertake a systematic study of certain factors influencing the development of color during lye treatments, and the loss of the color by leaching during washing out of the lye after lye treatments are completed. The thought behind these experiments is that if we can more definitely establish the factors affecting color formation during lye treatments and color loss during leaching we would be in a better position to explain what occurs during these two stages of pickling, and could better control them, with benefit to color of the canned or glassed product.

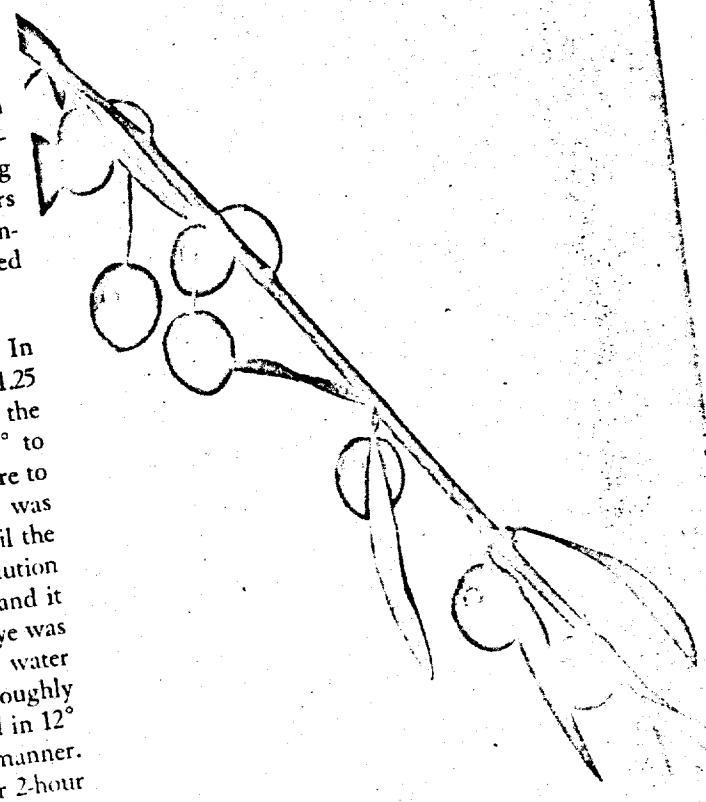
Effect of Depth or Length of Lye Treatment: In the first experiment on this problem a lye solution of 1.25 per cent sodium hydroxide was used. In one case the length of application at room temperature (about 60° to 65° F.) was 1½ hours in each application and exposure to air between lyes was 5 to about 16 hours. The lye was applied for 1½ hour periods after each exposure until the lye had penetrated to the pits. The original lye solution was used repeatedly until full color had developed, and it was then reinforced to about 1.0 per cent or a fresh lye was used in some cases. They were then soaked in water changed three times daily until the lye was thoroughly removed; about 6 to 7 days. They were then stored in 12° salometer brine for 3 days and canned in the usual manner.

Another lot received 1.25 per cent lye solution for 2-hour periods and air exposures, etc., as for the 1½-hour lot.

A third lot was treated for periods of 3 hours each in 1.25 per cent lye.

A fourth lot was treated for 4 hours in each application. Naturally, fewer applications were required to reach the pits with the longer periods of application (3 and 4 hours) than with the shortest period, (1½ hours).

The color of the olives treated 1½ hours in each lye application was best. The final product consisted of about 72 per cent black olives, 16 per cent dark brown and about 12 per cent light brown. For those treated 2 hours in each lye application there were about 68 per cent black; about 21 per cent dark brown and about 11 per cent light brown. For those treated for 3-hour periods the values were about 59 per cent black (but not as black as lots 1



Olives on the branch

* 25th Annual Olive Conference, Berkeley, Calif., ... ciation to M. F. H.

and 2); about 24 per cent dark brown and about 17 per cent light brown. For the 4-hour applications the results were about 57 per cent dark brown to black (not as dark as lots 1 and 2); about 25 per cent dark to medium brown and about 18 per cent light brown.

To the eye the general average color of lot 1 was much darker than of lots 3 and 4; lot 2 was nearly as good as lot 1.

The conclusion is that a larger number of short applications give a better color than a smaller number of longer lye applications; not a particularly new observation, nevertheless of such importance that verification on a more or less quantitative basis is of value.

These experiments were repeated using 1 hour, 2, 3 and 4-hour lye aplications. The results were similar to those obtained in the first series. The only other difference in procedure was use of a 0.5 per cent final lye instead of 1.25 per cent to remove bitterness.

In several experiments during the 1944-1945 season, W. V. Halverson and the writer treated Manzanillo, Mission and Sevillano olives with 1.25 per cent sodium hydroxide solutions in two ways: In one series of tests the first lye was allowed to penetrate practically all the olives to about 1/16 to 1/32 inches; the second was allowed to peterate to a total depth of about 1/16 to 1/8 inches; the third about 3/4 to the pit; and the fourth to the pits; in some cases a fifth lye for a short period was necessary. Exposure between lyes was 24-48 hours. Other lots of the same varieties, were treated for very short periods, 1 to 1 1/2 hours, in 1.25 per cent lye with exposures during the day and evening of 3 to 4 hours, and from about 10:00 P.M. to about 8:00 A.M. until good color was attained. Then the lye was allowed to penetrate to the pits. The two lots were washed and canned in the usual manner. All three varieties treated with the 1.25 per cent lye solutions for very short periods attained a much better color than those treated with fewer lyes for longer periods. In fact the short lye process gave with all three varieties a very large proportion of black olives; whereas the longer treatments produced canned olives that were for the most part dark brown to brown in color. After a year's storage in glass jars and enameled cans at 80° to 85° F. the differences in color were even more striking.

In another experiment (by Natarajan) 1.25 per cent lye was applied to Manzanillo olives for periods of 1 hour each with exposures of two hours between lyes, plus overnight exposure; and for comparison other olives were given longer lye treatments and longer exposures.

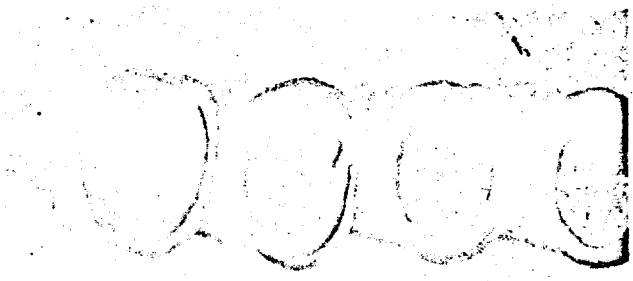
In both lye series the final lye contained only 0.5 per cent sodium hydroxide in order that leaching of the color would be at a minimum. Washing, brining and canning were according to usual procedures. The olives given the short lye treatments were superior in color to those given longer treatments.

Thus the results of experiments by three different investigators during two seasons on "many-short" versus "fewer-long" lye applications were in agreement. The short lye treatments plus short exposures gave much better color than longer lye applications plus longer periods of exposure.

Using short periods of lye application for definite periods of 1 to 1 1/2 hours plus short exposures it is possible to put lye treatments and exposures on a definite time schedule. Also, by use of such a schedule it should be possible to use less experienced help.

Use of Bicarbonate Between Lyes: In one series of experiments one lot of Manzanillo olives was treated with 1.25 per cent and 0.5 per cent lye solutions by "standard" air exposure procedure with the first lye penetrating most of the olives; the second about 1/16 inch into the flesh; the third about 1/8 inch, the fourth about 3/4 to the pits, and the fifth to the pits, the fifth being 0.5 per cent. Exposures of 24 hours each between lyes were given. Another lot was given a similar series of lye treatments and exposures, but was also soaked in 2 per cent sodium bicarbonate solution overnight after each lye, except the last, and before exposure. The bicarbonate treatment improved the color.

In another series the check lot of olives (no bicarbonate) was aerated in water for 24-hour periods between lyes; a second lot was aerated in 1 per cent sodium bicarbonate solution; a third lot was aerated in 1 per cent bicarbonate solution containing 5 per cent of salt and the fourth lot in 1 per cent bicarbonate plus 10 per cent salt. Olives of the fourth lot were of best color; the third lot was second best and the first lot poorest; although all were of good color. The bicarbonate treated olives appeared to be somewhat firmer after canning.



Olives penetrated about one-third to pit with lye.

Comparison of Sodium Bicarbonate, Sodium Silicate and Tri-Sodium Phosphate Solutions: In this experiment, conducted twice, respective lots of the olives were aerated in water, and in dilute solutions of sodium bicarbonate, sodium silicate and sodium phosphate between lye treatments. Color was slightly best in the bicarbonate treatment; but probably the silicate and phosphate solutions were too strong (1 and 2 per cent) for best results; requires further study.

In another experiment sodium carbonate, sodium silicate and tri-sodium phosphate as 2 per cent solutions were used as final lyes only; in comparison with 0.5 per cent sodium hydroxide, to remove final bitterness. The results were not conclusive although in one test the olives treated with 2 per cent sodium silicate were of better color than the others; in another case the phosphate was best.

The purpose of this experiment is to find a milder lye treatment than sodium hydroxide for the final lye for penetration to the pit, in order that leaching of color will not be excessive.

Use of Calcium Chloride Before and Between Lye Treatments: Calcium salts have been used successfully in dilute concentration to "set the color" during final washing of the olives and as this practice is so well known it was not used in this experiment. One objection to use of calcium salts such as gypsum or calcium chloride during washing is that sufficient calcium is apt to remain in the olive flesh to impart a brackish flavor on retorting.

In experiments this season 0.6 per cent of calcium chloride was added to the holding solution, and the olives were allowed to stand for about 4 weeks before pickling. Manzanillos previously cured about four months in holding solution by the Bell Packing Company were used. Calcium chloride, 0.20 grams per 100 c.c. in 20° salometer brine was added in another case to cover the olives after color had been attained by the short lye process and air exposures as previously described. They were left in this solution 48 hours, and were then given final lye treatment to the pits. A third lot received no calcium chloride at any stage of the process. The calcium treated olives were firmer and lost less color during washing in water than did the untreated lot. The olives treated in 0.6 per cent calcium chloride before pickling developed the best color. Neither lot had a brackish taste.

Use of Salt in Lye Solutions and Between Lyes: We have reported upon these treatments at previous conferences and they have been discussed by processors. Also, both methods have been used commercially and are probably still in use in some plants. One plant now no longer in operation used brine-lyes in pickling all of its Mission and Manzanillo olives.

In one of this year's experiments with Manzanillo olives a brine of 30° salometer was applied for 48 hours after color had been well attained and then the final lye treatment was given. In another case 6 per cent of salt was added to all lyes; that is the lyes were made up in 24° salometer brine. The brine between lyes gave a slightly better color. Brine lyes were slow in penetrating and gave a firmer final product. Some shriveling occurred in both lots.

Wooden pickling vats for olives

In a previous season similar experiments were made with Mission olives; in which case the brine lyes appeared to give somewhat better color than brine between lyes. There was no shriveling in this case. Previous seasons' experiments demonstrated very definitely that brine lyes greatly reduce the danger of bacterial spoilage. However, with Sevillano, Ascolano and Manzanillo olives there is considerable risk of salt shriveling.

Summary

1. Best color was attained most regularly by use of short lye applications and short exposures until full depth of color was developed.

2. Use of bicarbonate solution between lyes plus air exposure, or aeration of the olives in dilute biacarbonate between lyes gave improvement in color.

3. Use of trisodium phosphate or sodium silicate solutions for final lye treatments gave promise of better color retention. This requires further study.

4. Addition of calcium chloride to holding solution or its application between the last two lye treatments gave better color retention than in the untreated olives; less leaching out of color. There was no noticeable brackish flavor.

A SURVEY OF CHEMICALS FOR MUTAGENIC ACTION ON E. COLI[1]

M. DEMEREC, G. BERTANI,[2] AND J. FLINT

Department of Genetics, Carnegie Institution, Cold Spring Harbor, N. Y.

INTRODUCTION

At present we have available two good methods, using *Escherichia coli*, for the quantitative study of spontaneous mutability and of the mutagenic action of various agents such as radiations and chemicals. The older of the two methods deals with mutational changes from phage sensitivity to phage resistance, and the more recently developed one (Demerec, 1951; Bertani, 1951) with back-mutations from streptomycin dependence to nondependence. In both cases the mutants can be easily classified; very large numbers of bacteria may be tested; and slight increases in mutation rate can be easily detected.

After the streptomycin method had been developed we undertook extensive tests of a number of chemicals for mutagenicity, using mainly this method because it is the simpler of the two.

SELECTION OF CHEMICALS

Previous work by Witkin with *E. coli* (Witkin, 1947; Demerec, Wallace, and Witkin, 1948) had indicated that compounds exhibiting quite different properties may all be mutagenic. Using the phage method, she found that sodium desoxycholate, pyronin, acriflavine, caffeine, and colchicine acted as mutagens, but detected no mutagenic effect in tests with methyl green, basic fuchsin, and methylcholanthrene endosuccinate.

In planning the present survey, therefore, we prepared a list of chemicals, including representatives of widely separated groups, from simple inorganic and organic compounds to complex organic chemicals. Before the scheduled tests had been completed, however, we found that certain ferrous and manganous salts are very potent mutagens, and our attention was thus diverted to a detailed study of these compounds. Since it will require some time to

[1] This study was conducted in part under grants from the American Cancer Society, through the Committee on Growth of the National Research Council, and from the Jane Coffin Childs Memorial Fund for Medical Research. The assistance of Miss Eileen Yougen in carrying out the experiments is acknowledged.

[2] Present address: Department of Bacteriology, University of Illinois, Urbana, Illinois.

carry this out, the data so far available from the survey are being summarized here.

MATERIALS AND METHODS

The "streptomycin" method for detection of induced mutations has been fully described by Bertani (1951). Only technical details will be given here.

Strains B/Sd-4/1,3,4,5 and B/Sd-4/3,4 of *E. Coli* were used throughout this work. For every experiment, bacteria were grown for 24 hours at 37°C in an aerated broth culture containing 10 micrograms of streptomycin per milliliter. They reached a saturation titer of approximately 2 to 3 × 10^9 cells per milliliter. Each culture was started from an inoculum, usually large, taken from streptomycin-agar slants kept in a refrigerator. Before treatment the bacteria were washed in saline and resuspended in distilled water. A sample of the new suspension was added to the desired solution of chemical in distilled water, and incubated at 37°C for a certain period of time; no growth occurs under these conditions. Another sample of the same suspension was added to an equal amount of distilled water and incubated for the same period of time, as a control. At the end of the treatment period, both treated and control suspensions were assayed by plating suitable dilutions on streptomycin-agar plates. At the same time they were plated (0.1 ml per Petri dish), either undiluted or diluted not more than 1:10 in plain broth, onto a number of streptomycin-free plates, using a glass spreader and a turntable. The assay plates were incubated for 48 hours, after which it was possible to count the colonies and calculate the titers of the two suspensions at the end of treatment, and the percentage of survivors. The streptomycin-free plates (mutant plates) were incubated for at least six days. After this time the colonies were scored, and the frequency of mutants calculated by dividing the number of colonies by the number of (viable) bacteria plated.

The length of the period of incubation in distilled water does not appreciably affect the proportion of mutations in the controls. In all but a few exceptional cases, nevertheless, the control suspensions were incubated for the same period of time as the chemically treated suspensions.

The degree of crowding of the dependent bacteria on the mutant plates would be expected to affect the observed frequency of mutants, as it influences the extent of residual growth and thus the chance that a mutant cell will develop into a visible colony. This effect is slight, however, when the bacteria plated have been raised in 10 micrograms of streptomycin per milliliter.

The chemical treatment also may limit the extent of residual growth, causing a spurious lowering of the frequency of mutants in the treated series. This may explain why, in experiments with some chemicals, the treated series showed frequencies lower than those of the controls.

Several of the chemicals used cause the formation of clumps of bacteria during treatment. If these clumps have not dissolved by the time the treated suspension is assayed to determine survival, the observed survival value

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will be too low, and consequently the calculated mutation frequency too high. Therefore, except in the very first experiments done (*i.e.*, those with sulfuric, nitric, and hydrochloric acids, which in any case were not found to be mutagenic), the treated suspensions were always examined under the microscope before plating, and discarded if clumping was detected. By varying the concentration of chemical and the length of treatment, it was possible to eliminate clumping in many cases.

In a few scattered experiments (and in most of those done with phenol and formaldehyde) the dependent bacteria were grown in 25 instead of 10 micrograms of streptomycin per milliliter. It is known that this results in greater residual growth and therefore a larger number of spontaneous mutants per plate. This explains why in some experiments the spontaneous mutant frequencies were higher than average.

RESULTS

The results of a number of experiments for each chemical are reported in table 1. The concentration of the chemical shown in column 2 is the effective concentration at the time of treatment (*i.e.*, corrected for the addition of the bacterial suspension in distilled water). Number of plates (column 5) is the number of streptomycin-free plates used for the scoring of mutants. Total number of bacteria (column 6) is the number of viable dependent bacteria put on each plate, multiplied by the number of plates used. Number of mutants per 10^9 bacteria (column 7) is the mutant frequency, calculated by dividing the total number of colonies scored in an experiment by the total number of bacteria. The total number of mutant colonies is not given in the table, but can easily be calculated by the inverse operation. From it, one can determine the average number of mutants per plate, which in the experiments reported here was rarely larger than 10–20. Assuming a Poisson distribution, the variance can be estimated and the statistical significance of the results evaluated. Sometimes the same controls were common to several treated series; and in these cases the number of plates and total number of bacteria are reported only once for the controls. Different experiments done with each chemical are listed in order of decreasing survival.

Inorganic acids. Tests made with sulfuric, orthophosphoric, nitric, and hydrochloric acids gave negative results. Under certain conditions, all four of these compounds produced clumping of the treated bacteria, and this explains why in some experiments the values obtained for mutant frequency were higher in the treated series than in the controls. Negative results for hydrochloric acid had also been obtained in earlier experiments with Drosophila (Geldar and Belinieva, 1935).

Boric acid was the only inorganic acid tested that gave positive results. In all experiments but one, the treated series showed an increase in mutation frequency. No clumping was ever observed among bacteria treated with boric acid.

Alkalies. Sodium hydroxide and potassium hydroxide were tested, with inconclusive results. Under the conditions of these experiments they never produced clumping.

Ammonia. Ammonia had been used in experiments with Drosophila by Lobashov and Smirnov (1934) and Lobashov (1937), and found to have mutagenic action. In the tests reported here, ammonia showed definite mutagenic activity in experiments where the proportion of survivors was lower than 2 per cent. Apparently similar positive results were obtained with ammonium chloride; they are not reported here, however, because clumping could not be avoided after treatment with this chemical.

Hydrogen peroxide. Attention was first drawn to the mutagenic activity of peroxides by the work of Stone, Wyss, and Haas (1947) on the indirect effect of mutation-inducing radiations on bacteria. Dickey, Cleland, and Lotz (1949), using Neurospora, studied the mutagenic potency of hydrogen peroxide and of a number of organic peroxides, and obtained positive results. In our experiments also, hydrogen peroxide was clearly shown to be mutagenic.

Metal salts. Mercuric chloride, silver nitrate, copper sulfate, and sodium silicate were tested. Only copper sulfate showed indications of mutagenic effect in the lower range of survival. Mutagenic potency had already been demonstrated for this salt by Magrzhikovskaja (1936) and Law (1938) in experiments with Drosophila. The results reported for mercuric chloride and silver nitrate do not exclude the possibility of their having mutagenic activity at the lowest survival levels, because it was extremely difficult to obtain survivals below 1.0 per cent and yet not so low as to fall beyond the range of sensitivity of the method.

Organic acids. Lobashov and Smirnov (1934) and Lobashov (1937) had treated Drosophila with acetic acid, but failed to detect any mutagenic activity of this substance. In our experiments, acetic, formic, and lactic acids were tested. The first two showed mutagenic activity. Some of the experiments with lactic acid indicated a weak mutagenic effect; but this is rendered doubtful by the fact that experiments with sodium lactate consistently gave negative results.

Formaldehyde. The mutagenic potency of this substance had been discovered by Rapoport (1946) and confirmed by W. D. Kaplan (1948) and Auerbach (1949), working with Drosophila. Clear-cut positive results were obtained in our experiments also. Auerbach's hypothesis that production of mutations by formaldehyde can occur only through some intermediate effect on substances contained in Drosophila food is not supported by our experiments, in which the treatment was carried out on washed bacteria.

Phenol. The mutagenic action of phenol had been discovered by Hadorn and Niggli (1946), working on Drosophila with a very special treatment technique. Other techniques had failed to give positive results. Negative results had been obtained by Dickey, Cleland, and Lotz (1949) with Neurospora. Our tests gave clear-cut positive results for this substance, and also for two closely related substances, alpha-dinitrophenol and trinitro-

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TABLE 1

RESULTS OF EXPERIMENTS TESTING INDUCTION OF BACK-MUTATIONS IN Sd-4 (STREPTOMYCIN-DEPENDENT) *E. COLI* TREATED WITH CERTAIN CHEMICALS

Chemical	Concentration %	Treatment (hours)	Survival %	Treated			Control		
				No. of plates	Total no. of bacteria	Mutants per 10^8 bacteria	No. of plates	Total no. of bacteria	Mutants per 10^8 bacteria
Sulfuric acid	0.003	3	13.	10	1.2×10^8	11.8	4	8.5×10^7	8.3
	0.002	3	12.	8	8.0×10^7	70.0	4	7.0×10^7	147.
	0.002	3	11.	8	1.1×10^8	28.7	4	8.4×10^7	35.4
	0.005	3	7.0	9	4.6×10^7	10.9	4	5.4×10^7	33.1
	0.002	3	1.9	8	2.2×10^7	74.	4	9.2×10^7	98.1
Phosphoric acid (ortho-)	0.008	3	29.	8	1.9×10^8	29.4	4	6.4×10^7	35.9
	0.008	3	17.	8	1.0×10^8	16.5	4	7.3×10^7	12.3
	0.010	3	1.2	8	9.3×10^7	2.2	4	8.9×10^7	21.3
Nitric acid	0.008	3	8.8	10	8.1×10^7	11.1	4	8.5×10^7	8.3
	0.005	3	8.3	8	5.8×10^7	90.2	4	7.0×10^7	147.
	0.005	3	6.9	8	6.8×10^7	16.2	4	8.4×10^7	35.4
	0.006	3	3.5	10	2.6×10^7	11.7	4	5.4×10^7	33.1
	0.005	3	2.7	8	2.2×10^7	111.1	4	9.2×10^7	98.1
Hydrochloric acid	0.0015	3	100.	5	6.0×10^7	6.7	4	4.8×10^7	10.4
	0.0015	3	33.	5	4.0×10^8	5.8	4	9.6×10^7	24.0
	0.00375	3	21.	5	2.5×10^8	6.8			24.0
	0.0075	3	11.	10	1.3×10^7	30.1			10.4
	0.00075	3	0.8	3	1.1×10^7	0.0	2	9.2×10^7	3.6
	0.0015	3	0.2	4	4.0×10^8	0.0			3.6
	0.0015	3	0.2	4	3.8×10^8	25.0	5	2.5×10^8	26.2
Boric acid	4.0	3	50.	8	1.0×10^8	27.3	6	7.6×10^7	6.6
	1.5	24	33.	8	6.0×10^8	28.9	5	1.1×10^8	7.9
	2.0	24	25.	8	4.6×10^8	28.7			7.9
	2.0	24	24.	8	3.3×10^8	82.3	6	1.0×10^8	5.8

TABLE 1—*continued*

Chemical	Concentration %	Treatment (hours)	Survival %	Treated			Control		
				No. of plates	Total no. of bacteria	Mutants per 10^8 bacteria	No. of plates	Total no. of bacteria	Mutants per 10^8 bacteria
Boric acid (*continued*)	2.0	24	17.	8	1.1×10^8	6.6	6	4.6×10^8	9.8
	2.0	24	11.	8	1.6×10^8	64.1	6	1.0×10^8	13.6
	1.5	24	11.	8	8.3×10^7	31.2	4	8.9×10^7	21.3
	1.7	24	10.	9	5.9×10^7	20.3	6	9.0×10^7	6.7
	2.0	48	9.7	8	9.2×10^7	16.4	4	7.0×10^7	5.7
	1.9	24	3.3	10	2.2×10^7	18.6			6.7
	1.8	24	2.8	8	2.1×10^7	56.6	4	7.5×10^7	30.6
Sodium hydroxide	0.013	3	86.	10	2.6×10^8	11.7	5	3.0×10^8	18.9
	0.015	3	37.	10	3.0×10^8	32.4	4	6.4×10^7	35.9
	0.02	3	27.	7	5.4×10^8	13.8	5	1.4×10^8	16.8
	0.011	3	24.	10	3.4×10^8	3.3	6	1.9×10^8	3.6
	0.0145	3	20.	10	1.9×10^8	20.2	4	8.5×10^7	8.3
	0.015	3	16.	10	1.2×10^8	16.3	4	7.3×10^7	12.3
	0.0125	3	12.	8	1.2×10^8	28.2	4	1.0×10^8	38.0
	0.0125	3	11.	8	1.0×10^8	130.7	4	9.2×10^7	98.1
	0.014	3	5.7	8	5.3×10^7	43.0	4	6.8×10^7	43.8
	0.022	3	1.3	8	2.3×10^7	21.6	5	1.1×10^8	12.1
Potassium hydroxide	0.00945	3	44.	10	2.8×10^8	10.3	6	9.0×10^7	6.7
	0.015	3	42.	8	3.8×10^8	55.5	4	9.2×10^7	98.1
	0.016	3	32.	8	3.0×10^8	43.0	4	6.8×10^7	43.8
	0.015	3	29.	8	2.0×10^8	79.2	4	7.0×10^7	147.
	0.019	3	20.	10	3.6×10^8	12.5	6	1.8×10^8	5.5
	0.018	3	18.	10	1.4×10^8	9.9	6	1.1×10^8	9.7
	0.018	3	19.	10	3.4×10^8	20.1			5.5
	0.0185	3	13.	10	1.9×10^8	6.4	6	1.9×10^8	3.6
	0.0165	3	13.	10	1.8×10^8	4.4			3.6
	0.017	3	10.	10	7.9×10^7	12.7			9.7

TABLE 1 (*continued*)

Chemical	Concentration %	Treatment (hours)	Survival %	Treated: No. of plates	Treated: Total no. of bacteria	Treated: Mutants per 10^8 bacteria	Control: No. of plates	Control: Total no. of bacteria	Control: Mutants per 10^8 bacteria
Potassium hydroxide (*continued*)	0.019	24	8.5	10	1.5×10^8	17.8			5.5
	0.018	24	8.3	10	1.5×10^8	9.4			5.5
	0.0175	3	2.9	8	2.9×10^7	37.6	4	1.0×10^8	38.0
Ammonia	0.025	3	66.	6	2.4×10^8	4.9	2	1.8×10^8	6.8
	0.100	3	26.	9	1.1×10^8	2.6	5	2.5×10^8	3.7
	0.250	3	13.	9	3.9×10^8	10.3	5	1.7×10^8	10.0
	0.250	3	12.	8	3.1×10^8	1.6	4	1.4×10^8	2.8
	0.300	3	9.6	5	2.4×10^8	3.4	5	2.5×10^8	2.6
	0.400	3	2.3	10	1.1×10^8	8.0			2.6
	0.250	3	1.2	8	2.4×10^7	37.5	5	1.3×10^8	12.9
	0.500	3	1.0	4	1.2×10^7	41.6	5	1.5×10^8	13.3
	0.500	3	0.4	10	2.0×10^7	121.			2.6
	0.500	3	0.2	10	5.6×10^6	90.			12.9
Hydrogen peroxide	0.150	4	78.	9	1.9×10^8	19.9	8	2.2×10^8	5.0
	0.195	4	59.	8	1.3×10^8	28.7			5.0
	0.195	½	45.	6	5.2×10^6	124.	8	1.5×10^8	6.9
	0.225	½	32.	8	4.9×10^8	121.			6.9
	0.195	½	3.1	8	5.4×10^7	175.	6	1.3×10^8	4.7
	0.240	4	1.4	9	3.5×10^7	120.			5.0
	0.195	½	0.6	8	1.1×10^7	26.7			4.7
Mercuric chloride	0.00005	6	61.	9	5.8×10^8	1.7	5	1.5×10^8	0.7
	0.00030	3	40.	10	4.1×10^8	4.2	4	4.1×10^8	12.3
	0.00025	3	21.	4	3.1×10^8	5.9	4	1.5×10^8	2.0
	0.00030	3	18.	3	2.5×10^8	2.9	2	9.2×10^7	3.3
	0.00005	9	11.	9	1.1×10^8	3.7			0.7
Silver nitrate	0.000005	24	84.	9	3.3×10^8	4.8	6	2.6×10^8	2.3
	0.000100	3	79.	10	1.6×10^8	2.5	5	1.0×10^8	8.0
	0.000030	3	75.	9	1.1×10^8	6.7	7	1.1×10^8	8.6

TABLE 1 (*continued*)

Chemical	Concentration %	Treatment (hours)	Survival %	Treated: No. of plates	Treated: Total no. of bacteria	Treated: Mutants per 10^8 bacteria	Control: No. of plates	Control: Total no. of bacteria	Control: Mutants per 10^8 bacteria
Silver nitrate (*continued*)	0.000025	3	59.	9	2.3×10^8	2.1			2.3
	0.000100	3	26.	9	3.8×10^8	7.4			8.6
	0.000100	24	4.3	9	6.1×10^7	8.2			8.6
Copper sulfate	0.00020	3	88.	9	1.4×10^8	5.7	6	1.0×10^8	5.1
	0.00040	3	34.	9	4.7×10^8	3.6	8	1.2×10^8	8.8
	0.00050	3	22.	10	5.0×10^8	4.4	5	1.1×10^8	8.9
	0.00050	3	14.	9	3.8×10^8	10.6	5	1.5×10^8	2.1
	0.00050	3	12.	3	1.1×10^8	20.7	4	1.2×10^8	20.8
	0.00075	3	2.4	9	3.7×10^7	40.5			5.1
	0.00100	3	0.36	9	7.2×10^6	55.5			8.9
	0.00125	3	0.22	10	4.9×10^6	0.0			8.9
Sodium silicate	0.025	3	66.	10	1.7×10^8	5.9	5	1.3×10^8	6.3
	0.100	3	33.	5	4.2×10^8	2.4	5	1.3×10^8	5.3
	0.050	3	27.	10	6.9×10^7	8.7			6.3
	0.100	3	16.	7	3.8×10^8	6.6	5	1.7×10^8	6.1
	0.100	3	4.6	9	7.9×10^7	11.4	7	1.3×10^8	6.2
	0.150	3	3.0	9	5.1×10^7	2.0			6.2
	0.300	3	0.11	5	1.3×10^6	0.0	5	1.2×10^8	7.0
Acetic acid	0.03	3	79.	10	1.3×10^8	8.3	5	1.6×10^8	10.0
	0.03	3	36.	8	9.9×10^8	36.4	5	1.7×10^8	9.2
	0.03	3	11.	10	8.5×10^7	15.3			10.0
	0.01	24	7.2	8	7.6×10^7	19.8	6	7.9×10^7	10.1
	0.03	3	6.0	10	9.5×10^7	25.2			10.0
	0.03	3½	0.9	8	8.8×10^6	56.8	6	7.6×10^7	6.6
Lactic acid	0.010	3	40.	10	6.4×10^8	12.8	5	1.6×10^8	10.0
	0.010	3	36.	10	2.9×10^8	10.7			10.0
	0.019	3	14.	8	2.0×10^8	16.7	5	8.9×10^7	4.5

TABLE 1 (*continued*)

Chemical	Concentration %	Treatment (hours)	Survival %	Treated			Control		
				No. of plates	Total no. of bacteria	Mutants per 10^8 bacteria	No. of plates	Total no. of bacteria	Mutants per 10^8 bacteria
Lactic acid (*continued*)	0.021	3	7.5	8	1.1×10^8	40.2			4.5
	0.020	3	6.8	8	1.7×10^8	17.0	5	1.6×10^8	15.3
	0.020	3	4.6	8	6.7×10^8	32.9	5	9.2×10^7	27.3
	0.020	4	2.5	8	2.9×10^7	34.7	6	8.5×10^7	9.4
	0.020	3	1.9	8	2.6×10^7	11.4	6	1.0×10^8	4.8
Sodium lactate	2.5	3	85.	8	1.5×10^8	7.3	5	1.1×10^8	12.1
	3.0	3	61.	8	1.7×10^8	16.4	5	1.6×10^8	15.3
	2.0	24	8.3	8	8.7×10^7	5.8	6	7.9×10^7	10.1
	2.5	24	1.4	8	3.8×10^7	5.2	5	1.7×10^8	9.2
Formic acid	0.0070	3	100.	8	1.5×10^8	18.1	6	1.1×10^8	2.7
	0.0060	3	93.	8	1.4×10^8	18.4			2.7
	0.0050	3	77.	8	1.3×10^8	20.2	5	1.1×10^8	12.1
	0.0065	3	30.	8	8.2×10^8	44.0	5	1.7×10^8	9.2
	0.0060	3	20.	8	5.0×10^8	43.3	5	1.6×10^8	15.3
	0.0065	3	19.	8	4.6×10^8	24.4	5	1.5×10^8	3.3
	0.0060	3	15.	8	1.4×10^8	27.7	6	7.1×10^7	2.8
	0.0075	3	3.4	8	8.2×10^7	38.0			3.3
	0.0070	3	2.8	8	2.6×10^7	38.5			2.8
Formaldehyde	0.01	3	61.	2	7.6×10^7	400.	2	1.3×10^8	26.2
	0.01	3	58.	5	6.8×10^8	133.	4	9.4×10^8	15.5
	0.02	3	58.	5	6.8×10^8	157.			15.5
	0.01	3	48.	4	3.7×10^7	245.	4	7.6×10^7	79.
	0.01	3	48.	4	3.7×10^8	470.	4	7.6×10^8	68.
	0.01	3	21.	8	1.3×10^8	120.	4	7.3×10^7	12.3
	0.02	3	16.	8	1.0×10^8	129.			12.3
	0.04	3	9.0	7	1.5×10^8	326.			15.5
	0.05	3	1.7	10	3.2×10^8	560.			79.

TABLE 1 (*continued*)

Chemical	Concentration %	Treatment (hours)	Survival %	Treated			Control		
				No. of plates	Total no. of bacteria	Mutants per 10^8 bacteria	No. of plates	Total no. of bacteria	Mutants per 10^8 bacteria
Phenol	0.10	3	90.	10	4.4×10^8	84.5	4	2.0×10^8	74.5
	0.10	3	69.	9	1.2×10^9	2.5	5	9.5×10^8	0.6
	0.08	3	48.	8	7.4×10^8	3.4			0.6
	0.20	3	43.	9	7.5×10^8	2.5			0.6
	0.10	12	1.7	10	7.3×10^8	124.	5	2.2×10^8	74.8
	0.10	24	1.1	10	3.1×10^8	192.	4	1.1×10^8	59.5
	0.20	3	0.5	9	2.3×10^8	820.			74.5
Alpha-dinitrophenol	0.005	3	87.	3	9.6×10^8	2.7	2	7.4×10^7	4.1
	0.020	3	72.	3	1.1×10^8	8.1	5	2.5×10^8	5.5
	0.050	3	58.	10	1.7×10^8	33.5	5	1.5×10^8	5.4
	0.020	3	46.	10	9.5×10^7	51.5	4	8.4×10^7	8.5
	0.005	3	37.	10	2.3×10^9	25.1	5	3.1×10^8	12.5
	0.050	3	32.	10	6.6×10^7	56.0			8.5
	0.025	3	31.	9	1.7×10^8	14.2			12.5
	0.040	3	23.	10	4.8×10^7	25.0			8.5
	0.050	3	18.	10	9.1×10^7	37.4			5.5
	0.100	3	3.0	9	8.2×10^7	44.0	5	1.5×10^8	7.2
	0.100	3	2.6	10	7.3×10^7	97.0	5	1.4×10^9	7.3
	0.100	3	2.5	9	1.2×10^8	6.0			5.5
	0.100	3	2.4	7	3.5×10^7	65.5	4	8.4×10^8	7.6
	0.100	3	1.7	8	4.8×10^7	79.0	4	1.4×10^8	2.0
Nitro-[illegible]	0.010	3	94.	8	1.9×10^8	14.9	5	1.3×10^8	14.3
	0.018	3	34.	8	4.9×10^8	97.3	6	1.1×10^8	9.5
	0.017	3	7.4	8	5.8×10^7	132.	6	5.8×10^7	5.4
	0.017	3	4.2	8	6.2×10^7	27.4	5	8.9×10^7	4.5
	0.018	3	1.9	8	1.9×10^7	68.4	6	7.8×10^7	7.7

TABLE 1 (*continued*)

Chemical	Concentration %	Treatment (hours)	Survival %	Treated			Control		
				No. of plates	Total no. of bacteria	Mutants per 10^8 bacteria	No. of plates	Total no. of bacteria	Mutants per 10^8 bacteria
Sodium salicylate	2.0	3	62.	5	4.2×10^7	16.7	5	7.0×10^7	20.8
	1.0	3	38.	3	9.5×10^7	5.3	5	4.0×10^8	5.8
	2.0	3	10.	8	3.2×10^8	0.0	5	2.0×10^8	0.7
	3.0	3	0.03	10	2.2×10^6	0.0	5	4.0×10^8	5.8
Carbamate (methyl)	4.0	48	100.	7	3.0×10^8	8.0	5	1.7×10^7	6.1
	8.0	3	69.	8	1.4×10^8	14.4	6	7.8×10^7	7.7
	7.0	25	10.	8	1.8×10^8	5.5	6	1.3×10^8	10.6
	8.0	24	5.5	8	1.1×10^8	9.1	6	8.6×10^7	19.9
	8.0	24	4.6	8	8.6×10^7	4.7	6	7.0×10^7	5.7
	7.0	29	2.2	5	2.4×10^7	12.6			10.6
Carbamate (ethyl) =Urethane	5.0	3	62.	10	1.4×10^8	18.6	5	1.1×10^8	7.9
	6.0	3	56.	8	1.3×10^8	26.5	5	1.4×10^8	16.8
	4.0	3	27.	10	4.8×10^8	8.4	6	1.8×10^8	5.5
	3.5	3	25.	10	4.5×10^8	6.6			5.5
	7.0	3	10.	8	1.7×10^8	41.4	5	1.1×10^8	12.1
	7.0	3	8.3	8	1.7×10^8	53.3	5	1.3×10^8	14.3
	7.0	4	8.2	8	2.3×10^8	22.5	5	1.7×10^8	9.2
	7.0	4	4.7	7	5.6×10^7	98.5	5	8.5×10^7	8.3
	7.0	4½	2.7	8	2.0×10^7	40.0	6	5.8×10^7	3.4
Carbamate (*n*-propyl)	2.15	3	52.	7	4.6×10^8	28.0	6	7.6×10^7	6.6
	2.00	3	50.	8	7.0×10^8	11.9	6	1.1×10^8	7.5
	2.00	3	35.	8	3.6×10^8	29.9	6	7.8×10^7	6.4
	2.00	3½	32.	8	6.6×10^8	8.3	6	1.6×10^8	3.9
	2.00	3½	30.	8	7.2×10^8	10.6	6	1.8×10^8	2.2
	2.10	3½	29.	6	5.2×10^8	16.4			2.2
	2.10	3	24.	8	2.7×10^8	23.0	6	8.5×10^7	9.4
	2.10	3½	4.1	8	9.8×10^7	17.4	5	1.5×10^8	3.3

TABLE 1 (*continued*)

Chemical	Concentration %	Treatment (hours)	Survival %	Treated			Control		
				No. of plates	Total no. of bacteria	Mutants per 10^8 bacteria	No. of plates	Total no. of bacteria	Mutants per 10^8 bacteria
Carbamate (*n*-propyl) (*continued*)	2.10	3½	3.9	8	8.1×10^7	9.9			3.9
	2.10	3	2.9	8	7.0×10^7	24.2			3.3
	2.10	3½	2.5	8	3.2×10^7	34.8	6	9.7×10^8	12.3
	2.10	4	1.4	8	3.4×10^7	23.8			3.3
Carbamate (*n*-butyl)	0.50	3	85.	8	8.6×10^8	21.4	6	7.8×10^7	7.7
	0.76	3½	44.	7	9.2×10^8	5.3	6	1.8×10^8	2.2
	0.75	3½	29.	8	7.0×10^8	5.6	5	1.5×10^8	3.3
	0.75	3½	20.	8	4.2×10^8	8.3	6	1.6×10^8	3.9
	0.75	3½	19.	7	1.7×10^8	20.9	6	7.6×10^7	6.6
	0.75	3½	17.	8	2.6×10^8	7.4	6	1.1×10^8	2.7
	0.75	3	16.	8	1.6×10^8	10.0	7	7.6×10^7	9.2
	0.80	3½	4.9	8	1.2×10^8	5.9			3.3
	0.75	3½	3.4	8	4.5×10^7	8.9	6	9.7×10^7	12.3
	0.75	3½	1.4	8	1.5×10^7	0.0	6	7.8×10^7	6.4
Carbamate (isoamyl)	0.25	3	100.	8	2.0×10^8	8.4	6	1.6×10^8	3.9
	0.30	3	100.	8	1.6×10^8	11.3	6	1.1×10^8	2.7
	0.35	3	57.	8	8.5×10^8	8.0			2.7
	0.35	3	54.	8	1.1×10^8	9.4			3.9
	0.36	3	26.	8	6.2×10^8	6.3	6	1.8×10^8	2.2
	0.38	3	6.3	8	1.5×10^8	7.2			2.3
	0.37	3	5.8	8	1.4×10^8	7.0			2.2
[illegible]ral red (in light)	0.00025	3	50.	9	7.6×10^8	57.3	9	1.5×10^8	6.6
	0.00100	3	43.	9	6.6×10^8	118.			[illegible]
	0.00050	3	24.	8	3.2×10^8	122.			[illegible]
	0.00050	12	0.2	8	1.2×10^8	178.	8	6.7×10^8	10.5
Neutral red (in the dark)	0.00050	24	90.	9	4.2×10^8	17.2	6	4.6×10^8	10.1
	0.00050	12	86.	8	9.6×10^7	10.6	7	9.8×10^7	[illegible]

TABLE 1 (*continued*)

Chemical	Concentration %	Treatment (hours)	Survival %	Treated: No. of plates	Treated: Total no. of bacteria	Treated: Mutants per 10^8 bacteria	Control: No. of plates	Control: Total no. of bacteria	Control: Mutants per 10^8 bacteria
Neutral red (in the dark) (*continued*)	0.00050	24	83.	12	6.5×10^7	15.4	10	6.5×10^7	15.4
	0.00100	24	71.	10	4.6×10^7	17.4			15.4
	0.00050	12	70.	15	9.0×10^8	10.8	8		10.8
Acriflavine	0.0015	3	39.	10	4.8×10^8	78.3	4	1.0×10^8	14.4
	0.0030	3	28.	10	3.4×10^8	59.1			14.4
Caffeine alkaloid	2.10	3	72.	7	1.3×10^8	15.5	6	7.6×10^7	6.6
	0.60	48	44.	7	7.8×10^8	31.1	6	1.5×10^8	9.8
	2.10	24	34.	8	1.7×10^8	30.3	6	3.7×10^7	10.9
	0.50	48	15.	8	3.9×10^8	21.8	5	1.1×10^8	7.9
	1.00	48	15.	8	1.3×10^8	32.1	6	6.7×10^7	17.9
	0.50	48	12.	10	7.0×10^7	13.0	6	7.1×10^7	5.6
	0.50	48	5.1	8	9.3×10^7	34.6			7.9
	1.09	48	3.8	8	2.0×10^7	20.4	6	3.9×10^7	2.5
Necrosin	0.20	3	100.	8	4.6×10^8	23.6	6	1.4×10^7	6.9
	0.05	3	100.	8	4.2×10^8	26.0			6.9
	0.40	3	100.	8	1.2×10^8	16.4	6	1.0×10^8	9.9
	1.30	3	44.	8	8.5×10^8	17.8	6	1.4×10^8	8.4
	0.65	3½	3.4	7	3.9×10^7	172.	6	9.3×10^7	4.1

phenol (picric acid). The mutagenicity of phenol, however, was evident only after treatments that left very few survivors.

Sodium salicylate. Not mutagenic.

Carbamates. It was known from Oehlkers' work (1943) that some carbamates were able to induce chromosomal aberrations. Vogt (1948) had discovered that ethyl carbamate (urethane) showed mutagenic action in Drosophila. Since then, carbamates had been used by Bryson (unpublished) to induce mutations to phage resistance in *E. coli.* In our experiments, methyl carbamate gave negative results; ethyl and *n*-propyl carbamate gave good positive results; and *n*-butyl carbamate and isoamyl carbamate showed much weaker mutagenic effect.

Neutral red. Luther (1939) had found that neutral red was able to produce chromosomal aberrations. Buzzati (1947), treating Drosophila eggs with this substance, had observed an increase in frequency of mosaic flies. In our tests it seemed at first impossible to obtain low survival ranges, because treatment with concentrations higher than 0.001 per cent produced heavy clumping, and increases in length of treatment did not appreciably affect the number of survivors. Therefore no clear-cut positive results were obtained with the standard procedure. Since neutral red is a photodynamic substance, however, we then tried exposing the bacteria to light during treatment (a 60-watt bulb at a distance of about 10 cm), and finally got the desired survival ranges and with them a very large increase in frequency of mutants. Light had no effect on the controls. Similar results were reported by Kaplan (1948, 1949, 1950a, 1950b), treating *Bacterium prodigiosum* and *E. coli* with erythrosine.

Acriflavine. This substance was known to be a mutagenic agent for both bacteria and Drosophila (Witkin, 1947). Our tests also showed clear-cut positive results.

Caffeine. Caffeine had been found to be mutagenic for Ophiostoma (biochemical mutations) by Fries and Kihlman (1948) and for *E. coli* (mutations to phage resistance) by Witkin (Demerec, Wallace, and Witkin, 1948). Our experiments confirmed these results.

Necrosin. Necrosin is the name given by Menkin (1943) to the potent injurious euglobulin fraction of the exudate of inflamed tissues of vertebrates. A similar substance has subsequently been found by him in severely damaged tissues of some invertebrates (Menkin, 1949). The sample of necrosin used in our experiments was received from Dr. Menkin. It was found to be mutagenic, particularly in experiments where the proportion of survivors was low.

DISCUSSION

Streptomycin-dependent (Sd-4) bacteria, when plated on broth-agar medium without any streptomycin, undergo several divisions (residual growth) and then stop growing. The concentration of streptomycin in the medium in which the parent bacteria are grown is the most significant of the known factors influencing the number of divisions. For example, [illegible] grown

in broth medium containing 10 micrograms of streptomycin per milliliter, when plated on medium without streptomycin, will pass through 2-3 divisions; whereas bacteria grown in medium containing 25 micrograms of streptomycin will pass through 3-4 divisions. It has been found that very few, if any, nondependent back-mutants (background mutants) are present in cultures grown in broth with streptomycin, and therefore almost all such back-mutants appearing on the streptomycin-free plates can be assumed to originate during the residual growth. The almost complete absence of background mutants reduces the possibility that differential killing may be responsible for the increased proportion of back-mutants found among the survivors of treatment with certain chemicals; and this, together with the facts that residual growth can be dependably regulated in untreated material and that it allows expression of the back-mutations induced by treatment, makes the Sd-4 method especially suitable for studies of induced mutability.

Since spontaneous back-mutations occur during residual growth, any factor that increases this growth should increase the number of mutants. Therefore, in experiments such as these described here, it is necessary to be sure that any observed increase in mutant frequency is an effect of the treatment on the treated bacteria and not an effect of the treatment on the extent of residual growth. It is not likely that the chemicals used in these experiments would increase the residual growth. In the first place, they were usually applied in nonphysiological, toxic doses. Furthermore, the streptomycin requirement of the dependent bacteria is highly specific (Demerec, 1950), and ability to substitute for it would be quite unexpected in an unrelated chemical. However, even supposing that this had occurred, it would have been detected, either by the appearance of heavy residual growth on the plates of treated bacteria or through the microscopic check of the extent of residual growth that was made in all doubtful cases.

The analysis of data on the mutation-producing chemicals, presented in table 2, indicates that there were considerable differences of behavior among them. Some (ammonia, copper sulfate, and necrosin) were mutagenic only in treatments that left less than 5 per cent survivors; whereas others (hydrogen peroxide, formaldehyde, alpha-dinitrophenol, and neutral red in light) showed effects in treatments that allowed more than 50 per cent survival. Formaldehyde appears to be the most potent mutagen on the list. A treatment that left 61 per cent survivors resulted in 400 mutants per 10^9 treated bacteria; and two other treatments, each leaving 58 per cent survivors, gave 133 and 157 mutants, whereas in the controls there were only 23.2 and 15.5 mutants per 10^9.

Of the 31 chemicals listed in table 1, 19 were found to be mutagenic (table 2), and the following 12 were not: sulfuric, phosphoric, nitric, and hydrochloric acids; sodium and potassium hydroxides; mercuric chloride; silver nitrate; sodium silicate; sodium lactate; sodium salicylate; and methyl carbamate. Since the potency of the different mutagenic chemicals ranges from slight to strong, and, at least in the case of one mutagen that has been studied intensively (manganous chloride), can be shifted from one

TABLE 2

SUMMARY OF THE DATA ON MUTAGENIC CHEMICALS, SHOWING HIGHEST AND LOWEST FREQUENCIES OF MUTANTS OBSERVED IN EXPERIMENTS USING DIFFERENT TREATMENTS (EXPRESSED IN PERCENTAGES OF SURVIVORS)

	Survivors (per cent)			Controls
	50–100	5–50	less than 5	
	No. of mutants per 10^9 bacteria			
Boric acid	27	16–82	19–57	6–31
Ammonia	5	2–10	38–121	3–13
Hydrogen peroxide	20–29	121–124	27–175	5–7
Copper sulfate	6	4–21	40–56	2–21
Acetic acid	8	15–36	57	7–10
Lactic acid	...	11–40	11–35	5–27
Formic acid	18–20	24–44	38–39	3–15
Formaldehyde	133–400	120–326	560	12–79
Phenol	3–85	...	124–820	1–75
Alpha-dinitrophenol	3–34	14–56	6–97	3–13
Trinitrophenol	15	9–132	27–68	3–14
Carbamate, ethyl	19–27	7–53	40–99	6–20
n-propyl	12–28	8–30	10–35	2–12
n-butyl	21	5–21	6–9	2–12
isoamyl	8–11	6	7	2–4
Neutral red, light	57	118–122	178	7–11
dark	11–17	...	...	7–15
Acriflavine	...	59–78	...	14
Caffeine	16	13–35	20	3–18
Necrosin	16–26	18	172	4–10

extreme to the other by simple pretreatments of the bacteria, it seems very likely that the chemicals listed above as nonmutagens might become mutagenic if the treatment were made in a certain way. At any rate, examination of the list of mutagens in the table shows that mutagenicity does not belong to any one group of chemicals but is distributed widely among the different groups tested. Chemicals with such different properties as boric acid, ammonia, hydrogen peroxide, copper sulfate, acetic acid, formaldehyde, and phenol are on the list of mutagens. This fact, together with the indicated possibility that chemicals now considered noneffective would become mutagenic if appropriate conditions of treatment were found, leads us to conclude that genetic changes may be induced by most agents that are able to enter a living cell, upsetting some of its metabolic functions. It seems very likely that the genetic effect of a chemical is not direct but induced through changes in the cytoplasm, which in turn affect the genes. If this is so, it should be very difficult to establish what is the final reaction producing a change in a gene. It may well be that similar final reactions may be initiated by very different primary changes.

In this study we were considering back-mutations from Sd-4; and there is evidence to indicate that a majority of these may occur at one gene locus (Demerec, 1950; Newcombe and Nyholm, 1950). Several experiments by

others, however, based on observation of mutations to T1 phage resistance, have tested the mutagenicity of a few of the chemicals we used. Witkin (Witkin, 1947; Demerec, Wallace, and Witkin, 1948) found that acriflavine and caffeine were mutagenic; and Bryson (in manuscript), in extensive experiments with several carbamate compounds, showed that ethyl carbamate (urethane) is mutagenic. It therefore appears very probable that the mutagenic effect observed by us was not specific, but that treatment with these chemicals increases the mutation rate of the whole genome.

It should be kept in mind that we are dealing here with genetic changes that occur spontaneously with a rate of the order of one per 10^8 cell generations, a considerably lower rate than could be detected directly in germ cells of multicellular organisms. Since each cell of a multicellular organism is a genetic entity, however, in which mutations may occur spontaneously or be induced by various agents, the likelihood of mutation in some of the somatic cells of a higher organism is good, if a large number of these cells are exposed to the action of a mutagenic chemical. This likelihood is further increased by the fact that each cell contains several thousand genes, in any one of which a mutation may be induced.

SUMMARY

Thirty-one chemicals, representing various organic and inorganic groups, were tested for ability to induce back-mutations from streptomycin dependence (Sd-4) to nondependence in *E. coli*. Nineteen were found to be mutagens. It was demonstrated that mutagenicity is not a specific property of any one group of chemicals, but appears among widely different groups. Chemicals having such different properties as boric acid, ammonia, hydrogen peroxide, copper sulfate, acetic acid, formaldehyde, and phenol were found to be mutagenic, indicating that genetic changes may be induced by many agents that are able to enter the living cell and upset its metabolic functions.

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Poisoning from Common Household Products

ALAN K. DONE, M.D.*

Common household products pose a difficult problem in poisoning: Although involved less commonly than drugs, they are usually accompanied by much less information. Frequently the only information available is the trade name of the product and the purposes for which it was intended.

Toxicity data are essential for prompt and intelligent decisions regarding the prognosis and the need for hospitalization, diagnostic tests, and treatment. While the necessary data can often be obtained from the files of Poison Control Centers and such excellent references as that by Gleason, Gosselin, and Hodge,[12] the rapid proliferation of new products makes it impossible for such information to be obtained readily in all instances. It is possible in most instances to make at least a rough approximation of expected toxicity on the basis of the nature of the product. While there will always be deviations from any such categorical characterization, generalizations can be made which are useful with cognizance of their limitations.

The purpose of this report is to consider the toxic potential of those common household products which are most frequently involved in childhood ingestions, not only for purposes of prognostication in cases of ingestion but also to alert physicians to hazards which are frequently ignored. There is no intention of being precise, for that is impossible without considering each individual product specifically. Nor can we attempt to cover all of the almost limitless possibilities for toxic exposure in the home.

Table 1 lists those nonmedicinal household products which are most commonly involved in ingestions by children under the age of 5 years. The importance of some of these lies mainly in the numbers of people involved; with others there is a disproportionate morbidity which is reflected in the hospitalization rate. For instance, cosmetics are numerically very important, even though their ingestion is rarely associated

* Professor of Pediatrics, Adjunct Professor of Pharmacology, and Director of the Poison Information and Therapy Center, University of Utah Medical Center, Salt Lake City, Utah

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with significant morbidity. On the other hand, lye and corrosives, petroleum products, and polishes and waxes (mainly furniture polish) are less frequently ingested, but have a very high hospitalization rate. Intermediate in terms of severity of poisoning are pesticides, soaps and cleaners, bleaches, and disinfectants and deodorizers. In total, these classes of agents account for 30 per cent of the accidental ingestions by children under the age of 5 years.[17] The purpose of this report is to summarize the toxic potential of these types of products.

Table 1. *Common Nonmedicinal Household Products Most Frequently Ingested by Children Under 5 Years of Age (1966)*[17]

	PER CENT OF INGESTIONS	* HOSPITALIZATION RATE PER CENT
Cosmetics	5.9	4
Pesticides	5.8	16
Petroleum products	5.0	34
Soaps and cleaners	4.0	9
Bleaches	3.5	14
Disinfectants and deodorizers	2.2	27
Polishes and waxes	2.1	36
Lye and corrosives	1.5	49

Estimated from earlier data of National Clearinghouse for Poison Control Centers.

ASSESSING TOXICITY

A system of "toxicity ratings" was established by Gleason, Gosselin, and Hodge[12, 13] based upon the probable lethal dose for human beings.

Table 2. *Dose Basis for Approximate Toxicity Ratings, and Factors Tending to Raise or Lower Rating*

TOXICITY RATING*	PROBABLE LETHAL DOSE: PER KG.	PROBABLE LETHAL DOSE: VOL. IN 2 YEAR OLD
Extreme	5 mg.	A swallow† or less
High	50–500 mg.	2–3 swallows†
Medium	0.5–5 gm.	½–6 oz.
Nil or Low	5 gm.	More than 6 oz.

Raise	*Lower*
Liquid	Solid or semisolid
Pellets	Powdered (unless caustic)
Flavorful	Distasteful
Large volume	Small volume
Low viscosity hydrocarbon	Viscous

Method of dispensing or packaging

*Modified from Gosselin,[13] and Gleason, Gosselin, and Hodge.[12]
†Based upon average swallow volume of 4 to 5 ml. in a 2 year old child.[18]

estimated from animal studies and clinical data. This means of assessing toxic potential has proved to be of great value in providing at least an approximate answer to the question, "How toxic is it?" Table 2 presents a similar rating system, but with fewer gradations. The dosage data are translated into practical equivalents of volumes which would be expected to be potentially lethal in the average 2 year old child (number of swallows or ounces). No attempt is made to be any more precise than to indicate whether a material is likely to be lethal in a dose even smaller than one swallow (extreme toxicity), would require more than one but less than about three swallows (high toxicity), 0.5 to 6 ounces (medium toxicity), or more than about 6 ounces (toxicity nil or low). Thus, an error of 100 per cent or even more would not be expected to change the assessment significantly.

In assessing the potential toxicity of various types of products, the author borrowed extensively from the published estimates of others[11, 16, 18] and combined these with the experiences of this center. An attempt was made to rate products not only on the basis of dose, but also the likelihood that harmful quantities would be ingested or that the available toxicity data do not truly reflect the potential for harm in human beings. Since the dose ingested by a child can rarely be estimated except very grossly, it seemed important to take such factors into account insofar as possible. The factors which were considered to be of importance are listed in the bottom half of Table 2. Experience has shown that liquids or pelleted materials are more likely to be ingested in large quantity than powders and solids or semisolids. Flavoring has little to do with determining whether a product is ingested, but does influence the amount taken. Some substances are present in such small volumes that a toxic dose cannot be obtained from a single container. As noted below under Petroleum Products, low-viscosity hydrocarbons pose a threat of chemical pneumonia, and intrapulmonary aspiration of such substances makes the ingested dose irrelevant.

A factor which should be taken into account in individual instances, but which could not be anticipated here because of variations from one product to another, is the method of dispensing or packaging. For example, a substance which is available in aerosol form is usually less likely than a liquid in an ordinary bottle to be ingested in large quantities. A bottle having a very small aperture, such as is used with many shaving lotions, perfumes, and toilet waters, is likely to be ingested in smaller quantities than the same product in a wide-mouth bottle.

These were weighed one against another to arrive, rather arbitrarily, at a final rating. In some instances, there were solid data upon which to base a rating, while in others it was necessary to use theoretical considerations. In all instances, however, the final arbiter was clinical experience. While the latter admittedly is inaccurate and often subjective, the resulting estimate should be sufficiently accurate for clinical purposes in placing products into one of the four broad toxicity categories.

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Table 3. *Cosmetics*

TOXICITY	PRODUCT	TOXIC INGREDIENT OR EFFECT	TREATMENT
High	Permanent wave neutralizer	May contain either: Perborate (boric acid poisoning) Bromate (irritation, collapse, hemolysis, kidney damage)	Supportive For boric acid poisoning Sodium thiosulfate by mouth; demulcent; consider dialysis early
	Fingernail polish remover	Toluene; aliphatic acetates (irritation; central nervous system depression)	Supportive
Medium	Fingernail polish	Same as fingernail polish remover	Supportive
	Hair dye, metallic	Metal salts, pyrogallol (metal poisoning; corrosive)	For metal (if severe);[7,8] demulcents
	Permanent wave lotion	Thioglycolate (irritation; possible hypoglycemia)	Supportive; demulcent
	Bath oil	Perfume; sulfated castor oil	Demulcent (milk)
	Shaving lotion	Alcohol	Supportive
	Hair tonic	Alcohol, others (variable)	Supportive;† demulcents
	Cologne, toilet water	Alcohol, essential oils	Supportive;† demulcents
Low	Perfume	Alcohol, essential oils (irritation; possible hypoglycemia)	Supportive;† demulcents
	Shampoo	Anionic detergent (irritation)	Demulcent (milk)
	Bubble bath	Sodium lauryl sulfate (gastrointestinal irritation)	Demulcent (milk)
	Depilatories	Thioglycolate (see above)	Supportive; demulcent
	Hair straightener	Glycols and alcohols; may be caustic	Supportive ± as for caustic
	Hair dye, oxidation	Various amines, etc. (gastrointestinal irritation; ?methemoglobinemia)	Demulcent; methylene blue for severe methemoglobinemia.[7,8]
	Deodorants	Alcohol; aluminum or zinc salts (gastrointestinal irritation; hypoglycemia possible)	Supportive;† demulcents
	Shaving cream	Soaps	Milk
	Bath salts	Polymeric phosphate; borax	For causticity or boric acid poisoning
Nil	Make-up, liquid Eye make-up Hair dye, vegetable (henna, indigo) Cleansing or conditioning cream Hair dressing (nonalcoholic) Hand lotion or cream Lipstick, tube rouge	None	None

*In addition to evacuation of stomach or removal from skin, or both, when indicated.
†Ethyl alcohol, in addition to being a depressant, may produce hypoglycemia in young children.[5] Related alcohols, with the exception of methanol, have qualitatively similar effects; [illegible] methanol.

COSMETICS

Table 3 indicates that the majority of cosmetics can be given a fairly low toxicity rating on the basis of the system used here. Exceptions are permanent wave neutralizers and fingernail polish removers. (Fingernail polish remover is no more toxic than the polish on a weight basis, but the volume of the latter is generally less.) There has been a tendency in recent years to replace bromate with the less toxic perborate in permanent wave neutralizers; the toxic potential remains relatively high, however. The solvents in fingernail polish remover or the polish itself produce principally narcotic effects on the central nervous system and some gastrointestinal irritation. Most of the remaining products listed owe what toxicities they have to gastrointestinal irritation or to the presence of alcohol. Alcohol, in addition to its central nervous depressant effects, may produce hypoglycemia, especially in young children.[5] Among the exceptions, metallic hair dyes may rarely produce various types of metal poisoning and may be somewhat corrosive upon ingestion. Permanent wave lotions frequently contain thioglycolate which, like alcohol, may produce hypoglycemia.

A few additional materials deserve comment. Perfume is more toxic, milligram-per-milligram, than most colognes, but the volume of the former is usually quite small. Depilatories formerly contained thallium, but the latter has been replace by relatively nontoxic thioglycolate salts similar to those described above for wave lotions. Some oxidation types of hair dyes, in addition to causing gastrointestinal irritation when ingested, would be expected to cause methemoglobinemia, but this has not been seen with any degree of frequency. The aluminum or zinc compounds in deodorants have theoretical toxicity, but the nature of the formulations and the types of packaging make the ingestion of hazardous quantities highly unlikely. Bath salts occasionally contain borax, which is potentially capable of causing boric acid poisoning, but because they are powders the amounts ingested are usually small and serious difficulty rarely arises.

Numerous other cosmetics contain materials which themselves have significant toxicities, but factors mentioned previously tend to mitigate the toxic hazard. Prime examples here are eye make-up, lipstick, or tube rouge. These and other products shown at the bottom of Table 3 are practically devoid of toxic reactions.

PESTICIDES

It can be seen in Table 4 that for most classes of pesticides the range of toxicity is great and relatively high. The reader is referred to other sources for more details about toxic manifestations and treatment.[6-8, 19, 20]

The inorganic compounds listed all have extreme toxicity. Fortunately, they also have fairly effective antidotes as noted. In some instances these products represent an unnecessary risk, and it is often possible to reduce the hazard to children by substituting products having

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Table 4. *Pesticides*

CHEMICAL CLASS	TOXICITY RATING: Medium (or low)	High	Extreme	TREATMENT
Inorganics			Cyanide Phosphorus [illegible] Thallium Fluoride	Nitrite, Thiosulfate* $CuSO_4$ [illegible] [illegible] [illegible] [illegible] Chelate*
Chlorinated hydrocarbons	Chlorobenzilate Kelthane Methoxychlor DDD Perthane 2,4-D 2,4,5-T	Dieldrin Toxaphene Lindane Chlordane Heptachlor Thiodan DDT	Endrin Aldrin	Supportive; combat CNS excitation
Organophosphate anticholinesterases	Chlorthion NPD Butonate Phostex Malathion	Mipafox Pirazinon Dibrom Dipterex Diazinon Ethion Navadel DDVP Co-ral	Trithion (more toxic) Dimefox Demeton Guthion Phosphamidon OMPA Pyrazothion Isolan EPN TEPP Paraoxon Parathion Di-syston Pyrazoxon Phosdrin Thimet	Atropine, Cholinesterase regenerators (e.g., 2-PAM)†
Carbamates	Zineb Captan Ferbam	Sevin		Atropine† cholinesterase regenerators not useful)
Botanicals	Pyrethrins Allethrin	Rotenone	Nicotine Strychnine	Supportive
Miscellaneous	Warfarin		Fluoroacetate	Vitamin K* Glycerol monoacetate*

*In addition to evacuation of stomach or removal from skin, when indicated, as well as supportive measures.
†Details of specific antidotal treatment can be found elsewhere.[illegible]
‡Arsenic acid or sodium arsenite. Relatively insoluble salts such as lead arsenate or arsenic trioxide, and organic arsenicals such as the methanearsonates (all of which are common constituents of herbicides) have a much lower ("medium") toxicity.[illegible]

lesser toxicity and comparable effectiveness. For instance, there is little reason for employing thallium or phosphorus as a rodenticide when warfarin, which has little or no acute toxicity in humans, can be used effectively.

The chlorinated hydrocarbons tend to have a lesser degree of toxicity and a wider range than the inorganics. Here, again, it is often possible to select for home use a product which is comparatively less toxic than some others without necessarily sacrificing efficiency.

Among organophosphate insecticides the preparations are legion and the toxicity range again is quite wide. Individually, the organophosphates in the "extreme" toxicity class are not included frequently in pesticides intended for lay use; as a group, however, they may be encountered with some degree of frequency as causes of poisoning due to household products. An additional example of the ability frequently to substitute a substance of lesser toxicity without prohibitive loss of effectiveness is provided by malathion. It is rapidly detoxified by mammals and so has a very low order of toxicity, but is an effective cholinesterase inhibitor in insects.[19]

The carbamates have a relatively low degree of toxicity in vertebrates. Zineb and Captan are fungicides; ferbam and sevin are mild cholinesterase inhibitors and are insecticidal. Unlike the organophosphate anticholinesterases, the cholinesterase inhibition of carbamates is not relieved by the cholinesterase regenerators, which may actually increase the severity of poisoning.

Among the botanicals, nicotine and strychnine are unnecessarily toxic; on the other hand, the pyrethrins and the synthetic relative, allethrin, have a very low degree of toxicity.

Warfarin is probably the most widely used rodenticide at the present time. Its action is to inhibit prothrombin formation and its effects are counteracted by vitamin K. Like the closely related medicinal, Dicumarol, warfarin produces hypoprothrombinemia only after repeated adminis-

Table 5. *Petroleum Products: Estimation of Aspiration Hazard and Systemic Toxicity*

PRODUCT	SOURCE(S)	SYSTEMIC TOXICITY	ASPIRATION HAZARD*
Petroleum ether or benzine	Industrial or rubber solvents	4+	–
Gasoline	Fuel	3+	2+
Naphtha	Solvent, lighter fluid, dry cleaner, thinner	3+	3+
Kerosene	Fuel, charcoal lighter fluid, thinner, pesticide solvent	2+	2+
Mineral seal oil	Furniture polish	3+	4+
Fuel or diesel oil	Fuel; heating oil	1+	1+
Mineral oil		–	–‡
Lubricating oil	Motor oil, cutting oil, transmission fluid	–	–‡

*Formulations with increased viscosity have decreased aspiration hazard.[11]
‡Low-grade lipoid pneumonia possible with aspiration.

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tration and not with a single dose even if large. Consequently, although animal studies suggest a higher toxicity, warfarin is ranked medium to low in terms of acute poisoning in humans. Fluoroacetate is extremely toxic, but is not generally available except to professional exterminators; there is experimental evidence that its toxic effects are inhibited by glycerol monoacetate.[5]

It is important to recognize that the solvents employed in pesticide formulations are sometimes at least as toxic as the pesticide itself. This fact could not be taken into account in the construction of Table 4 because the solvents differ widely among various preparations of the same pesticide. However, the solvents usually used are petroleum products such as those listed in Table 5. Xylene is also sometimes used. The nature of the solvent will usually be specified on the label, which should always be consulted in cases of potential pesticide poisoning.

PETROLEUM PRODUCTS

A departure from the approach used thus far is necessary in the discussion of petroleum products (Table 5). Here it is difficult to be categorical about toxic potential because there are two possible effects which are unrelated: systemic toxicity and aspiration hazard. The latter is related principally to the viscosity or surface tension of the compound,[11] while systemic effects are related to other chemical properties. Thus, a material which has only mild or moderate systemic toxicity may be extremely dangerous if aspirated into the lungs even in very small quantity. Conversely, a preparation such as petroleum ether or benzine may have striking systemic toxicity and no appreciable aspiration hazard. The matter is complicated further by the fact that in many formulations a material which otherwise would be a striking aspiration hazard may not be such if the viscosity has been increased by various means. Among materials which pose an aspiration hazard, intrapulmonary aspiration of at least small quantities is nearly assured by the low surface tension of the material. As a result, ingestion of such products as kerosene and mineral seal oil is nearly always followed by at least some chemical pneumonia.

Gasoline, naphtha, kerosene, and mineral seal oil produce both systemic and pulmonary effects. The latter effect is especially pronounced with mineral seal oil, which is a constituent of many furniture polishes. Fuel or diesel oil produce little systemic or pulmonary effect. Mineral oil and lubricating oil are too viscid to produce the typically severe "hydrocarbon" or "chemical" pneumonia, but they are capable of producing a low-grade lipoid pneumonia if aspirated in large quantities.

The pneumonia produced by some of these agents can be fulminating and pose a difficult therapeutic problem. The management is well discussed elsewhere.[18] Systemic toxicity of these materials usually is manifest by a state resembling alcoholic intoxication, together with restlessness, incoordination, confusion, coma (sometimes with con-

Many petroleum products contain additives which have significant toxicities of their own, but their concentrations are usually so low as to not contribute appreciably to the acute effects of the parent compound.[19]

SOAPS, DETERGENTS, CLEANERS, AND BLEACHES

As can be seen in Table 6, what toxicity is exhibited by soaps, detergents, and cleaners is most often related to irritant or caustic properties. Materials which are frankly caustic will usually have a warning to that effect on the label.

Table 6. *Soaps, Detergents, Cleaners, and Bleaches*

TOXICITY	PRODUCT	TOXIC INGREDIENT OR EFFECT	TREATMENT
High	Electric dishwasher granules†	Caustic (may be severe)	Treat as caustic burn†
	Ammonia†	Caustic; coma and convulsions	As caustic†; supportive
	Bleach, commercial	Boric acid or oxalate poisoning	Milk; calcium; supportive
	Bleach, oxygen	Boric acid poisoning	Supportive
	Disinfectant cleaners: See Disinfectants and Deodorizers (Table 7)		
Medium	Bleach, chlorine	Gastrointestinal irritation, some causticity	Demulcents ± treat as caustic burn
	Borax	Boric acid poisoning	Supportive
	Water softeners† (soluble)	Some caustic; hypocalcemia and acidosis possible	Milk; as for caustic†; supportive
	Liquid general cleaners:		
	Kerosene	Pneumonia, systemic toxicity	As for petroleum distillates†
	Pine oil	Gastrointestinal and genitourinary irritation; depression and weakness	Supportive; demulcents
	Detergent granules† for laundry, dishes and general use	Gastrointestinal irritation to causticity (some frankly caustic and have higher toxicity)	Demulcents; treat as caustic burn†
Low	Detergent powders†	Gastrointestinal irritation (causticity possible, but unlikely)	Demulcents, soap; ± treat as caustic burn†
	Liquid detergents	Gastrointestinal irritation	Demulcents, soap
	Toilet soap	Gastrointestinal irritation	Demulcents
	Fabric softeners	None	None
	Window cleaners (liquid)	Alcohol	See footnote to Table 3
Inhalation hazard	*Chlorine bleach mixed with:*		
	Strong acid (bowl cleaner)	Chlorine gas (intense respiratory irritation)	Bicarbonate aerosol; oxygen
	Ammonia	Chloramine fumes (respiratory irritation, nausea)	Terminate exposure; supportive

*In addition to evacuation of stomach (except with caustic burn) or removal from skin, when indicated.

†Products threatening caustic effects will be identified with a *caution* label. Details of

Electric dishwasher granules may be severely caustic and have, on occasion, caused complete destruction of the stomach. Most of the hospitalizations following ingestions of cleaning preparations are attributable to ammonia, which is highly caustic in its concentrated form. It is important, however, to appreciate that products which simply contain ammonia, but in highly diluted form, have only the toxic potential of the basic cleaning compound. In other words, one must distinguish between ammonia per se and materials which are simply ammoniated. The treatment of such caustic burns is well described elsewhere.[2] Most other types of detergent preparations have medium or low toxicity ratings, but it is important to realize that there are rare exceptions; occasional products will be disproportionately caustic.

The toxic potential of bleaches varies according to the type. Generally speaking, the oxygen-type household bleaches and the commercial ones have the highest toxicities, both being capable of producing boric acid poisoning or (in the case of the commercial products) sometimes oxalic acid poisoning. Chlorine bleaches, which are usually dilute solutions of sodium hypochlorite, are generally overrated so far as toxicity is concerned. Systemic toxicity is low and, while there is some caustic effect, it is rarely sufficiently severe to cause fatality or stricture.

Borax may cause boric acid poisoning, but the likelihood is diminished when the product is in powder form. Soluble water softeners which contain polymeric phosphates are not only potentially caustic, but may also produce hypocalcemia and acidosis. (Insoluble water softener resins have low or nil toxicity). Liquid cleaners intended for general purposes usually owe their moderate toxicities principally to such additives as pine oil or kerosene, as noted in Table 6. It is important, however, not to overestimate the toxicity of the many preparations which simply have a pine oil scent; the effects produced would be those of the basic detergent or other cleaning agent.

An unusual hazard exists when chlorine bleaches are mixed with strong acids (such as those contained in some toilet bowl cleaners) or strong ammonia solutions. As noted in Table 6, strong acid causes the release of chlorine gas, and ammonia combines with hypochlorite to produce chloramine. The fumes of either are intensely irritating to eyes and respiratory trace, and occasional individuals have been "overcome."

A new and very serious hazard is the liquid preparations of caustic alkalis which have recently been marketed and unfortunately widely advertised and cause serious esophageal burns.

DISINFECTANTS AND DEODORIZERS

Referring to Table 7, those deodorizers in cake form (usually intended for bathrooms, toilets, or garbage cans) which contain naphthalene have high degrees of toxicity, while those which contain *p*-dichlorobenzene or sodium bisulfate are much less toxic, yet the latter are equally effective for their intended purposes. Among disinfectants, the acidic or phenolic ones have the highest toxicities. Acid disinfectants may [illegible] but the anion (borate, formate, etc.)

may have additional toxicity. The phenolic disinfectants may cause irritation or corrosion, shock, coma, and renal damage which may progress to failure.

Table 7. *Disinfectants and Deodorizers*

TOXICITY	PRODUCT	TOXIC INGREDIENT OR EFFECT	TREATMENT
High	Naphthalene deodorizer (bathroom, toilet, garbage can)	Irritation, coma, convulsions, hemolysis, kidney damage	Supportive; alkalinize urine; transfuse as needed
	Acid disinfectant (boric, chloroacetic, formic, salicylic, etc.)	Corrosive, plus systemic effects of anion	Supportive and as caustic burns
	Phenolic disinfectant	Phenols; hexachlorophene (gastrointestinal irritation, shock, coma; corrosion or kidney damage possible)	Treat as caustic burns or anticipate renal failure
Medium to high‡	Alkali disinfectant (sodium or ammonium hydroxides)	Potentially caustic	Demulcents ± treat as caustic burns
	Benzalkonium and other QAC† disinfectants	Gastrointestinal irritation, convulsions, coma, respiratory distress, collapse	Supportive; demulcents; mild soap solution or milk
	Pine oil disinfectant	Gastrointestinal and genitourinary irritation; depression and weakness	Supportive; demulcents
	Halogen disinfectants	Hypochlorites or chlorinated hydrocarbons (irritation; excitation)	Demulcents ± treat as caustic burns; sedation as needed
Medium	Wick deodorizers	Formaldehyde and hydrocarbons (gastrointestinal irritation, abdominal pain, shock, hematuria, coma, convulsions)	Supportive; demulcents
	Deodorizing cleansers	Pine oil or QAC†	(See above)
	p-Dichlorobenzene or sodium bisulfate deodorizer (bathroom, toilet, garbage can)	Irritation, abdominal pain, narcosis; liver, kidney damage possible	Supportive; demulcents; sodium bicarbonate
Low	Iodophor disinfectant	Detergent-iodine complex (gastrointestinal irritation)	Demulcents
Nil	Spray deodorizers	(Variable)	Symptomatic
	Refrigerator deodorizer	Charcoal (inert)	None

*In addition to evacuation of stomach (except with caustic burn) or removal from skin, when indicated.
†Quaternary ammonium compounds.
‡Depending upon constitution and concentration.
¢Details of treatment can be found elsewhere.[2]

The toxicities of alkali, quaternary ammonium, pine oil, and halogen disinfectants vary on the basis of concentration of the active ingredients (see Table 7). Wick deodorizers and deodorizing cleansers have variable composition and are generally rated in the "medium" group. The iodo-

phor disinfectants are among the safest. Spray deodorizers cause little difficulty because the obtaining of a large dose is improbable.

OTHERS

Two additional classes of compounds, polishes and waxes and lye and other corrosives, are important not only numerically but also because they produce disproportionate morbidity. Generally speaking, polishes and waxes create little problem except for furniture polishes containing petroleum hydrocarbons, which have already been discussed. Corrosive materials in addition to those previously discussed are usually strong alkalis most commonly used in drain pipe cleaners, paint removers, and some cleaning agents. Reagent tablets for detecting sugar in urine have also represented a caustic hazard. The toxicologic problem of principal concern with respect to caustic alkalis is the local tissue injury involving esophagus and sometimes stomach which can result in shock, asphyxia, perforation, or late formation of esophageal strictures. These problems and their treatment are discussed in more detail elsewhere.[2]

SUMMARY

A review of hazardous household products is not only potentially helpful in the management of cases of acute poisoning, but also provides opportunities for practicing preventive medicine. Much more effective than generalizing about the importance of keeping things out of the reach of small children are specific recommendations concerning particular materials. These can be in the form of suggestions either to avoid certain highly toxic materials or to surround their use with effective safeguards. An attempt was made here to characterize the potential hazards of the household products which are most frequently ingested by children.

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University of Utah Medical Center
Salt Lake City, Utah 84112

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EFFECT OF ADMINISTRATION OF HOT WATER, ACIDS, ALKALI, MECHOLYL CHLORIDE, OR ATROPINE SULFATE UPON THE GASTRIC MUCOSA OF MICE[1]

By HELEN M. DYER, *biochemist*, MARGARET G. KELLY, *assistant biochemist*, and THELMA B. DUNN, *research fellow pathology*, *National Cancer Institute, National Institute of Health, United States Public Health Service*

INTRODUCTION

The papers (*1*) that were presented at a conference on gastric cancer[2] in 1940, supplemented by those (*2*) from a conference[3] held in 1944, cover the literature adequately on both clinical and experimental gastric cancer. The high incidence of gastric cancer in man, its diagnosis, treatment, etiology, and the question of a genetic influence were discussed in detail. No attempt is made at this time, therefore, to review the literature.

Voegtlin (*3*) emphasized the value of the production of a disease under well-defined experimental conditions as an important contribution to an understanding of the process of the disease. At the present time, no confirmed report has been found of the production of gastric adenocarcinoma in animals with the oral administration of any agent. Stewart (*4*) induced neoplasms of the forestomach of mice with the oral administration of methylcholanthrene or 1, 2, 5, 6-dibenzanthracene. Stewart and Lorenz (*5*) succeeded in producing gastric adenocarcinoma in several strains of mice by the injection of emulsions of these two carcinogens directly into the wall of the glandular portion of the stomach. Production of adenocarcinomas in the stomachs of mice by a combination of selective breeding and parenteral injections of methylcholanthrene was recently reported by Strong (*6*).

The etiology of gastric cancer, however, is not known. The present investigation was undertaken with the hope of producing cancers or precancerous lesions in the gastric mucosa of mice by the repeated administration of tissue irritants and of agents which had been shown to modify the secretion of gastric juice. In addition to this treatment, some groups of animals were given an emulsion of 1, 2, 5, 6-dibenzanthracene repeatedly by esophageal intubation (*7*).

The various agents failed to produce chronic gastric lesions under the conditions of the experiment. The report is therefore presented with a minimum of detail.

MATERIALS AND EXPERIMENTAL PROCEDURE

Inbred mice of strains A, C3H, and I, and hybrid LA mice (strain L mice crossed with mice of strain A) of the F_1, F_2, and F_3 generations were selected for the experiment. Both males and females were used, except in the case of mice of strain C3H, of which only males were selected because of the high incidence of spontaneous mammary tumors in the females. The mice were separated according to sex and were housed in metal, raised-bottom cages in groups of five.

Pellets similar in composition to diet 77 (*8*) were fed ad libitum, except on treatment days when the food was removed from all cages approximately 4 hours before the injections and was returned within the hour following treatment. When the material was administered in the drinking water, the food was removed from these cages also for the same periods as those in which the injected mice fasted. Water in bottles with special outlet tubes was available at all times.

The agents administered were hot distilled water, hydrochloric acid, lactic acid, sodium hydroxide, mecholyl chloride (acetyl-β-methylcholine chloride), and atropine sulfate. Groups of mice from each of the strains were evenly distributed with regard to age and sex and then subjected to treatment with each agent. The mice from those groups that also received repeated injections of an emulsion of 1,2,5,6-dibenzanthracene were matched with mice that received a control emulsion of mineral oil and sodium alginate supplementary to the specific agent.

The carcinogenic hydrocarbon was emulsified by the gradual addition of a solution of 1,2,5,6-dibenzanthracene in hot mineral oil to a hot aqueous solution of sodium alginate. A mechanical stirrer was used during the addition of the hydrocarbon solution, and the dissolved alginate was kept in a water bath at a temperature below boiling. The final emulsion contained, per cubic centimeter, approximately 2 mg. of dibenzanthracene and 8 mg. of sodium alginate.

During the course of the treatments, the mice suffered such a loss of appetite and of body weight that the regimen as originally adopted had to be changed. The dosage was decreased; the periods of treatment were shortened; the intervals between treatments were increased; and whenever the majority of the mice showed loss of weight or other unfavorable symptoms, the injections were discontinued temporarily. During the first 3 months of the experiment injections were made 5 times weekly from Monday through Friday, except those with mecholyl and atropine sulfate, which were administered 10 times weekly (twice a day on each of the 5 days). The dibenzanthracene

[1] Received for publication August 23, 1946.

[2] Under the auspices of the National Advisory Cancer Council, held at the National Institute of Health, Bethesda, Md., on October 11 and 12, 1940.

[3] Under the auspices of the Committee on Cooperation in Cancer Research, National Advisory Cancer Council, United States Public Health Service, held at The Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York City, on November 14, 1944.

and control emulsions were begun later. At this early period, because of the great mortality among the treated mice, additional animals were started in each group, and the number of injections was decreased to 3 a week on alternate days. When the dibenzanthracene and control emulsions were given during a later period, they were administered once a week. Lactic acid and atropine sulfate were furnished continuously in the drinking water except during short periods when great loss of weight was observed and the mice were given drinking water without the supplements.

For the treatment with hot water care was taken to cool the needle by immersion in cold water before inserting it in the mouth of the animal.

The effect of subcutaneous injections of mecholyl into mice upon the secretion of gastric juice was investigated before the repeated injections were begun. The procedure described by Roe and Dyer (*9*) was followed except that urethane was used to anesthetize the mice and the peptic activity was measured by a modification (*10*) of the method of Anson. An attempt was made to prolong the action of mecholyl chloride by incorporating it into a beeswax-liquid petrolatum emulsion and injecting it intramuscularly in amounts up to 2.0 mg. of mecholyl per mouse. Profuse salivation and bulging of the eyes and occasional gastric hemorrhages resulted, but the action of the mecholyl was not prolonged significantly. After three such injections into the experimental mice, the subcutaneous injections of aqueous solutions of the gastric stimulant were resumed.

After 2 months of subcutaneous administration of atropine sulfate, intramuscular injections of a beeswax-liquid petrolatum emulsion of this substance were tried. Abscesses appeared at the sites of injection, so that the subcutaneous injections of the aqueous solution were resumed after 10 days. When the number of injections of the various agents was reduced to three a week, the surviving mice in this group and the new mice added to the group were given atropine sulfate in their drinking water instead of subcutaneously.

Preliminary tests with esophageal intubations of the alginate-mineral oil emulsion to mice that were killed at intervals of 0.5, 2.0, and 6.0 hours following the injection demonstrated the persistence of a white gel in the stomach. The gel adhered to the gastric mucosa and frequently appeared to form a plug in the pyloric region of the stomach. Supplementary injections of hydrochloric acid or of hot water had no observable effect upon the persistence of the emulsion in the stomach; sodium hydroxide changed the white gel of the injected emulsion to a yellowish, mucilaginous fluid which was found to remain in the stomach for some hours.

The loss of mice was so great during the first year of the investigation that it was decided thereafter to discontinue treatment of all mice that had been treated for as long as 6 months (65 to 75 injections) and to keep the surviving animals under observation until they began to show signs of abnormality, or loss of weight, or had reached the age of 12 to 15 months.

The dosages of the agents and the numbers of injections are summarized in table 1.

TABLE 1.—*Summary of treatment of mice*[1]

Agent	Concentration[2]	Number of injections per mouse[3]		Number of mice		Age of Mice at—				Final weights of mice		Appearance of stomachs at necropsy			
						First treatment		Necropsy				Gross		Microscopic	
		Range	Average	Died	Killed	Range	Average	Range	Average	Range	Average	Normal	Hemorrhagic	Normal	Pathologic[4]
						Months	*Months*	*Months*	*Months*	*Grams*	*Grams*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Water, approximately 60° C.		15-141	57	34	28	1-11	4	3-22	11	11-41	23	92	8	93	0
Control emulsion		4-23	13			1-13	6								
Water, approximately 60° C., and Dibenzanthracene emulsion.	2 mg. per cc.	11-129	67	14	18	1-6	3	3-17	11	10-36	24	94	6	72	6
		3-16	11			1-8	5								
Water, room temperature		16-131	62	20	14	1-8	4	7-18	12	17-34	26	93	7	93	7
Control emulsion		3-23	13			1-10	5								
Water, room temperature, and Dibenzanthracene emulsion.	2 mg. per cc.	27-138	75	15	17	1-6	2	5-18	11	15-46	29	97	3	82	18
		2-17	13			1-6	3							–	
Hydrochloric acid	1.0 to 0.25N	10-92	41	35	23	1-7	3	2-18	9	10-45	21	88	12	65	4
Control emulsion		3-21	12			1-10	4								
Hydrochloric acid, and Dibenzanthracene emulsion.	1.0 to 0.25N	19-99	54	18	22	1-7	3	4-17	11	13-52	25	93	7	86	0
	2 mg. per cc.	2-21	12			1-8	3								

See footnotes at end of table.

TABLE 1.—*Summary of treatment of mice*[1]—Continued

Agent	Concentration [2]	Number of injections per mouse [3]		Number of mice		Age of Mice at—				Final weights of mice		Appearance of stomachs at necropsy			
						First treatment		Necropsy				Gross		Microscopic	
		Range	Average	Died	Killed	Range	Average	Range	Average	Range	Average	Normal	Hemorrhagic	Normal	Pathologic [4]
						Months	*Months*	*Months*	*Months*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*
Sodium hydroxide	0.5 to 0.1N	20-132	51	19	17	1-8	3	3-18	10	17-37	28	90	10	70	12
Control emulsion		1-24	13			1-10	4								
Sodium hydroxide, and	0.5 to 0.1N	12-65	38	23	11	1-8	3	4-19	9	11-42	24	89	11	82	9
Dibenzanthracene emulsion.	2 mg. per cc	1-16	10			1-6	4								
Mecholyl chloride	0.05 to 0.5 mg. per cc.	56-306	151	19	15	1-5	2	5-17	9	13-38	25	91	9	73	0
Control emulsion		1-22	12			1-5	4								
Mecholyl chloride, and	0.05 to 0.5 mg. per cc.	62-286	168	15	11	1-5	2	5-15	11	17-37	26	89	11	91	0
Dibenzanthracene emulsion.	2 mg. per cc	3-22	13			2-5	4								
Atropine sulfate, s. c., or	0.1 to 1.0 mg. per cc.	50-266	216	23	12	1-10	2	2-17	9	15-43	26	83	17	92	0
Atropine sulfate in drinking water.	0.25 mg. per cc	21-100	69			1-10	5								
Control emulsion		4-18	11												
Atropine sulfate, s. c., or	0.1 to 1.0 mg. per cc.	25-265	200	17	14	1-7	2	6-20	11	15-40	25	87	13	100	0
Atropine sulfate in drinking water, and.	0.25 mg. per cc	53-100	86												
Dibenzanthracene emulsion.	2 mg. per cc	5-17	12			1-7	1								
Lactic acid	0.2 to 0.1N	31-153	91	18	17	1-9	3	8-16	13	15-38	25	91	9	82	12
Control emulsion		7-28	15			1-9	1								
Lactic acid, and	0.2 to 0.1N	45-160	93	5	13	1-6	3	5-15	11	13-37	21	78	22	100	0
Dibenzanthracene emulsion.	2 mg. per cc	8-32	14			1-6	4								

[1] Mecholyl chloride was administered subcutaneously; atropine sulfate, subcutaneously and in drinking water; lactic acid in drinking water; and all other agents by esophageal intubation.
[2] The volumes administered were 0.01 cc. of agent per gram of body weight for all esophageal intubations and subcutaneous injections, and ad libitum when agent was administered in the drinking water.
[3] When agent was administered in drinking water the values are for days of treatment per mouse.
[4] Miscellaneous pathologic changes other than typical lesions in mice of strain I.

The mice were weighed biweekly during the period of treatment and once a month between the last injection and the time of killing. Many of the mice died during the night, and post mortem changes were too advanced for necropsy. Where post mortem changes were not too great, gross examination was made of each animal that died, special care being exercised in the examination of the gastrointestinal tract. The remaining mice were killed as stated previously. They were necropsied, and histologic studies were made.

In the routine examination of mice that were killed at an early date, the stomach was removed at once, quickly examined for gross lesions, cut into three longitudinal strips, and fixed in absolute alcohol for Mayer's mucicarmine stain, Regaud's solution for Bowie's pepsinogen stain, and Zenker-formol for routine hematoxylin-eosin stain. The rest of the organs were then examined, and those showing gross abnormalities were taken for routine hematoxylin-eosin stain. Later in the experiment, special stains on the stomach were usually omitted, but the routine hematoxylin-eosin stain was made of the stomachs of all the experimental mice that were killed, and the special stains and the hematoxylin-eosin stains were used for histologic studies of the stomachs of 15 untreated control mice of strains A, C3H, and I, and LA hybrids.

RESULTS

Table 1 summarizes the experiments and the findings with regard to the stomachs of all the mice that were necropsied. In the case of the animals that died during treatment, the only abnormality observed in any of the stomachs of any of the groups was hemorrhage. Petechial hemorrhages when present were invariably located in the glandular portion of the gastric mucosa and extended over a very small area. From preliminary studies of the acute toxic effects of the various agents on mice, it seems likely that the stomach hemorrhages were caused by the acute, irritating effects of the agents injected. Hemorrhages were observed in small areas of the intestinal tracts of 9 percent of the treated mice and were found in all groups.

No significant difference in the amount of mucin

or pepsinogen in the gastric mucosa could be detected in any of the groups of experimental or control animals, although occasional mice showed a considerable variation for which no cause was found. Positive mucin staining is found characteristically in a few glands near the limiting ridge (vestiges of a cardiac portion in the mouse), on the surface of the gastric gland or fundic portion, and in cells located at the base of the pyloric glands. The mucicarmine stain did not outline so distinct a layer of mucous cells at the necks of gastric glands as is usually encountered in the rat. Stomachs of strain I mice were of particular interest, since these special staining procedures disclosed a disarrangement of cell types and showed that mucous staining cells were present in the fundic glands before the typical strain I lesion, as described by Stewart and Andervont (*11*), had fully developed.

Except for those of the strain I animals, the stomachs examined were remarkably normal. A few miscellaneous pathologic changes were recorded which did not appear significant. Only 1 mouse, a hybrid LA female, which had received 92 injections of hydrochloric acid and 11 injections of dibenzanthracene over a period of 6.5 months and was killed at the age of 8.5 months, showed an inflammatory reaction about the base of the glands, and some hyperplastic cells. Another hybrid animal treated with atropine sulfate and dibenzanthracene developed an epidermoid carcinoma of the forestomach. Stomachs of the strain I animals showed the changes typical for this strain, which did not seem to be modified by treatment.

Numerous pathologic changes were found in other organs examined; but none of them could be ascribed to any specific experimental procedure, since they occur commonly in any groups of old experimental mice. Lesions in the lungs were very frequent, especially in animals subjected to esophageal injections. These pulmonary changes usually consisted of areas of consolidation caused by a leukocytic exudate or accumulations of large vacuolated cells in the alveoli, bronchitis, peribronchial leukocytic infiltrations, and areas of atelectasis. These pulmonary conditions were probably the most frequent immediate cause of death.

Several types of tumors were found, but they were not significantly more frequent in any particular group. Among them were mammary tumors, leukemias, and lung tumors. Nine of fourteen mice that developed tumors had received dibenzanthracene.

SUMMARY

Repeated direct alimentary administration of hot water, hydrochloric acid, sodium hydroxide, lactic acid, or atropine sulfate, with or without an emulsion of 1, 2, 5, 6-dibenzanthracene, failed to produce cancer or precancerous lesions in the gastric mucosa of mice of strains A, I, and C3H, and in hybrid LA mice (strain L crossed with mice of strain A).

Repeated parenteral injections of mecholyl chloride or of atropine sulfate, with and without direct alimentary administration of 1, 2, 5, 6-dibenzanthracene, were likewise ineffective in producing any specific pathologic changes in the gastric mucosa of mice.

That the stomachs showed so little injury following very drastic treatment is evidence of the remarkable resistance and recuperative power of the normal gastric mucosa.

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German Translation

Changes in Blood Chemistry in Experimental Sodium Hydroxide Poisoning

I.G. v. Fazekas

(From the Institute of Legal Medicine of the Royal Hungarian Franz-Joseph University in Szeged, Hungary - Director: Prof. Dr. J. v. Balo)

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In the various toxicology handbooks it is generally stated that, along with the local and corrosive effect exerted by caustic alkalis in the case of caustic poisoning, the remote effect of these toxic products plays a secondary role. In previous publications [Jankovich, Incze, Fazekas (23) and Jankovich, Incze (24)] we were able to point out, on the basis of histological changes detected in various human organs such as liver, spleen, brain, etc., the remote effect exerted by sodium hydroxide poisoning which previously had hardly been considered or even completely overlooked. In recent publications we reported histological changes, especially in the brain, observed during poisoning with another caustic alkali, namely ammonia, both in human patients who died from ammonia poisoning (10, 25) and in animal experiments (11). These results prompted us to continue our research in this direction, namely we wanted to clarify the toxic effect exerted by these products (NH_4OH, NaOH) on the living organism by chemical means. The tests carried out by me on rabbits showed that acute ammonia poisoning causes extensive changes in blood chemistry, such as lipemia (12), hyperglycemia, hyperphosphatemia, hypocalcemia, and acidosis (13). The ability of ammonium

salts, particularly of NH_4Cl, to produce acidosis was already known on the basis of studies performed by Pohl and Munzer (36); however, this remote effect during ammonia poisoning has been established only recently. At first it may seem strange that NH_4OH, in spite of its strong alkaline character, can produce acidosis of the blood in the same way as compounds with an acid reaction, such as NH_4Cl, $CaCl_2$, $SrCl_2$, NaH_2PO_4, or inorganic acids (e.g. HCl, etc.), since it is generally assumed that caustic alkalis cause a shift to the alkaline side in blood chemistry. The results reported above raise certain doubts as to the general validity of this assumption, especially in regard to caustic alkali poisoning; for this reason, further clarification of this question is not only of specific but also of general importance. A recognition of changes in the chemistry of the organism, caused by a caustic alkali or during poisoning with such compounds, is not only important from a pharmacodynamic and toxicological standpoint, but also allows the treatment of poisoned people in the sense of causal therapy and thus exerts a more favorable influence on their fate. On the basis of these considerations, the chemical effect of experimental sodium hydroxide poisoning became the object of our further studies, following our previous research on ammonia poisoning.

Tests were carried out on rabbits (body weight 2500-3500g) divided into three groups. Group I (16 rabbits): determination of the sugar, inorganic phosphorus and serum calcium content (total calcium) of blood. Group II (16 rabbits): inorganic phosphorus in blood and alkali reserves in serum. Group III (14 rabbits): blood serum chlorine and sodium values.

All values were determined prior to poisoning and at various times after poisoning. Calculation of the normal values was done one week prior to poisoning after a 20-24 hour fasting period. The data given here always refer to the

average value calculated from two parallel tests.

Methods of Determination. Blood sugar was determined by the micro method of Hagedorn-Jensen (18); inorganic phosphorus: Table 1, by the macro method of Fiske-Subbarow (14), Table 2, by the micro method of Kuttner-Cohen (26) in the total blood. Serum calcium was determined by the titration process (oxidimetry) of Clark and Collip (7). Alkali reserves were determined by the CO_2 capacity calculation method of Van Slyke (44). The serum chlorine content was determined according to Rusznyak and Kellner (38), and the serum sodium content according to D.M. Rourke (37). In determining the alkali reserves, blood was obtained by heart puncture, as prescribed, and a blood layer was stored under paraffin oil, while in other studies blood was taken from the ear vein.

Studies. Group I, normal values: in the blood of healthy rabbits, blood sugar 100-123 mg%; inorganic P, according to Fiske-Subbarow, 3.03-4.25 mg%; according to Kuttner-Cohen (see Group II) 3.10-5.50 mg%. Serum calcium: 14.10-18.10 mg%. Our normal values for P agree in general with those reported by Harnes (20), for Ca with those reported by Herman and Zentner (21) and Bourne and Campbell (2), as well as with those reported for P and Ca by Sjollema and Seekles (40) and by Brown and Howard (4).

Test animals in Group I received, after 1 day of fasting, a 1, 2, 3, 5 and 10% NaOH solution introduced into the stomach with a stomach probe. Each animal received 0.50, 0.75, 1.00, 1.25, 1.50, 2.25, and 2.50g NaOH; these amounts were dissolved in 12.5, 25, 50 and 75 cc water. Administration of these different NaOH doses was intended to inform us about the relationship between the degree of changes established in the blood chemistry and the volume, concentration and the amount in grams of the administered poison.

Symptoms: the animals became restless already 1-2 minutes after administration of the poison. At first, they stretched out, pressing their stomach against the floor, then suddenly jumped up, or rolled from one side to another (pain!); 5-10 minutes later, they excreted urine and droppings, rolled in their excreta and dirtied their pelt, and a greatly accelerated breathing was also observed. This condition gradually ceased after 1-1.5 hours, the animals drew their feet under their body and remained almost motionless in their cage; they moved very reluctantly when exposed to external stimuli. Breathing gradually became slow and superficial. In those animals which died after 1-2 days, a general weakness and steadily increasing loss of strength was observed already 2-3 hours after poisoning: they kept their eyes half closed and exhibited a definite staggering when they moved. This condition deteriorated visibly, breathing and heart beat became weaker, until the animals died with (or without) convulsions. In those animals which died later than after 3 days, the initial symptoms were milder, and those that survived gave a quite healthy impression already a few hours after the early light symptoms had ceased. Results of tests with Group I are given in Table 1.

From Table 1 we can see that blood sugar values always increase under the effect of NaOH poisoning. The increase in blood sugar can assume such large proportions already 20 minutes after poisoning that sometimes they are more than twice as high as the normal values (see, for example, rabbit #2). In general, the values are doubled after 1-1.5 hours (e.g., in rabbit #12), and sometimes they are 2.5 times as high as the initial values (see rabbit #3); in most cases, the increase in blood sugar is most clearly expressed 6-7 hours after poisoning. This increase can still be detected in most cases during the first day, but shows

Table 1

Changes in the Sugar, Inorganic Phosphorus and Calcium Content of Blood in Rabbits during Experimental Sodium Hydroxide Poisoning.

(1) No.	Körpergewicht g	(2) Dosis des NaOH ccm	%	(4) g	(3) Blutzucker vor der Vergiftung mg%	(5) nach der Vergiftung Min.	(6) Std.	(7) Tage	mg%	(8) Differenz %	(11) Phosphor im Gesamtblut vor der Vergiftung mg%	(5) nach der Vergiftung Min.	(6) Std.	(7) Tage	mg%	(8) Differenz %	(11) vor der Vergiftung mg	(5) nach der Vergiftung Min.	(6) Std.	(7) Tage	mg%	(8) Differenz %	(11) Lebensdauer nach der Vergiftung
1	2720	75	3	2,25	110		6	–	280	+ 155	4,00		6		10,09	+ 150	17,4		6		11,88	– 32	7 Std. hours
						–	7	–	237	+ 115			7	–	16,66	+ 317		–	7		10,92	– 37	
2	2650	25	10	2,5	105	20	–	–	228	+ 117	3,62	20	–		5,64	+ 56	16,3	20	–	–	14,38	– 12	10¼
						–	9¾	–				–	9¾	–	6,67	+ 84		–	9¾	–	–	–	
						–	–	–	–			–	10¼	–	11,42	+ 215		–	10¼	–	12,56	– 23	
3	2415	75	2	1,5	120	–	1	–	298	+ 148	3,84	–	1½	–	6,06	+ 58	17,80	–	1½		6,30	– 65	12
4	2100	25	5	1,25	110	–	1¼	–	197	+ 79	3,52	–	1¼	–	4,44	+ 26	16,7	–	1¾	–	13,93	– 17	22½
						–	–	–	–	–		–	9	–	7,27	+ 107			9	–	11,70	– 30	
						–	22½	–	80	+ 27		–	22½	–	14,28	+ 306		–	22½	–	9,72	– 42	
5	2630	75	2	1,5	123	25	–	–	149	+ 37	3,21	25	–	–	3,33	+ 4	17,17	25	–	–	10,40	– 39	26
							26	–	140	+ 14		–	26	–	20,00	+ 523		–	26	–	10,00	– 42	
6	2470	75	3	2,25	110	–	6	–	202	+ 84	3,20	–	6	–	4,94	+ 54	15,7	–	6	–	12,5	– 20	28
						–	–	1	150	+ 36		–	–	1	7,27	+ 127		–	–	1	8,91	– 43	
						–	28	–	87	+ 21		–	28	–	14,81	+ 363		–	28	–	7,83	– 50	
7	3255	75	2	1,5	120	45	–	–	130	+ 8	4,00	45	–	–	4,35	+ 9	16,53	45	–	–	13,80	– 17	28
						–	–	1	184	+ 53		–	–	1	5,78	+ 45		–	–	1	12,54	– 24	
8	2200	25	5	1,25	100	–	1½	–	138	+ 38	3,22	–	1½	–	4,70	+ 46	17,3	–	1½	–	13,75	– 21	37
						–	–	–	–	–		–	9½	–	5,56	+ 73		–	–	–	–	–	
						–	–	1	103	3		–	–	–		–		–	–	–	–	–	
9	4275	75	2	1,5	116	30	–	–	142	+ 22	3,47	30	–	–	4,00	+ 15	16,2	30	–	–	16,00	– 1	40
						–	–	1	156	+ 34		–	–	1	3,33	– 4		–	–	1	13,23	– 18	
10	2830	75	2	1,5	120	–	1	–	165	+ 38	3,03	–	1	–	4,04	+ 33	16,0	–	1	–	12,2	– 24	3½ Tage days
						–	–	1	141	+ 18		–	–	1	3,63	+ 20		–	–	1	10,58	– 34	

1 - Body weight

2 - NaOH dose

3 - Blood sugar

4 - Before poisoning

5 - After poisoning

6 - Hours

7 - Days

8 - Difference

9 - Phosphorus in total blood

10 - Serum calcium

11 - Duration of life after poisoning

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Table 1 (continued)

Changes in the Sugar, Inorganic Phosphorus and Calcium Content of Blood in Rabbits during Experimental Sodium Hydroxide Poisoning.

(1) Nr.	Körpergewicht g	(2) Dosis des NaOH ccm	%	g	(4) vor der Vergiftung mg%	(3) Blutzucker (5) nach der Vergiftung (6) Min.	(6) Std.	(7) Tage	mg%	(8) Differenz %	(4) vor der Vergiftung mg%	(9) Phosphor im Gesamtblut (5) nach der Vergiftung (6) Min.	(6) Std.	(7) Tage	mg%	(8) Differenz %	(4) vor der Vergiftung mg%	(10) Serumcalcium (5) nach der Vergiftung (6) Min.	(6) Std.	(7) Tage	mg%	(8) Differenz %	(11) Lebensdauer nach der Vergiftung
11	3160	75	1	0,75	112	45	—	—	112	—	3,40	45	—	—	4,00	+ 18	16,10	45	—	—	12,40	− 23	5 Tage
						—	—	3	132	+ 18		—	—	3	4,00	+ 18		—	—	3	18,01	+ 12	
						—	—	5	123	+ 10		—	—	5	14,80	+ 335		—	—	5	11,18	31	
12	2565	12,5	10	1,25	109	—	1½	—	218	+ 100	4,03	—	1½	—	5,79	+ 44	16,7	—	1½	—	15,61	7	9
						—	—	—	—	—		—	8½	—	5,97	+ 48		—	—	—	—		
						—	—	1	116	+ 6		—	—	—	—	—		—	—	—			
						—	—	—	—	—		—	56	—	5,55	38		—	56	—	17,01	2	
						—	—	8	69	− 37		—	—	8	8,00	99		—	—	8	13,44	20	
13	2550	12,5	10	1,25	112	—	1½	—	176	+ 57	4,00	—	1½	—	5,00	25	16,5	—	1½	—	14,47	12	14½
						—	8	—	—	—		—	8	—	6,67	+ 67		—	—	—	—	—	
						—	—	1	89	− 21		—	—	—	—	—		—	—	—			
						—	—	—	—	—		—	56	—	5,47	+ 37		—	56	—	15,5	6	
						—	—	14	74	− 34		—	—	14	8,00	+ 100		—	—	14	13,53	18	
						—	—	14½	49	64		—		14½	15,38	+ 285				14½	12,62	24	
14	2350	50	2	1	118	—	1¾	—	127	+ 8	4,25	—	1¾	—	5,52	+ 31	17,80	—					
						—	29	—	143	+ 26		—	—	—	—	—		—	—				
						—	—	3½	112	− 5		—	—	3½	5,71	+ 34				3½	13,53	24	
						—	—	9	112	− 5		—	—	9	4,21	—				9	13,31	25	
						—	—	15	115			—	—	15	4,23	—		—	—	15	17,20		
15	3560	75	1	0,75	107	—	1¼		107	—	4,08		1¼	—	4,34	+ 6	14,10		1¼		12,00	15	
						—		3	147	+ 37		—		3	5,00	+ 23				3	15,09	7	
						—		10	96	− 10		—		10	5,00	+ 23		—		10	14,20	—	
								15	105					15	4,00					15	14,20		
16	3100	50	1	0,5	100		1		98	− 2	3,47	—	1	—	4,08	+ 18	18,10	—	1		13,40	26	
						—	—	3	145	+ 45		—	—	3	4,00	+ 15		—		3	16,26	10	
						—	—	10	121	+ 21		—	—	10	4,44	+ 28		—		10	19,88	10	
						—	—	15	102	—		—	—	15	3,50				—	15	18,20	—	

days

survived

1 - Body weight

2 - NaOH dose

3 - Blood sugar

4 - Before poisoning

5 - After poisoning

6 - Hours

7 - Days

8 - Difference

9 - Phosphorus in total blood

10 - Serum calcium

11 - Duration of life after poisoning

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a clearly decreasing tendency, as compared to the initial rapid rise, so that in some cases (e.g., in rabbits #4, 6, 12 and 13) lower values than those prior to poisoning are found shortly before death. Since the animals hardly eat (or do not eat at all) as a result of poisoning, the hypoglycemia observed several days after poisoning must be considered as a hunger symptom. In general, the greatest rise in blood sugar values was recorded in those cases (rabbits #1, 2, 3, 6 and 12) where NaOH was given in large amounts or in high concentration. A 2% NaOH solution can also produce a marked hyperglycemia (rabbit #3) when sufficiently large amounts of NaOH reach the stomach with this solution. The increasing effect of a 1% NaOH solution on the blood sugar level is, however, quite small. The increasing effect of NaOH poisoning on the blood sugar levels thus depends on the amount (in g) of administered NaOH and on the concentration of the solution used (in %), but does not appear to depend on the solvent (water) or the volume (cc).

The inorganic phosphorus content of the blood is higher in every case of NaOH poisoning, particularly when the outcome is fatal. This increase can, in exceptional cases, amount to 56% already 20 minutes after poisoning (e.g., in rabbit #2: from 3.62 mg% to 5.64 mg%); in the great majority of cases, however, even in those with a fatal outcome, a noticeable increase in the inorganic P content of blood was noted only in the second hour after poisoning. In most cases, the organic P content of the blood shows an increase of 25-60%, in comparison to normal values, 1-1.5 hours after poisoning (rabbits #3, 4, 8, 10, 12 and 13), and a 50-150% increase after 6-10 hours (rabbits #1, 2, 4, 6, 8, 12 and 13). The P content then continues to increase until death sets in and can exhibit an increase of up to 200-500% over the initial value. Our results show that in all those cases where the P content had increased by 50% or more over

the initial value not later than 10 hours after poisoning, the latter was always fatal. The more rapidly the P content increased and the higher was the level of this increase, the more quickly did the test animal die. We can therefore draw the following conclusion: the rapid (within 10 hours after poisoning) increase of inorganic P in the blood by at least 50% of the normal value indicates a severe poisoning and means a poor prognosis. In rabbits #14, 15 and 16, this increase amounted to only 34 and 28% and normal P values were gradually re-established: these animals remained alive. An increase of blood phosphorus of less than 50% does not allow us to draw the conclusion that the animals will stay alive, since rabbits #7, 9, 10 and 11, in which only a moderate or slight increase in the P content of the blood was observed (45, 15, 33 and 18%, respectively) still finally died from poisoning. In the case of rabbit #11, an increase of the P content (335%) took place suddenly immediately before the death of the animal; probably, this was also the case in rabbits #7, 9 and 10, although we were unfortunately unable to observe these animals shortly before their death, since the latter occurred at night when no observer was present. The effect of the concentration of the NaOH solution used exerted an influence on the increase of the P content to the extent that the latter was generally greater in those cases where an NaOH solution of higher concentration was administered. However, exceptions were also noted here; for example, in rabbit #5, in which a very sharp increase (523%) of the P content was noted after administration of a 2% NaOH solution. In those cases where the P content increased slightly, an NaOH solution with a concentration greater than 1-2% was never used.

The serum calcium content was reduced in every case. Already 20, 25 and 45 minutes after poisoning, the serum calcium content was 12, 39 and 23% lower than the initial value (rabbits #2, 5 and 11). The calcium content of serum

continued to decrease in the next few hours: the lowest figures were found at the onset of death. The greatest decrease amounted to 65% of the initial value (rabbit #3), followed by a 50% decrease in rabbit #6; in the remaining cases with a fatal outcome, an average decrease of 20-40% in the Ca content was noted. In those rabbits which survived (#14, 15 and 16), the greatest decrease in Ca content observed was equal to 15-26%, and initial Ca values were gradually restored in these animals after several days. In those cases where the decrease in serum calcium occurred to a greater extent and more rapidly, the animals generally died more quickly. We could not establish any relationship between the degree of Ca decrease and the concentration and volume of the NaOH solution used; the degree of Ca decrease appears to be influenced primarily by the amount (in g) of NaOH used.

We can draw the following conclusions from the test results shown in Table 1; after experimental NaOH poisoning, an increase in blood sugar (hyperglycemia), lasting about 1 day, takes place, followed by a drop in blood sugar content (hunger hypoglycemia). Further, a gradual increase of inorganic total blood phosphorus content is observed, accompanied by a simultaneous steady decrease of the serum calcium content.

Group II (16 rabbits). At different times after NaOH poisoning, the content of inorganic phosphorus in the blood and the alkali reserves were investigated. In order to spare the animals' blood, phosphorus determination in this group was carried out with 0.2 cc total blood by the micro method of Kuttner-Cohen (26). By this method we obtained initial (normal) values of 3.10-5.50 mg%, similar to those obtained by the Fiske-Subbarow method. Values of the alkali reserves prior to poisoning (normal values) were determined by the Van Slyke method; they varied from 59.12 to 54.42 vol.%, and thus almost agreed with the

original data (63-46 vol.%) of Van Slyke (44) and with the normal values (77-53 vol.%) obtained by Volhard (46), calculated by Van Slyke's method and referring to man, as well as with the figures (69.1-54.3) reported by Hollo' and Weiss (22).

Animals in this group received NaOH in the form of a 2, 3 and 5% solution, namely 0.41, 0.47, 0.50 and 0.75g NaOH per kg body weight. Results of these tests are shown in Table 2.

From the data listed in Table 2 we can see that also in these cases, similarly to animals of Group I, the inorganic blood phosphorus content gradually increases after poisoning, reaching its highest level at the time of death (see rabbits #1-14). Among the fatal cases, the highest figure (264%) of the initial value was recorded for rabbit #13, and the lowest value was 30%. In the animals which survived poisoning, a gradual drop of the P values was noted after a temporary increase (37-54%). Simultaneously with an increase in P values a greater or weaker decrease (drop) of alkali reserve values was noted in every case without any exceptions. We want to emphasize here the fact that in no case did an increase of alkali reserves take place. A moderate drop of these values occurred in two cases (#9 and 13) already 25-45 minutes after poisoning; this drop amounted to 23% of the initial value (from 56.4 to 43.3 vol.% and from 58.25 to 44.3 vol.%). In one case (rabbit #1), a 57% decrease of alkali reserves in 1-3/4 hour was noted (from 56.2 to 24.2 vol.%). In cases with a fatal outcome, we were able to observe an average decrease of 30-60%, as compared to the initial values. In those cases where we had the opportunity to make several determinations, a certain amount of variation was recorded. In the course of the same case, after a clear initial drop of the alkali reserves, a slight increase, followed again by a drop, could be noted; in the case of such variations, however, the initial value was never reached again. In cases with no fatal outcome, the decrease in alkali

Table 1

Changes in the Inorganic Phosphorus Content of the Blood and in Alkali Reserves of the Blood Serum of Rabbits during Experimental Sodium Hydroxide Poisoning.

Nr. No.	(1) Körpergewicht g	(2) Dosis des NaOH ccm	%	g	(3) auf 1 kg Körpergewicht	(4) Vor der Vergiftung mg %	(5) Phosphor im Gesamtblut (6) nach der Vergiftung Min.	Std. (7)	Tage (8)	mg %	(9) Differenz %	(10) Alkalireserve des Blutserums (4) Vor der Vergiftung Vol. %	(6) nach der Vergiftung Min.	Std. (7)	Tage (8)	Vol %	(9) Differenz	(11) Lebensdauer nach der Vergiftung
1	2790	73	3	2,19	0,75	3,1		1¾		5,05	+ 63	56,2	—	1¾		24,2	57	2½ Std. hours
2	2700	67,5	3	2,03	0,75	4,63	10	—	—	5,88	+ 27	54,92	—	—	—	—	—	7½ „
								7	—	5,99	+ 29		—	7	—	34,7	— 37	
3	2600	65	3	1,95	0,75	4,34	—	6	—	5,00	+ 15	57,2	—	6	—	39,5	— 31	12 „
4	3800	95	3	2,85	0,75	3,57		14		6,88	+ 93	58,5		14	—	39,34	— 33	15 „
							—	15	—	11,63	+ 226		—	15	—	31,77	— 46	
5	2920	73	3	2,19	0,75	5,5	—	15		16,13	+ 193	57,8	—	15	—	22,21	— 62	15 „
6	3130	78,3	3	2,35	0,75	4,5	—	16		9,8	+ 118	57,8	—	16	—	29,00	— 50	16 „
7	3200	80	3	2,40	0,75	5,3	—	14	—	6,9	+ 30	57,2		14	—	47,9	— 16	18 „
8	3040	45	5	2,25	0,75	5,00	15		—	5,55	+ 11	57,65	—	—	—	—	—	23 „
							—	7	—	7,14	+ 43		—	7	—	35,7	— 30	
							—	23	—	12,82	+ 156		—	23	—	42,4	— 26	
9	2980	74,5	3	2,23	0,75	4,76	45		—	—	—	56,4	45	—	—	43,3	— 23	24½ „
								7½	—	4,77	—		—	7½	—	49,00	— 13	
							—	24	—	8,34	+ 75		—	24	—	33,8	— 40	
10	2835	71	3	2,13	0,75	4,16	—	6	—	6,25	+ 50	56,5	—	6	—	45,3	— 20	27½ „
							—	21½	—	5,88	+ 41		—	21½	—	52,8	— 7	
							—	27½	—	12,82	+ 208		—	27½	—	34,6	— 39	
11	2650	45	3	1,35	0,50	5,00	30	—	—	5,00	—	57,26	30	—	—	55,7	— 3	28 „
							—	7	—	5,88	+ 18		—	—	—	—	—	
								23	—	7,69	+ 54			23	—	41,4	— 28	
							—	28	—	17,80	+ 256			28	—	24,2	58	

1 - Body weight
2 - NaOH dose
3 - per kg body weight
4 - Before poisoning
5 - Phosphorus in total blood
6 - After poisoning
7 - Hours
8 - Days
9 - Difference
10 - Blood serum alkali reserves
11 - Duration of life after poisoning

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Table 2 (continued)

Changes in the Inorganic Phosphorus Content of the Blood and in Alkali Reserves of the Blood Serum of Rabbits during Experimental Sodium Hydroxide Poisoning.

Nr. / No.	1 Körpergewicht g	2 Dosis des NaOH ccm	%	g	3 auf 1 kg Körpergewicht	5 Phosphor im Gesamtblut: 4 vor der Vergiftung mg%	6 nach der Vergiftung: Min.	Std.	Tage	mg%	9 Differenz %	10 Alkalireserve des Blutserums: 4 vor der Vergiftung Vol.%	6 nach der Vergiftung: Min.	Std.	Tage	Vol.%	9 Differenz %	11 Lebensdauer nach der Vergiftung
12	3600	100	2	2	0,56	3.25	—	1¾	—	4,00	+ 23	56,47	—		—	—	—	35 Std. (hours)
							—	7½	—	5,88	+ 81		—	7½	—	45 16	— 20	
							—	29	—	8,10	+ 149		—	29	—	28,88	— 49	
13	2550	43	3	1,28	0,50	5.5	25	—	—	5,95	8	58,25	25	—	—	44.3	— 24	3 Tage (days) 1 Std. (hour)
							—	7	—	6,25	+ 14		—	—	—	—		
								—	1	6,62	+ 20		—		1	54,8	6	
							—	—	2	7,14	+ 30		—	—	—	—	—	
							—	—	3	7,81	+ 42		—	—	3	44,71	23	
							—	1	3	20,00	+ 264		—	1	3	26.75	— 54	
14	3200	75	2	1,5	0,47	4,52	—	1	—	4.76	+ 5	56.8	—	1		48.5	— 15	12 Tage (days)
							—	42	—	7,58	+ 68		—	42	—	55.7	2	
							—	—	3	6.10	+ 35		—	—		—		
								—	4	5.55	+ 23		—	—	—			
							—	—	7	4.85	+ 7		—	—				
								—	10	8,34	+ 85		—		10	31.3	44	
15	2900	75	2	1,5	0.47	4.35	—	1	—	4,76	+ 9	54,52	—					am Leben geblieben (survived)
							—	42	—	5,82	+ 34			42		44 3	19	
							—	—	3	5,27	+ 21							
									4	5,95	+ 37		—				—	
							—	—	7	4.76	+ 9					—		
							—		10	5.00	+ 15				10	57.5	6	
16	2460	50	2	1	0,41	4,85	—	1	—	7.46	54	59,12						am Leben geblieben
							—	43	—	7,35	+ 52		—	43	—	58,6	1	
							—		3	6,25	+ 29			—				
							—		4	6,85	+ 41			—		—		
							—		7	5,95	+ 23		—					
									10	6,25	+ 29				10	58.2	2	

1 - Body weight
2 - NaOH dose
3 - per kg body weight
4 - Before poisoning
5 - Phosphorus in total blood
6 - After poisoning
7 - Hours
8 - Days
9 - Difference
10 - Blood serum alkali reserves
11 - Duration of life after poisoning

071

No.	(1) Körpergewicht g	(2) Dosis des NaOH ccm % g	(3) Blutzucker (4) vor der Vergiftung mg%	(5) nach der Vergiftung Min.	(6) Std.	(7) Tage mg%	(8) Differenz %	Phosphor im Gesamtblut (9) (4) vor der Vergiftung mg%	(5) nach der Vergiftung Min.	(6) Std.	(7) Tage mg	(8) Differenz %	(4) vor der Vergiftung mg%	Serumcalcium (10) (5) nach der Vergiftung Min.	(6) Std.	(7) Tage mg	(9) Differenz %	(11) Leber nach der Vergiftung

reserve values did not reach any greater degree. Although no numerical relationship between the increase of inorganic blood phosphorus content and the drop in alkali reserve values could be established, we can still say that a greater P increase corresponds in most cases to a clear decrease of alkali reserve values. Those animals which received 0.75g NaOH/kg body weight exhibited more severe injuries and died earlier than those which received 0.50g/kg body weight or less. The same observation also applies to the stronger or weaker concentration of the NaOH solution.

From the data given in Table 2 we can therefore conclude that, during experimental NaOH poisoning, a decrease of serum alkali reserves takes place along with a gradual increase of inorganic blood phosphorus, i.e. blood chemistry exhibits a shift in the acid direction.

Group III (14 rabbits): determination of the chlorine and sodium content of blood serum at different times after poisoning.

Normal values: our investigations showed that healthy rabbits had a serum chlorine content of 363-418 mg%. Reported average values for serum Cl content in healthy rabbits are 363 mg%, according to Denis (8), and 365 mg%, according to Glass (17). Our average is somewhat higher, namely 384 mg%. Corresponding studies carried out on man and dogs by different authors allow us to conclude that the blood serum chlorine content is subject to certain individual variations, even under the same conditions. Thus, for example, Laudat and Grandsire (30) found 363-380 mg% and Manzini (33) 342-390 mg% in man, while in dogs Manzini found 379-396 mg% and Parhon and Derevici (35) 384-410 mg%.

In our studies, the sodium content of the blood serum of healthy rabbits varied from 334 to 408 mg%, with an average value of 370 mg%. The average value reported by Denis for rabbits is 355 mg%. Also here the average value found by

us is somewhat higher, and this is probably connected with the higher chlorine values. As is generally known, both Cl and Na are present in blood mostly in the form of NaCl. Under normal conditions, therefore, a higher Na value will always correspond to a higher Cl value. The following serum Na values were found in studies performed on human subjects: Laudat and Grandsire 334-346 mg%, McVicar and H. Ross (45) 365 mg%, Manzini (33) (humans and dogs) 335-340 mg%.

For poisoning purposes, animals of Group III received 0.75g NaOH (rabbits #1 and 12) and 0.50g NaOH/kg body weight (rabbits #13 and 14), introduced into the stomach as a 2-3% NaOH solution after a 1-day fasting. Results of these tests are given in Table 3.

From Table 3 we can see that the serum Cl content shows only a slight decrease (7-8%) in the first few hours (1-4 hours) after NaOH poisoning, even in cases with a fatal outcome. Later, this decrease gradually becomes more marked, but was not always particularly great even in severe cases of poisoning which resulted in the death of the animals after 1-2 days. The most marked decrease in the serum Cl content was observed in rabbit #8; here, the Cl content was 33% lower than the initial value (from 390 to 262 mg%) already 7 hours after poisoning. In rabbit #1, the decrease amounted to 22%, in rabbit #4, 16%, and in rabbit #7, 19% in 20 hours. Amount the other cases with a fatal outcome, a decrease of 10% was noted in 3 cases (rabbits #5, 9 and 11), while in 5 other cases (rabbits #2, 3, 6, 10 and 12) this decrease was less than 10%. In those cases where the animals survived, this decrease was so slight that the values recorded did not exceed the limits of possible error sources.

Along with a decrease of the chlorine content an increase of the blood sodium content was noted, which gradually rose until death in cases with a fatal outcome. In those animals which survived (rabbits #13 and 14) the initial rise in the Na

Table 3

Changes in the Chlorine and Sodium Content of Blood Serum during Experimental Sodium Hydroxide Poisoning.

No.	(1) Körpergewicht g	(2) Dosis des NaOH ccm	%	g	(3) auf 1 kg Körpergewicht	(4) Vor der Vergiftung mg%	(5) Serumchlor nach der Vergiftung (6) Min.	Std. (7)	Tage (8)	mg%	(9) Differenz %	(4) Vor der Vergiftung mg%	(10) Serumnatrium nach der Vergiftung (6) Min.	Std. (7)	Tage (8)	mg%	(9) Differenz	(11) Lebensdauer nach der Vergiftung
1	2755	69	3	2,06	0,75	418	25	7	—	399 326	− 7 − 22	376	25	7		412 442	+ 10 + 17	7 Std. hours
2	2275	57	3	1,70	0,75	376	30 20	7 7	— —	372 369 344	− 1 − 2 − 8	334	30 20	7 7		412 410 422	+ 23 + 22 + 27	7 Std. + 20 Min.
3	2710	67½	3	2,02	0,75	383	—	4	—	376	− 2	357		4		391	+ 9	12 Std.
4	2685	67	3	2,01	0,75	404	20	— 7¼	— —	376 337	− 7 − 16	357	20	7¼		420 416	+ 18 + 17	15 "
5	2730	68	3	2,04	0,75	365	—	14 16½	—	358 327	− 2 − 11	380		14 16½		381 421	+ 11	16½ "
6	2770	69	3	2,07	0,75	377	— —	1½ 7½ 22	— —	347 355 345	− 8 − 6 − 8	382		1½ 7½ 22		394 426 449	+ 3 + 11 + 17	22 "
7	3065	76,6	3	2,29	0,75	376	— — —	4 20 22½	— — —	369 305 305	− 2 − 19 − 19	373		4 20 22½		391 382 393	+ 5 + 2 + 5	22½ "
8	2900	72½	3	2,17	0,75	390	10 + — —	1 7 —	— — 1	340 262 262	− 10 − 33 − 33	408	10 +	1 7	1	418 429 432	+ 3 + 5 + 6	29 "
9	3400	85	3	2,55	0,75	370	10 —	1 7	— —	365 340 333	− 1 − 8 − 10	374	10 +	1 7	1	414 338 438	+ 11 + 6 + 17	29 "

1 - Body weight
2 - NaOH dose
3 - per kg body weight
4 - Before poisoning
5 - Serum chlorine
6 - After poisoning
7 - Hours
8 - Days
9 - Difference
10 - Serum sodium
11 - Duration of life after poisoning

Table 3 (continued

Changes in the Chlorine and Sodium Content of Blood Serum
during Experimental Sodium Hydroxide Poisoning

Nr / No.	(1) Körpergewicht g	(2) Dosis des NaOH ccm	%	g	(3) auf 1 kg Körpergewicht	(4) vor der Vergiftung mg%	(5) Serumchlor nach der Vergiftung (6) Min.	Std. (7)	Tage (8)	mg%	(9) Differenz %	(4) vor der Vergiftung mg%	Serumnatrium (10) nach der Vergiftung (6) Min.	Std. (7)	Tage (8)	mg%	(9) Differenz %	(11) Lebensdauer nach der Vergiftung
10	3125	78	3	2.34	0.75	364	—	14	—	390	+ 7	356		14		368	+ 3	30 Std. — hours
							—	18½	—	377	+ 4			18½		366	+ 3	
							—	1 +	1	340	— 6			1	1	394	+ 10	
11	3055	76	3	2.28	0.75	388	—	4	—	363	— 7	379		4		382	+ 1	35 „
							—	20	—	310	— 13			20		439	+ 16	
							—	30	—	347	— 11			30		456	+ 20	
12	2660	66½	3	1.99	0.75	365	—	14		383	+ 5	365		14		382	+ 5	2½ Tage — days
							—	18½	—	369	+ 1			18½		409	+ 12	
							—	1	1	340	— 7			1	1	392	+ 8	
13	2500	62	2	1.25	0.50	372	—	4	—	376	+ 1	362		4		418	+ 15	am Leben geblieben — Survived
							—	—	1	355	— 5				1	393	8	
								—	3	372					3	389	+ 7	
14	2700	67	2	1.35	0.50	363		7	—	363	—	374		7		407	+ 9	
								—	1	347	— 4				1	389	+ 4	
								—	3	376	3				3	384	+ 3	

1 - Body weight
2 - NaOH dose
3 - per kg body weight
4 - Before poisoning
5 - Serum chlorine
6 - After poisoning
7 - Hours
8 - Days
9 - Difference
10 - Serum sodium
11 - Duration of life after poisoning

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content gradually dropped down again. The increase of the Na content was slow in most cases, but could also take place very rapidly, as, for example, in rabbits #1, 2 and 4, in which the increase amounted to 18, 10 and 23% of the initial value already 20, 25 and 30 minutes after poisoning. The greatest increase was noted in rabbit #2, where it reached 27% of the initial value (from 334 to 422 mg% (rabbits #1, 4, 6, 9, 11 and 13), in 5 cases (rabbits #3, 5, 10, 12 and 14) it was equal to 9-12%, and in 2 animals (rabbits #7, 8) it was merely 5-6%. Noteworthy is the fact that the greatest decrease of the Cl values was recorded together with the smallest increase of the Na content (rabbits #7, 8), whereas the greatest increase of the Na content was accompanied by a particularly small Cl decrease (rabbit #2). A moderate Cl decrease with a greater Na increase was noted in rabbits 6, 9 and 11, a greater Na increase (+) together with a greater Cl decrease (-) in rabbits #1 and 4, and an almost equal degree of Na increase and Cl decrease was noted in animals #5, 10 and 12.

The shifts in Na and Cl values mentioned here were more marked in cases with a fatal outcome, which may be due to the fact that these animals received NaOH solutions of higher concentration (3%) and larger amounts (0.75g) NaOH per kg body weight than the survivors (2% NaOH solution and 0.50 g/kg body weight).

The results listed in Table 3 thus lead to the conclusion that a mostly moderate and seldom great, but always detectable, decrease of the serum chlorine content, as well as an increase of the serum sodium content of similar degree can be observed during experimental NaOH poisoning. Since a shift of the serum Cl and serum Na contents in an opposite direction (±) takes place, the noticeable change in the relationship between Cl and Na ions signifies that a noteworthy equilibrium disturbance occurs in the mineral metabolism of the organism.

Our tests thus demonstrate that acute NaOH poisoning does not merely cause a local caustic burn, in contrast to previously held concepts, but also leads to

a chemical shift in the organism; this general toxic effect has so far hardly been considered or has even been completely overlooked. The general pathological changes are expressed in the form of a disruption of sugar metabolism (hyperglycemia), a disruption of the blood ionic equilibrium (hyperphospatemia, hypocalcemia, hypochloremia, hypersodioemia) and in a reduction of alkali reserves (CO_2 capacity), namely as symptoms characteristic for acidosis.

The results of our tests are in agreement with all the data (available to us) reported by other authors, referring to findings obtained from humans suffering from caustic alkali poisoning or similar animal experiments.

Thus, Lowy (32) found higher blood sugar values in humans subject to caustic alkali poisoning; the reason for this is considered to be the resorption of cellular protein caused by tissue destruction, since hyperglycemia can also take place after parenteral administration of different types of protein. In man, Labbe, Nepveux and Rohacek (28) found hyperglycemia following peroral administration of sodium bicarbonate, whereas hypoglycemia was noted by Underhill et al. (43). In animal experiments, Kusaka (27) observed a hyperglycemia after intravenous injection into rabbits of a strong (pH = 1.5-10.08) carbonate-bicarbonate mixture, whereas hypoglycemia was noted after administration of a weaker mixture (pH = 8.35). The apparent contradiction between the various results obtained can most likely be explained by the difference in the amounts and concentrations of caustic alkalis used, and also by the different times at which blood samples were taken.

J. Gaizler (16) was able to detect in children (1923) and adults (1924) poisoned by caustic alkalis an acetonuria, which was the more severe the greater the tissue destruction caused by the alkali. Gaizler believed that this acetonuria was a consequence of the effect exerted by protein degradation pro-

ducts formed during tissue destruction. As proof of this assumption, Gaizler reported the fact that the urine nitrogen content was greatly increased in patients suffering from alkali poisoning and that the plasma colloid lability reaction (Gerloczy) was strongly positive, indicating extensive protein breakdown in the organism. In some cases, the urine also gave a strongly positive butyric acid reaction. Since acetonuria is a sign of acidosis, Gaizler assumed, on the basis of these findings, that "in severe cases of poisoning, the cause of death must be closely connected with acidosis."

The correctness of this concept is supported not only by our results, but also by the results of tests performed by other authors. Thus, Alpern and Sorkin (1) studied (1925) the effect on dogs exerted by acids and alkalis on the action of adrenaline, and found a decrease of the blood alkali reserves after intravenous injection of a ln-NaOH solution; they assumed that the cause was an excessive compensation reaction of the organism, which they attributed to central reasons (causes). Suzuki (41) administered NaOH per os to rabbits (1926) and also found a decrease (drop) of the blood alkali reserves, which is a sign of acidosis. At the same time, however, he observed a shift of the pH into an alkaline direction; he explained this curious phenomenon with the aid of Haldane's concept of "paradoxal anoxemia." This concept states that oxyhemoglobin is more stable in an alkaline medium, thereby making the CO_2 and O_2 exchange between tissues and blood more difficult, and that larger amounts of organic acids are formed as a result of the poor oxygen supply to tissues in the intermediate metabolism. In our tests, the pH values were not studied on a regular basis, and we are therefore unable to express an opinion on this part of the question. Sekoulitch (39) also found (1930) a lowering of alkali reserves, together with actonuria, in human patients suffering from alkali poisoning.

Literature data, as well as our results, show therefore that acidosis occurs both during NH_4OH and NaOH poisoning, and in general under the effect of certain salts.

Reports which could provide some information on the blood content of inorganic P, Ca, Cl and Na during NaOH poisoning could not be found in the literature available to us, although knowledge about the behavior of these ions, especially from the standpoint of acid-base equilibrium, would be of considerable importance. As is generally known, these blood components always exhibit constant values under normal conditions and play an extremely important role in the maintenance of the acid-base equilibrium. Any change in the ratio of these components results in a disruption of the acid-base equilibrium.

Numerous reports can be found in the literature which indicate that changes in the blood chemistry occur during acidosis caused by various acids and salts; these changes are similar to those observed during our previous study of NH_4OH poisoning and also during the study of NaOH poisoning described here. Thus, for example, hyperglycemia arises during peroral and intravenous administration of various mineral or organic acids [Elias (9), Lesser (31)]. After administration of lactic acid to dogs, a decrease of the serum calcium content [Floris (15)] and of the blood chlorine content [Van Caulaert, Petrequin and Bauer (6)] was noted. The chlorine content of blood in dogs also drops after uric acid injections [Capani (5)]. After intravenous injection of mono- and trisodium phosphate [Zamorani (47)] and of sodium glycerophosphate [Boyd, Hines and Stearns (3)] an increase of P and a decrease of Ca in the serum of dogs and rabbits could be observed. A decrease of alkali reserves and of the blood chlorine content was observed during sublimate (mercuric chloride) poisoning in man [E. Muntwyler, Ch.T. Way and E. Pomerene (34)] and in dogs [Trusler, Fisher and Richardson (42)], as well as during poisoning with hydrargyrum oxycyanatum [Laudan, Glass and Beiles (29)].

The nature of the acidosis caused by NH_4Cl is explained by Pohl and Munzer (36) and also by Haldane (10) as being due to the fact that hydrochloric acid is formed by NH_4Cl and CO_2 in the blood, according to the equation:

$$2NH_4Cl + CO_2 = CO(NH_2)_2 + 2HCl + H_2O;$$

this process causes a shift in the acid direction. This explanation appears to be appropriate in the case of acidosis caused by Cl-containing salts and acids, but is not sufficient to explain the acidosis produced during NH_4OH and NaOH poisoning, since in this case no chlorine is supplied to the organism. One could, however, assume here that the chlorine required for the formation of hydrochloric acid is provided by blood or other organs (for example, skin); in this case, however, an increase (rise) of the serum Cl content would be expected. As was mentioned several times above, however, a decrease (drop) of the serum Cl content is observed during NaOH poisoning; in such cases, therefore, another explanation must be sought for the arising of acidosis. As was already mentioned above, other researchers believe that acidosis during NaOH poisoning is caused primarily by protein breakdown products (Gaizler, Sekoulitch) and(or) large amounts of organic acids formed as a result of poor CO_2 supply (Suzuki), and even by processes occurring in the nervous system. Although these concepts may eventually appear to be justified, we still believe, on the basis of our results, that we can assume that the disruption of the equilibrium of phosphorus, calcium, chlorine and sodium blood components plays a predominant role in the acidosis occurring during NaOH poisoning; acid phosphates must obviously be mentioned here in the first place. Proof of this fact is the considerable increase in the inorganic P content of blood, the bulk of which most probably comes from the red blood corpuscles. It is known that rather large amounts of red blood corpuscles are destroyed at the site of the local action of the alkali (stomach), and the alkali entering the

blood system by way of resorption also exerts an hemolytic effect. In this manner, the phosphates of red blood corpuscles are set free and contribute to a great extent to the shift of blood chemistry in an acid direction. At the same time, the part played by injury of other organs should naturally also not be overlooked.

After a joint appraisal of our test results and of the literature information, we therefore come to the conclusion that, in alkali poisoning, not only the local and caustic effect of the alkali must be taken into account, but also severe (extensive) changes in the chemistry of the organism. These changes lead, by way of severe disturbances of the sugar metabolism and ionic equilibrium, to the appearance of acidosis, which can cause irreparable damage in different organs and thus result in death.

Summary

Summary

During experimental NaOH poisoning of rabbits, the following changes in blood chemistry were noted:

1. Disruption of the sugar metabolism in the form of hyperglycemia, which sometimes changed over to hypoglycemia (hunger!) in animals that survived poisoning by more than 1 day.

2. Extensive increase of inorganic phosphorus in whole blood.

3. Decrease of serum calcium

4. Moderate decrease of serum chlorine

5. Slight decrease of serum sodium

6. Marked acidosis in the form of a decreased serum alkali reserve (CO_2-capacity).

From these results we can see that, in cases of alkali poisoning, not only the local and caustic effect of the alkali must be taken into account, but also severe damage in the chemical system of the organism.

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Translated by A. Schidlovlty

(JITCO)
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Aus dem Institut für gerichtliche Medizin der kgl. ung. Franz-Joseph-Universität in Szeged, Ungarn.
(Direktor: o. ö. Prof. Dr. J. v. Baló.)

Die Veränderungen des Blutchemismus bei experimenteller Laugenvergiftung.

Von

Dr. I. Gy. v. Fazekas, Adjunkt.

(Eingegangen am 2. Dezember 1936.)

In den verschiedenen Toxikologien wird über die Laugenvergiftungen meistens gesagt, daß neben der örtlichen, ätzenden Wirkung der Laugen die Fernwirkung dieser Gifte vollkommen in den Hintergrund gedrängt werde. In bisher erschienenen Mitteilungen [Jankovich-Incze-Fazekas (23), Jankovich-Incze (24)] konnten wir auf Grund der in den verschiedensten Organen — Leber, Milz, Gehirn usw. — gefundenen histologischen Veränderungen (an menschlichem Material) auf die bis dahin kaum oder gar nicht beachtete Fernwirkung der Natronlaugenvergiftung hinweisen. In späteren Veröffentlichungen berichteten wir über die histologischen Veränderungen — insbesondere des Gehirns — bei der Vergiftung mit einer anderen Lauge, dem Ammoniumhydroxyd, die sowohl bei an dieser Vergiftung verstorbenen Menschen (10, 25) wie auch im Tierversuch (11) gefunden waren. Durch diese Ergebnisse sahen wir uns veranlaßt, die begonnene Forschung fortzusetzen; wir wollten die Wirkung der Vergiftung mit diesen Stoffen (NH_4OH, NaOH) auf den lebenden Organismus auf chemischem Wege klären. Meine Versuche an Kaninchen führten zu dem Ergebnis, daß es bei der akuten NH_4OH-Vergiftung zu schweren Veränderungen des Blutchemismus kommt: Lipämie (12), Hyperglykämie, Hyperphosphatämie, Hypocalcämie, Azidose (13). Die Fähigkeit der Ammoniumsalze, insbesondere des NH_4Cl, Azidose zu erzeugen, war durch die Untersuchungen von Pohl und Münzer (36) auch bisher bekannt, diese Fernwirkung der NH_4OH-Vergiftung lernte man aber erst neuerdings kennen. Auf den ersten Blick muß es befremdend erscheinen, daß NH_4OH trotz seines stark alkalischen Charakters im Blute ebenso eine Azidose erzeugen solle wie die Verbindungen mit saurer Reaktion (z. B. NH_4Cl, $NiCl_2$, $BiCl_3$, NaH_2PO_4) oder die anorganischen Säuren (z. B. HCl usw.); herrscht doch in bezug auf die Laugen allgemein die Auffassung, daß durch diese der Chemismus eine Verschiebung nach der alkalischen Seite erleide. Die oben erwähnten Ergebnisse lassen jedoch gegen die allgemeine Gültigkeit dieser Auffassung — insbesondere im Sinne der Laugenvergiftungen — gewisse Zweifel wach werden; die weitere Klärung dieser Frage hat daher nicht bloß besondere, sondern auch allgemeine Bedeutung. Die Erkenntnis der Veränderungen im Chemismus des Organismus, die

durch Laugen bzw. durch die Vergiftung mit diesen hervorgerufen werden, ist nicht nur in pharmakodynamischer und toxikologischer Hinsicht wichtig, sondern gestattet auch, die Vergifteten im Sinne der Kausaltherapie zu behandeln und somit ihr Schicksal günstiger zu beeinflussen. Auf Grund dieser Überlegung machten wir nun — nach den Forschungen über die NH_4OH-Vergiftung — die chemische Wirkung der experimentellen NaOH-Vergiftung zum Gegenstand unserer weiteren Untersuchungen.

Die Versuche wurden an Kaninchen von 2500—3500 g Körpergewicht in drei Gruppen ausgeführt.

Gruppe I (10 Tiere). Bestimmung des Zuckers, anorganischen Phosphors und des Serumcalciumgehalts (Gesamtcalcium) im Blute.

Gruppe II (16 Tiere). Anorganischer Phosphor im Blute und Alkalireserve im Serum.

Gruppe III (11 Tiere). Serumchlor- und Serumnatrium-Werte.

Sämtliche Werte wurden vor und zu verschiedenen Zeiten nach der Vergiftung bestimmt. Die Berechnung der Normalwerte erfolgte stets eine Woche vor der Vergiftung nach 20—24 Stunden langem Fasten. Die einzelnen Angaben beziehen sich stets auf den aus zwei parallel ausgeführten Untersuchungen berechneten Mittelwert.

Untersuchungsverfahren.

Blutzucker mit Hilfe des Mikroverfahrens nach Hagedorn-Jensen (18); anorganischer Phosphor, Tabelle 1: Makroverfahren nach Fiske-Subbarow (14). Tabelle 2: Mikroverfahren nach Kuttner-Cohen (26) im Gesamtblut. Serumcalcium: Titrationsverfahren (Oxydimetrie) nach Clark und Collip (7). Alkalireserve mit Hilfe der CO_2-Kapazitätsberechnung nach Van Slyke (11). Serumchlor nach Rusznyák und Kellner (38), Serumnatrium nach D. M. Rourke (37). Zur Bestimmung der Alkalireserve wurde das Blut laut Vorschrift mittels Herzpunktion gewonnen und unter Paraffinöl geschichtet, zu den übrigen Untersuchungen wurde das Blut aus der Ohrvene entnommen.

Untersuchungen.

Gruppe I, Normalwerte: Im Blute der gesunden Kaninchen fanden wir Blutzucker: 100—123 mg%; anorganischen Phosphor nach Fiske-Subbarow 3,03 bis 4,25 mg%, nach Kuttner-Cohen (siehe Gruppe II) 3,10—5,50 mg%, Serumcalcium 14,10—18,10 mg%. Unsere Normalwerte stimmen im allgemeinen mit den von Harnes (20) für P, von Hermann und Zentner (21), Bourne und Campbell (2) für Ca sowie mit den von Sjollema und Seekles (40), Brown und Howard (4) für P und Ca angegebenen Normalwerten überein.

Die Versuchstiere der Gruppe I erhielten nach einem Tag Hungern 1-, 2-, 3-, 5-, 10%ige NaOH-Lösung mit Hilfe der Magensonde. Auf ein Kaninchen entfielen je 0,50, 0,75, 1,00, 1,25, 1,50, 2,25, 2,50 g NaOH; diese Mengen waren in 12,5, 25, 50, 75 ccm Wasser gelöst worden. Die Verabreichung des NaOH in den verschiedensten Dosierungen sollte uns Aufschluß geben, inwiefern der Grad der im Blutchemismus sich einstellenden Veränderungen mit dem Volumen, der Konzentration und der in g ausgedrückten Menge des verabreichten Giftes zusammenhänge.

Erscheinungen: Schon 1—2 Minuten nach der Einverleibung des Giftes wurden die Tiere unruhig. Zunächst nahmen sie eine gestreckte Lage ein, wobei sie den Bauch an den Boden drückten, sprangen dann

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plötzlich auf, oder wälzen sich von der einen Seite auf die andere (Gehirnreizung). 5—10 Minuten später lecken sie Harn und Stuhl unter sich, wälzen sich in den Exkrementen herum und beschmutzten ihr Fell; dadurch tritt sich stark beschleunigtes Atmen ein. Dieser Zustand hörte in 1—15 Stunden allmählich auf, die Tiere zogen die Beine unter den Leib und lockten nahezu unbeweglich im Käfig; auch auf äußere Reize bewegten sie sich nicht mehr. Die Atmung wurde allmählich langsam und oberflächlich. Bei den Tieren, die in 1—2 Tagen zugrunde gingen, trat schon 2—3 Stunden nach der Vergiftung allgemeine Schwäche sowie stets zunehmender Kältegefühl ein; sie hielten die Augen halb geschlossen und schleppten sich zugleitend, die Atmung und Herzarbeit wurden immer schwächer, bis die Tiere unter Zuckungen — oder auch ohne diese — verendeten. Bei jenen Kaninchen, die erst später als in 3 Tagen verendeten, waren auch die Anfangserscheinungen milder und die am Leben geblieben Tiere verhielten schon wenige Stunden nach Ablauf der leichten Anfangserscheinungen einen ganz gesunden Eindruck.

Die Beobachtungen der Versuche bei der Gruppe I sind in Tabelle 1 zusammengestellt.

Aus Tabelle 1 ist zu ersehen, daß die Blutzuckerwerte auf die Einwirkung der NaOH-Vergiftung stets steigen. Die Vermehrung des Blutzuckers erfolgt schon 20 Minuten nach der Vergiftung so beträchtliche Maße annehmend, daß bei unter mehr als das Doppelte des Normalwertes erreicht wird (siehe z. B. Kaninchen Nr. 2). Im allgemeinen wird in 1—1½ Stunden das Maximum erreicht (z. B. Kaninchen Nr. 12), manchmal auch das 2½fache des Anfangswertes erreicht (siehe Kaninchen Nr. 3); meist ist die Zunahme des Blutzuckers in der 6. Stunde nach der Vergiftung am ausgeprägtesten. Sie ist in den meisten Fällen im Laufe des ersten Tages noch nachweisbar, zeigt aber im Vergleich zu dem anfänglichen meisten Anstieg deutlich abnehmende Tendenz, so daß in einem Teile der Fälle (z. B. bei den Kaninchen Nr. 4, 6, 12 und 13) kurz vor dem Tode niedrigere Werte zu finden sind als vor der Vergiftung. Da die Tiere infolge der Vergiftung kaum etwas oder keine Nahrung zu sich nehmen, darf die sich einstellende Hypoglykämie als Hungerwirkung angesprochen werden. Im allgemeinen war in jenen Fällen der eingetretene Tod der Vergiftung einstellende Hypoglykämie als Hungerwirkung angesprochen werden. Im allgemeinen war in jenen Fällen der Grad der Blutzuckerwerte zu vergleichen (Kaninchen Nr. 1, 2, 3, 6 und 12), wenn das NaOH in großen Mengen bzw. starker Konzentration einverleibt worden war. Auch die 25%ige NaOH-Lösung kann deutliche Hyperglykämie erzeugen (Kaninchen Nr. 3), wenn mit derselben genügende Mengen NaOH in den Magen gelangt sind. Die steigernde Wirkung der 10%igen NaOH-Lösung auf den Blutzucker ist jedenfalls recht gering. Die geringste Wirkung der Natronlaugenvergiftung auf den Blutzucker hängt somit von der Menge (g) des verabreichten NaOH und von der Konzentration der verwendeten Lösung (%) ab, scheint aber vom Lösungsmittel (Wasser) und vom Volumen (ccm) unabhängig zu sein.

Tabelle 1. Änderung des Zucker-, anorganischen Phosphor- und Calciumgehaltes des Blutes von Kaninchen bei experimenteller Natriumhydroxydvergiftung.

Nr.	Körpergewicht	Dosis des NaOH			Blutzucker						Differenz	Phosphor im Gesamtblut					Differenz	Serumcalcium					Differenz	Lebensdauer nach der Vergiftung
					vor der Vergiftung	nach der Vergiftung						vor der Vergiftung	nach der Vergiftung					vor der Vergiftung	nach der Vergiftung					
	g	ccm	%	g	mg%	Min.	Std.	Tage	mg%	%	%	mg%	Min.	Std.	Tage	mg%	%	mg%	Min.	Std.	Tage	mg%	%	
1	2720	75	3	2,25	110	—	6	—	280	+ 155	4,00		—	6	—	10,00	+ 150	17,4	—	6	—	11,88	− 32	7 Std.
						—	7	—	237	+ 115			—	7	—	16,66	+ 317		—	7	—	10,92	− 37	
2	2650	25	10	2,5	105	20	—	—	228	+ 117	3,62	20	—	—	—	5,64	+ 56	16,3	20	—	—	14,38	− 12	10¼ -
						—	9¾	—	—	—		—	9¾	—	—	6,67	+ 84		—	9¾	—	—	—	
						—	—	—	—	—		—	10¼	—	—	11,42	+ 215		—	10¼	—	12,56	− 23	
3	2415	75	2	1,5	120	—	1	—	298	+ 148	3,84	—	1½	—	—	6,06	+ 58	17,80	—	1½	—	6,30	− 65	12 -
4	2100	25	5	1,25	110	—	1¼	—	197	+ 79	3,52	—	1¼	—	—	4,44	+ 26	16,7	—	1¾	—	13,93	− 17	22½ -
						—	—	—	—	—		—	9	—	—	7,27	+ 107		—	9	—	11,70	− 30	
						—	22½	—	80	− 27		—	22½	—	—	14,28	+ 306		—	22½	—	9,72	− 42	
5	2630	75	2	1,5	123	25	—	—	149	+ 37	3,21	25	—	—	—	3,33	+ 4	17,17	25	—	—	10,40	− 39	26 "
						—	26	—	140	+ 14		—	26	—	—	20,00	+ 523		—	26	—	10,00	− 42	
6	2470	75	3	2,25	110	—	6	—	202	+ 84	3,20	—	6	—	—	4,94	+ 54	15,7	—	6	—	12,5	− 20	28 -
						—	—	1	150	+ 36		—	—	1	1	7,27	+ 127		—	—	1	8,91	− 43	
						—	28	—	87	− 21		—	28	—	—	14,81	+ 363		—	28	—	7,83	− 50	
7	3255	75	2	1,5	120	45	—	—	130	+ 8	4,00	45	—	—	—	4,35	+ 9	16,53	45	—	—	13,80	− 17	28 -
						—	—	1	184	+ 53		—	—	1	1	5,78	+ 45		—	—	1	12,54	− 24	
8	2200	25	5	1,25	100	—	1½	—	138	+ 38	3,22	—	1½	—	—	4,70	+ 46	17,3	—	1½	—	13,75	− 21	37 -
						—	—	—	—	—		—	9½	—	—	5,56	+ 73		—	—	—	—	—	
						—	—	1	103	+ 3		—	—	—	—	—	—		—	—	—	—	—	
9	4275	75	2	1,5	116	30	—	—	142	+ 22	2,47	30	—	—	—	4,00	+ 15	16,2	30	—	—	16,00	− 1	40 -
						—	—	1	156	+ 34		—	—	1	1	3,33	− 4		—	—	1	13,23	− 18	
10	2830	75	2	1,5	120	—	1	—	165	+ 38	3,03	—	1	—	—	4,04	+ 33	16,0	—	1	—	12,2	− 24	3½ Tage
						—	—	1	141	+ 18		—	—	1	1	3,63	+ 20		—	—	1	10,58	− 34	

Tabelle 1 (Fortsetzung).

Nr.	Kaninchen Gewicht g	Dosis der NaOH-Lösung ccm	%	Verwendete NaOH-Lösung %	Blutzucker vor der Vergiftung mg%	Blutzucker nach der Vergiftung Min.	Blutzucker nach der Vergiftung Std.	Blutzucker nach der Vergiftung Tage	Blutzucker nach der Vergiftung mg%	Differenz %	Phosphor im Gesamtblut vor der Vergiftung mg%	Phosphor im Gesamtblut nach der Vergiftung Min.	Phosphor im Gesamtblut nach der Vergiftung Std.	Phosphor im Gesamtblut nach der Vergiftung Tage	Phosphor im Gesamtblut nach der Vergiftung mg%	Differenz %	Serumcalcium vor der Vergiftung mg %	Serumcalcium nach der Vergiftung Min.	Serumcalcium nach der Vergiftung Std.	Serumcalcium nach der Vergiftung Tage	Serumcalcium nach der Vergiftung mg%	Differenz %	Lebensdauer nach der Vergiftung
11	3500	75	1	0,75	112	45	—	—	112	—	3,40	45	—	—	4,00	+ 18	16,10	45	—	—	12,40	− 23	5 Tage
						—	3	3	132	+ 18		—	3	3	4,00	+ 18		—	3	3	18,01	+ 12	
						—	5	5	123	+ 10		—	5	5	14,80	+ 335		—	5	5	11,18	− 31	
12	2505	12,5	1	1,25	109	—	1½	—	218	− 100		—	1½	—	5,79	+ 44		—	1½	—	15,61	− 7	9 „
						—	—	1	116	+ 6	4,06	—	8½	—	5,97	+ 48	16,7	—	—	—	—	—	
						—	—	8	69	− 37		—	—	56	5,55	+ 36		—	56	—	17,01	+ 2	
						—	—	8	176	+ 57		—	—	8	8,04	+ 99		—	8	—	13,44	− 20	
13	2570	12,5	1	1,25	112	—	1½	—	89	− 21	4,00	—	1½	—	5,00	+ 25		—	1½	—	14,47	− 12	14½ „
						—	8	1	—	—		—	8	—	6,67	+ 67		—	—	—	—	—	
						—	—	14	74	− 34		—	56	—	5,47	+ 37	16,5	—	56	—	15,5	− 6	
						—	—	14½	41	− 64		—	—	14	8,00	+ 100		—	—	14	13,53	− 18	
						—	—					—	—	14½	15,38	+ 285		—	—	14½	12,62	− 24	
14	2330	50	2	1	118	—	1¾	—	127	+ 8		—	1¾	—	5,52	+ 31		—	—	—	—	—	am Leben geblieben
						—	29	—	143	+ 26		—	—	—	—	—		—	—	—	—	—	
						—	31½	31½	112	− 5	4,25	—	31½	—	5,71	+ 34	17,80	—	31½	—	13,53	− 24	
						—	9	9	112	− 5		—	9	9	4,21	—		—	9	9	13,31	− 25	
						—	15	15	115	—		—	15	15	4,23	—		—	15	15	17,20	—	
15	3500	75	1	0,75	107	—	1¼	—	107	—	4,08	—	1¼	—	4,34	+ 6		—	1¼	—	12,00	− 15	
						—	3	3	147	+ 37		—	3	3	5,00	+ 23	14,10	—	3	3	15,09	+ 7	
						—	10	10	96	− 10		—	10	10	5,00	+ 23		—	10	10	14,20	—	
						—	15	15	105	—		—	15	15	4,00	—		—	15	15	14,20	—	
16	3140	50	1	0,5	109	—	1	—	98	− 2		—	1	—	4,08	+ 18		—	1	—	13,40	− 26	
						—	3	3	145	+ 45	3,47	—	3	3	4,60	+ 15	18,10	—	3	3	16,26	− 10	
						—	10	10	121	+ 21		—	10	10	4,44	+ 28		—	10	10	19,88	+ 10	
						—	15	15	102	—		—	15	15	3,50	—		—	15	15	18,20	—	

Der Gehalt des Blutes an **anorganischem Phosphor** ist in jedem Falle von NaOH-Vergiftung, insbesondere bei tödlichem Verlauf, **vermehrt**. Diese Vermehrung kann ausnahmsweise schon 20 Minuten nach der Vergiftung 56% betragen (z. B. Kaninchen Nr. 2: von 3,62 mg%; auf 5,64 mg%), in der weitaus größeren Anzahl der Fälle aber, auch in den tödlich verlaufenen, war ein nennenswerter Anstieg erst in der zweiten Stunde nach der Vergiftung zu verzeichnen. In den meisten Fällen zeigt der Gehalt des Blutes an organischem Phosphor 1—1,5 Stunden nach der Vergiftung im Vergleich zum Normalwert eine Zunahme um 25—60% (Kaninchen Nr. 3, 4, 8, 10, 12 und 13), in 6—10 Stunden eine solche um 50—150% (Kaninchen Nr. 1, 2, 4, 6, 8, 12 und 13). Hierauf nimmt der Phosphorgehalt bis zum Eintreten des Todes noch weiter zu und kann eine Steigerung bis zu 200—500% des Anfangswertes erfahren. Aus unseren Ergebnissen geht hervor, daß in allen jenen Fällen, wo die Vermehrung des Phosphorgehalts in spätestens 10 Stunden nach der Vergiftung 50% oder mehr des Anfangswertes erreicht hatte, die Vergiftung sicher zum Tode führte. Je rascher die Zunahme des Phosphors vor sich ging und je höhere Grade sie erreichte, um so schneller ging auch das Versuchstier zugrunde. Man kann demnach folgenden Schluß ziehen: Die rasche (innerhalb 10 Stunden nach der Vergiftung erfolgte) Vermehrung des anorganischen Blutphosphors um zumindest 50% des Normalwertes weist auf eine schwere Vergiftung hin und bedeutet eine schlechte Prognose. Bei den Kaninchen Nr. 14, 15 und 16 betrug die Vermehrung bloß 34 bzw. 28%, und allmählich stellten sich wieder Normalwerte ein; diese Tiere blieben am Leben. Eine Vermehrung des Blutphosphors um weniger als 50% gestattet aber nicht den Schluß, daß die Tiere am Leben bleiben, denn die Kaninchen Nr. 7, 9, 10 und 11, bei denen es bloß zu einer mäßigen bzw. geringen anfänglichen Steigerung gekommen war (45, 15, 33 und 18%), verendeten schließlich dennoch. Bei Kaninchen Nr. 11 stellte sich unmittelbar vor dem Tode plötzlich die Zunahme des Phosphorgehalts ein (335%); wahrscheinlich war dies auch bei den Kaninchen Nr. 7, 9 und 10 der Fall, doch waren wir in diesen Fällen leider nicht in der Lage, die Tiere kurz vor ihrem Ende zu beobachten, da dieses in Abwesenheit des Beobachters nachts eingetreten war. Die Wirkung der Konzentration der verwendeten NaOH-Lösung zeigte insofern einen Einfluß auf die Vermehrung des Phosphors, als die letztere im allgemeinen in jenen Fällen stärker war, wo eine NaOH-Lösung von stärkerer Konzentration verabreicht wurde. Doch gab es auch hier Ausnahmen: z. B. Kaninchen Nr. 5, mit sehr starkem Ansteigen des Phosphorwertes nach Verabreichung der 2%igen Lösung (523%). Bei geringer Vermehrung des Phosphorgehalts war niemals eine Lösung von stärkerer Konzentration als 1—2% verwendet worden.

Das Serumcalcium war in jedem Falle verringert. Schon 20, 25, 45 Minuten nach der Vergiftung war der Serumcalciumgehalt um 12, 39, 23% niedriger als der Anfangswert (Kaninchen Nr. 2, 5

und 11). Die Senkung des Calciumgehalts nahm in den nächsten Stunden weiter zu; die niedrigsten Werte fanden sich bei Eintreten des Todes. Die stärkste Senkung betrug 65% (Kaninchen Nr. 3) des Anfangswertes, hierauf folgte eine solche von 50% (Kaninchen Nr. 6); in den übrigen tödlich verlaufenen Fällen war im Durchschnitt eine Senkung von 20—40% zu beobachten. Bei den am Leben gebliebenen Kaninchen (Nr. 14, 15 und 16) betrugen die stärksten Senkungen 15—26%, doch wurden hier in mehreren Tagen allmählich wieder die Ausgangswerte erreicht. In den Fällen, wo die Verminderung des Serumcalciums in stärkerem Maße und rascher erfolgt war, gingen die Tiere meist rascher zugrunde. Zwischen dem Grade der Calciumsenkung und der Konzentration sowie des Volumens der verwendeten NaOH-Lösung konnte kein Zusammenhang festgestellt werden; der Grad der Senkung scheint in erster Linie durch die NaOH-Menge (g) beeinflußt zu werden.

Aus den in Tabelle 1 zusammengestellten Versuchsergebnissen läßt sich demnach feststellen: **Nach experimenteller NaOH-Vergiftung stellt sich eine etwa einen Tag andauernde Vermehrung des Blutzuckers (Hyperglykämie) ein, auf die eine Senkung des Blutzuckergehalts folgt (Hunger-Hypoglykämie). Ferner kommt es zur allmählichen Zunahme des anorganischen Gesamtblutphosphors und gleichzeitig zur ständigen Senkung des Serumcalciums.**

Gruppe II (16 Kaninchen). Zu verschiedenen Zeiten nach der NaOH-Vergiftung wird der Gehalt des Blutes an anorganischem Phosphor sowie die Alkalireserve untersucht. Um das Blut der Tiere zu schonen, wurde bei dieser Gruppe die Phosphorbestimmung nach der Mikromethode von Kuttner-Cohen (26) mit (0,2 ccm Gesamtblut) ausgeführt. Mit diesem Verfahren erhielten wir ähnliche Anfangs- (Normal-) werte (3,10—5,50 mg%) wie mit dem Verfahren nach Fiske-Subbarow. Die Werte der Alkalireserve vor der Vergiftung (Normalwerte) bestimmten wir nach dem Verfahren von Van Slyke; sie schwankten zwischen 59,12 und 54,42 Vol.% und stimmten somit mit den ursprünglichen Angaben (63—46) von Van Slyke (44) sowie mit den nach dem Van Slykeschen Verfahren bei Menschen bezogenen Normalwerten von Volhard (46) (77—55), ferner Hollós und Weiß (22) (69,1—54,3) nahezu überein.

Die Tiere dieser Gruppe erhielten NaOH in der Form einer 2-, 3-, 5%igen Lösung und zwar 0,41, 0,47, 0,50, 0,75 g NaOH je 1 kg Körpergewicht. Die Ergebnisse erhellen aus Tabelle 2.

Aus den Angaben der Tabelle 2 geht hervor, daß auch in diesen Fällen in ähnlicher Weise wie bei den Tieren der Gruppe I — der anorganische Blutphosphor nach der Vergiftung allmählich ansteigt, um die höchsten Werte bei Eintreten des Todes zu erreichen (siehe die Kaninchen Nr. 1—11). Unter den tödlich verlaufenen Fällen betrug der höchste Wert 261% des Anfangswertes (Kaninchen Nr. 13), der niedrigste 30%. Bei den Tieren, die nach der Vergiftung mit dem Leben davongekommen sind, war nach

Tabelle 2. Änderung des anorganischen Phosphorgehalts des Blutes und der Alkalireserve des Blutserums von Kaninchen bei experimenteller Natriumhydroxydvergiftung.

Nr.	Körpergewicht	Dosis des NaOH				Phosphor im Gesamtblut						Alkalireserve des Blutserums						
		ccm	%	g	auf 1 kg Körpergewicht	vor der Vergiftung mg%	nach der Vergiftung Min.	Std.	Tage	mg%	Differenz %	vor der Vergiftung Vol.%	nach der Vergiftung Min.	Std.	Tage	Vol.%	Differenz %	Lebensdauer nach der Vergiftung
1	2790	73	3	2,19	0,75	3,1	—	1¾	—	5,05	+ 63	36,2	—	1¾	—	24,2	— 57	2½ Std.
2	2700	67,5	3	2,03	0,75	4,63	10	—	—	5,88	+ 27	34,92	—	—	—	—	—	7½ „
							—	7	—	5,99	+ 29		—	7	—	34,7	— 37	
3	2600	65	3	1,95	0,75	4,34	—	6	—	5,00	+ 15	57,2	—	6	—	39,5	— 31	12 „
4	3800	95	3	2,85	0,75	3,57	—	14	—	6,88	+ 93	58,5	—	14	—	39,34	— 33	15 „
							—	15	—	11,63	+ 226		—	15	—	31,77	— 46	
5	2920	73	3	2,19	0,75	5,5	—	15	—	16,13	+ 193	57,8	—	15	—	22,21	— 62	15 „
6	3130	78,3	3	2,35	0,75	4,5	—	16	—	9,8	+ 118	57,8	—	16	—	29,00	— 50	16 „
7	3200	80	3	2,40	0,75	5,3	—	14	—	6,9	+ 30	57,2	—	14	—	47,9	— 16	18 „
8	3040	45	5	2,25	0,75	5,00	15	—	—	5,55	+ 11	57,65	—	—	—	—	—	23 „
							—	7	—	7,14	+ 43		—	7	—	35,7	— 30	
							—	23	—	12,82	+ 156		—	23	—	42,4	— 26	
9	2980	74,5	3	2,23	0,75	4,76	45	—	—	—	—	56,4	45	—	—	43,3	— 23	24½ „
							—	7½	—	4,77	+ 75		—	7½	—	49,00	— 13	
							—	24	—	8,34			—	24	—	33,8	— 40	
10	2835	71	3	2,13	0,75	4,16	—	6	—	6,25	+ 50	56,5	—	6	—	45,3	— 20	27½ „
							—	21½	—	5,88	+ 41		—	21½	—	52,8	— 7	
							—	27½	—	12,82	+ 208		—	27½	—	34,6	— 39	
11	2650	45	3	1,35	0,50	5,00	30	—	—	5,00	—	57,26	30	—	—	55,7	— 3	28 „
							—	7	—	5,88	+ 18		—	—	—	—	—	
							—	23	—	7,69	+ 54		—	23	—	[illegible]	— 28	
							—	28	—	17,80	+ 246		—	[illegible]	—			

Tabelle 2 (Fortsetzung).

Nr.	Körpergewicht g	von	g	auf 1 kg Körpergewicht	Phosphor im Gesamtblut: vor der Vergiftung	nach der Vergiftung: Min.	Std.	Tage		Differenz	Alkalireserve des Blutserums: vor der Vergiftung Vol.%	nach der Vergiftung: Min.	Std.	Tage	Vol.%	Differenz	Lebensdauer nach der Vergiftung
12	3600	100	2	0,56	3,25	—	1¼	—	4,00	+ 23	56,47	—	7½	—	—	— 20	35 Std.
						—	29	—	5,88	+ 81		—	29	—	45,16	— 49	
						—	—	—	8,10	+ 149		—	—	—	28,88		
					25	—	7	—	5,95	+ 8		25	—	—	44,3	— 24	
13	2550	43	3	1,28	5,5	—	—	—	6,25	+ 14	58,25	—	—	—	—	— 6	3 Tage + 1 Std.
						—	1	—	6,62	+ 20		—	1	—	54,8		
						—	2	—	7,14	+ 30		—	—	—	—	— 23	
						3	—	—	7,81	+ 42		—	—	3	44,71	— 54	
						3	—	—	20,00	+ 264		1	—	3	26,75		
					1	—	—	—	4,76	+ 5	1	—	—	—	48,5	— 15	12 Tage
					42	—	—	—	7,58	+ 68	42	—	—	—	55,7	— 2	
14	3200	75	2	1,5	4,52	—	3	—	6,10	+ 35	56,8	—	—	—	—	—	
						—	4	—	5,55	+ 23		—	—	—	—	—	
						—	7	—	4,85	+ 7		—	—	—	—	— 44	
						—	10	—	8,34	+ 85		—	—	10	31,3		
					1	—	—	—	4,76	+ 9		—	42	—	—	— 19	am Leben geblieben
					42	—	—	—	5,82	+ 34		—	—	—	44,8		
15	2900	75	2	1,5	4,35	—	3	—	5,27	+ 21	54,52	—	—	—	—	—	
						—	4	—	5,95	+ 37		—	—	—	—	—	
						—	7	—	4,76	+ 9		—	—	—	—		
						—	10	—	5,00	+ 15		—	—	10	57,5	+ 6	
					1	—	—	—	7,46	+ 54		—	—	—	—	— 1	am Leben geblieben
					43	—	—	—	7,35	+ 52		—	43	—	58,6		
						—	3	—	6,25	+ 29		—	—	—	—		
16	2450	56	2	1	4,85	—	4	—	6,85	+ 41	59,12	—	—	—	—	—	
						—	7	—	5,95	+ 23		—	—	—	—	— 2	
						—	10	—	6,25	+ 29		—	—	10	58,2		

einer vorübergehenden Vermehrung (37—54 %) eine allmähliche Senkung der Phosphorwerte zu verzeichnen. Zugleich mit dem Ansteigen der Phosphorwerte stellte sich ausnahmslos in jedem Falle eine stärkere oder schwächere Senkung der Alkalireservewerte ein. Wir wollen hier mit Nachdruck betonen, daß es in keinem Falle zum Anstieg der Alkalireservewerte kam. Eine mäßige Senkung dieser Werte trat in zwei Fällen (9 und 13) schon 25—45 Minuten nach der Vergiftung ein; sie betrug 23 % des Anfangswertes (von 56,4 auf 43,3 bzw. von 58,25 auf 44,3 Vol.%). In einem Falle (Kaninchen Nr. 1) zeigte die Alkalireserve eine Verminderung von 57 % (von 56,2 auf 24,2 Vol.%) in 1¾ Stunden. In den tödlich verlaufenen Fällen konnten wir im Vergleich zu den Anfangswerten eine durchschnittliche Senkung von 30—60 % beobachten. In jenen Fällen, wo wir Gelegenheit hatten, mehrere Bestimmungen auszuführen, war eine gewisse Schwankung zu verzeichnen. Im Verlaufe desselben Falles zeigte sich nämlich nach deutlicher anfänglicher Senkung geringe Vermehrung und hierauf abermals Senkung der Alkalireserve; bei diesen Schwankungen wurde jedoch der Anfangswert niemals erreicht. Bei den nicht tödlich verlaufenden Fällen erreichte die Senkung der Alkalireservewerte keinen stärkeren Grad. Zwischen der Vermehrung des anorganischen Blutphosphors und der Senkung der Alkalireserve konnte zwar kein zahlenmäßiger Zusammenhang nachgewiesen werden, doch darf man sagen, daß der stärkeren Phosphorvermehrung meist auch eine deutlichere Verminderung der Alkalireserve entspricht. Jene Tiere, die je kg Körpergewicht 0,75 g NaOH erhalten hatten, ließen schwerere Schädigungen erkennen und verendeten früher als jene, die 0,50 g/kg oder noch weniger erhielten. Dieselbe Feststellung gilt auch in bezug auf die stärkere bzw. schwächere Konzentration der NaOH-Lösung.

Aus den Angaben der Tabelle 2 folgt demnach, daß bei der experimentellen NaOH-Vergiftung zugleich mit der allmählichen Vermehrung des anorganischen Blutphosphors die Senkung der Serumalkalireserve einhergeht, d. h. daß der Blutchemismus eine Verschiebung in der sauren Richtung erleidet.

Gruppe III (14 Kaninchen): Bestimmung des Blutserum- Chlor- und Natriumgehalts zu verschiedenen Zeitpunkten nach der Vergiftung.

Normalwerte: Bei gesunden Kaninchen ergaben unsere Untersuchungen einen Serum-Chlorgehalt von 363—418 mg%. Als Mittelwert für den Chlorgehalt des Serums gesunder Kaninchen wird von Denis (8) 363, von Glass (17) 365 mg% angegeben. Unser Mittelwert ist etwas höher: 384 mg%. Die an Menschen und Hunden ausgeführten diesbezüglichen Untersuchungen verschiedener Verfasser lassen darauf schließen, daß der Blutserumchlorgehalt auch unter gleichen Verhältnissen gewissen individuellen Schwankungen unterworfen ist. So fanden z. B. beim Menschen

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Laudat und Grandsire (30) 363—380 mg%, Manzini (33) 342—390 mg%, bei Hunden Manzini 379—396 mg%, Parhon und Derevici (35) 384—410 mg%.

Der Natriumgehalt des Blutserums gesunder Kaninchen schwankte bei unseren Untersuchungen zwischen 334 und 408 mg%, als Mittelwert ergab sich 370 mg%. Denis gibt als Mittelwert — für Kaninchen — 355 mg% an. Auch hier ist der von uns gefundene Mittelwert etwas höher; dies dürfte mit den höheren Chlorwerten zusammenhängen. Wie allgemein bekannt, ist sowohl Cl wie auch Na zum großen Teil in der Form von NaCl im Blut vorhanden. Unter normalen Verhältnissen wird daher dem höheren Cl-Wert stets auch ein höherer Na-Wert entsprechen. Bei Untersuchungen am Menschen wurden folgende Serum-Na-Werte gefunden: Laudat und Grandsire 334—346 mg%, Mc Vicar und H. Ross (45) 365 mg%, Manzini (33) (Menschen und Hunde) 335—340 mg%.

Zum Zwecke der Vergiftung erhielten die Tiere der Gruppe III 0,75 g (Kaninchen Nr. 1 und 12) bzw. 0,50 g (Kaninchen Nr. 13 und 14) NaOH je kg Körpergewicht in 2—3%iger Lösung durch die Magensonde nach einem Tag langem Fasten. Die Ergebnisse siehe in Tabelle 3:

Die nachstehende Tabelle zeigt, daß der Serumchlorgehalt auch in den tödlich verlaufenen Versuchen in den ersten Stunden (1.—4. Stunde) nach der NaOH-Vergiftung bloß eine geringe (7—8%) Senkung aufweist. Diese Senkung wird später allmählich ausgeprägter, war aber auch in den schweren, in 1—2 Tagen zum Tode führenden Vergiftungen nicht immer besonders stark. Die deutlichste Senkung fand sich im Falle 8; hier war der Chlorgehalt des Serums schon 7 Stunden nach der Vergiftung um 33% niedriger als der Anfangswert (von 390 auf 262 mg%). Im Falle 1 betrug die Senkung 22%, im Falle 4 16% und im Falle 7 in 20 Stunden 19%. Von den übrigen tödlichen Fällen wurde noch in dreien eine Senkung von 10% erreicht (Kaninchen Nr. 5, 9 und 11), in weiteren 5 Fällen (Kaninchen Nr. 2, 3, 6, 10 und 12) blieb sie unter 10%. In den nicht tödlich verlaufenen Fällen war die Senkung so gering, daß die Werte die Grenze der möglichen Fehlerquellen nicht überschreiten.

Neben der Senkung des Chlorgehalts war die Vermehrung des Blutnatriums zu beobachten, die in den tödlichen Fällen allmählich anstieg und bis zum Eintreten des Todes anhielt. In den am Leben gebliebenen Fällen (Kaninchen Nr. 13 und 14) nahm die anfängliche Vermehrung des Na allmählich wieder ab. Die Vermehrung des Na-Gehalts ging in den meisten Fällen langsam vor sich, konnte aber auch sehr rasch erfolgen, wie z. B. bei den Kaninchen Nr. 1, 2 und 4, wo sie schon 20, 25 und 30 Minuten nach der Vergiftung 18, 10 und 23% des Anfangswertes betrug. Am stärksten war sie bei Kaninchen Nr. 2, wo eine Zunahme von 27% des Anfangswertes erreicht wurde (von 334 auf 422 mg%). In 6 Fällen betrug die Vermehrung 15—20% (Kaninchen Nr. 1, 4, 6, 9, 11 und 13), in 5 Fällen (Nr. 3, 5, 10, 12 und 14) 9—12% und bei zwei Tieren bloß 5—6% (Ka-

Tabelle 3. Änderung des Chlor- und Natriumgehalts des Blutserums bei experimenteller Natriumhydroxydvergiftung.

Nr.	Körpergewicht g	Dosis des NaOH cem	Dosis des NaOH %	Dosis des NaOH g	Dosis des NaOH auf 1 kg Körpergewicht	Serumchlor: vor der Vergiftung mg%	Serumchlor: nach der Vergiftung Min.	Serumchlor: nach der Vergiftung Std.	Serumchlor: nach der Vergiftung Tage	Serumchlor: nach der Vergiftung mg%	Serumchlor: Differenz %	Serumnatrium: vor der Vergiftung mg%	Serumnatrium: nach der Vergiftung Min.	Serumnatrium: nach der Vergiftung Std.	Serumnatrium: nach der Vergiftung Tage	Serumnatrium: nach der Vergiftung mg%	Serumnatrium: Differenz %	Lebensdauer nach der Vergiftung
1	2755	69	3	2,06	0,75	418	25	—	—	399	— 7	376	25			412	+ 10	7 Std.
							—	7	—	326	— 22			7		442	+ 17	
2	2275	57	3	1,70	0,75	376	30	—	—	372	— 1	334	30			412	+ 23	7 Std. + 20 Min.
							—	7	—	369	— 2			7		410	+ 22	
							20 +	7	—	344	— 8		20 +	7		422	+ 27	
3	2710	67½	3	2,02	0,75	383	—	4	—	376	— 2	357		4		391	+ 9	12 Std.
4	2685	67	3	2,01	0,75	404	20	—	—	376	— 7	357	20			420	+ 18	15 „
							—	7¼	—	337	— 16			7¼		416	+ 17	
5	2730	68	3	2,04	0,75	365	+	14	—	358	— 2	380		14		381	—	16½ „
							—	16½	—	327	— 11			16½		421	+ 11	
6	2770	69	3	2,07	0,75	377	—	11½	—	347	— 8	382		11½		394	+ 3	22 „
							—	7½	—	355	— 6			7½		426	+ 11	
							—	22	—	345	— 8			22		449	+ 17	
7	3065	76,6	3	2,29	0,75	376	—	4	—	369	— 2	373		4		391	+ 5	22½ „
							—	20	—	305	— 19			20		382	+ 2	
							—	22½	—	305	— 19			22½		393	+ 5	
8	2900	72½	3	2,17	0,75	390	10 +	1	—	340	— 10	408	10 +	1		418	+ 3	29 „
							—	7	—	262	— 33			7		429	+ 5	
							—	1	—	262	— 33				1	432	+ 6	
9	3400	85	3	2,55	0,75	370	10 +	1	—	365	— 1	374	10 +	1		414	+ 11	20 „
							—	7	—	340	— 8			7		[illegible]	[illegible]	
							—	[illegible]	1	338	10			[illegible]				

Tabelle 3. (Fortsetzung.)

Nr.	Körpergewicht	Dosis des NaOH: gesamt	g	auf 1 kg Körpergewicht	Serumchlor: vor der Vergiftung	nach der Vergiftung: Min.	Std.	Tage	mg%	Differenz %	Serumnatrium: vor der Vergiftung mg%	nach der Vergiftung: Min.	Std.	Tage	mg%	Differenz %	Lebensdauer nach der Vergiftung
10	3125	78	3	2,34	0,75	364	—	—	390	+ 7	356	—	14		368	+ 3	30 Std.
							—	—	377	+ 4			18½		366	+ 3	
							—	1	340	— 6			1 +	1	394	+ 10	
11	3055	76	3	2,28	0,75	388	—	—	363	— 7	379		4		382	+ 1	35 „
							—	—	340	— 13			20		439	+ 16	
							—	—	347	— 11			30		456	+ 20	
12	2660	66½	3	1,99	0,75	365	—	—	383	+ 5	365		14		382	+ 5	2½ Tage
							—	—	369	+ 1			18½		409	+ 12	
							—	1	340	— 7			1 +	1	392	+ 8	
13	2500	62	2	1,25	0,50	372			376	+ 1	362		4		416	+ 15	am Leben geblieben
							—	—	355	— 5					393	+ 8	
							—	1	372					1	389	+ 7	
								3						3			
14	2700	67	2	1,35	0,50	363			363	— 4	374		7		407	+ 9	
							—	1	347	— 4				1	389	+ 4	
							—	3	376	+ 3				3	384	+ 3	

ninchen Nr. 7 und 8). Erwähnenswert ist der Umstand, daß zugleich mit der geringsten Na-Vermehrung (Kaninchen Nr. 7 und 8) die stärkste Senkung der Chlorwerte zu verzeichnen war, während die stärkste Na-Vermehrung mit besonders geringer Cl-Senkung einherging (Kaninchen Nr. 2). Mäßige Cl-Senkung bei stärkerer Na-Vermehrung war bei den Kaninchen 6, 9 und 11, stärkere Na-Vermehrung (+) und zugleich stärkere Cl-Senkung (—) bei 1 und 4 und Na-Vermehrung bzw. Cl-Senkung nahezu gleichen Grades bei den Tieren Nr. 5, 10 und 12 zu beobachten.

Die hier angeführten Verschiebungen der Cl- sowie der Na-Werte waren bei tödlich verlaufenen Fällen deutlicher, was damit zusammenhängen mag, daß diese Tiere Lösungen stärkerer Konzentration (3%) und je kg Körpergewicht größere Mengen (0,75 g) NaOH erhalten hatten als die am Leben gebliebenen Tiere (2%ige Lösung bzw. 0,50 g/kg).

Die in Tabelle 3 angeführten Ergebnisse führen somit zu dem

Schluß, daß bei der experimentellen NaOH-Vergiftung eine meist mäßige, selten stärkere, aber stets nachweisbare Senkung des Serum-Chlorgehalts sowie eine Vermehrung des Serumnatriums ähnlichen Grades zu finden ist. Da eine Verschiebung des Serumchlors und Serumnatriums in entgegengesetzter Richtung (↓↑) zustande kommt, bedeutet die wahrnehmbare Veränderung des Verhältnisses der Cl- und Na-Ionen zueinander eine beachtenswerte Gleichgewichtsstörung im Mineralstoffwechsel des Organismus.

Aus unseren Versuchen geht demnach hervor, daß die akute NaOH-Vergiftung im Gegensatz zu der bisherigen Auffassung nicht bloß eine örtliche Verätzung verursacht, sondern auch zur Verschiebung des Chemismus des Organismus führt; diese allgemeine Giftwirkung wurde bisher kaum oder gar nicht beachtet. Die pathologischen allgemeinen Veränderungen gelangen in der Störung des Zuckerstoffwechsels (Hyperglykämie), in der Störung des Blutionengleichgewichts (Hyperphosphatämie, Hypocalcämie, Hypochlorämie, Hypernatriumämie) sowie in der Verminderung der Alkalireserve (CO_2-Kapazität), also in den Erscheinungen, die für die Azidose bezeichnend sind, zum Ausdruck.

Die Ergebnisse unserer Versuche stimmen mit allen jenen — uns zur Verfügung stehenden — Angaben anderer Verfasser überein, die sich auf die Befunde bei laugenvergifteten Menschen oder auf derartige Tierversuche beziehen:

Löwy (32) fand bei laugenvergifteten Menschen erhöhte Blutzuckerwerte. Als Ursache wird die infolge der Gewebezerstörung verursachte Resorption des Zelleiweißes angesehen, denn auch nach der parenteralen Verabreichung verschiedener Eiweißarten kann sich Hyperglykämie einstellen. Beim Menschen fanden Labbé, Nepveux und Rohacek (28) nach der peroralen Verabreichung von Natriumbicarbonat Hyperglykämie, Underhill (43) u. a. hingegen Hypoglykämie. Im Tierversuch konnte Kusaka (27) nach der intravenösen Injektion eines stärkeren (p_H = 9,5 bis 10,08) Carbonat-Bicarbonatgemenges an Kaninchen Hyperglykämie, bei schwächerem Gemenge (p_H = 8,35) hingegen Hypoglykämie beobachten. Der scheinbare Widerspruch der verschiedenen Ergebnisse dürfte höchstwahrscheinlich auf die Unterschiede der Menge und Konzentration der Laugenverbindungen sowie auf den verschiedenen Zeitpunkt der Blutentnahme zurückzuführen sein.

J. Gaizler (16) konnte bei laugenvergifteten Kindern (1923) und Erwachsenen (1924) Azetonurie nachweisen, die um so stärker war, je schwerer sich die durch die Lauge verursachte Gewebezerstörung gestaltete. Gaizler sah in der Azetonurie eine Folge der Wirkung der bei der Gewebezerstörung entstandenen Eiweißabbauprodukte. Als Beweis führt er an, daß bei Laugenvergifteten der Nitrogengehalt des Harns stark vermehrt

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und die Plasma-Kolloid-Labilitätsreaktion (Gerlóczy) stark positiv ist, was für einen starken Eiweißzerfall im Organismus spricht. In einigen Fällen gab der Harn auch stark positive Buttersäurereaktion. Da die Azetonurie ein Zeichen der Azidose darstellt, nahm Gaizler auf Grund dieses Befundes an, daß „. . . in schweren Fällen die Todesursache in engem Zusammenhang mit der Azidose stehe . . .".

Für die Richtigkeit dieser Auffassung sprechen nicht bloß unsere, sondern auch die Versuchsergebnisse anderer Verfasser: Alpern und Sorkin (1) untersuchten (1925) an Hunden den Einfluß von Säuren und Laugen auf die Adrenalinwirkung und fanden nach der intravenösen Injektion einer n-NaOH-Lösung die Verminderung der Alkalireserve des Blutes. Als Ursache nahmen sie die übermäßige Kompensationsreaktion des Organismus an, die sie auf zentrale Ursachen zurückführten. Suzuki (41) verabreichte (1926) Kaninchen NaOH per os und fand ebenfalls die Senkung der Alkalireserve des Blutes, also Zeichen der Azidose; zugleich konnte er jedoch die Verschiebung der p_H in alkalischer Richtung beobachten; er erklärt diese eigenartige Erscheinung mit der Haldaneschen „paradoxen Anoxämie". Dies soll besagen, daß im alkalischen Medium das Oxyhämoglobin stabiler und dadurch der CO_2- und O_2-Austausch zwischen Geweben und Blut erschwert ist und daß infolge der schlechten Oxygenversorgung der Gewebe im intermediären Stoffwechsel organische Säuren in erhöhtem Maße gebildet werden. Bei unseren Versuchen wurde die p_H nicht regelmäßig untersucht, wir können daher über diesen Teil der Frage keine Meinung äußern. Sékoulitch (39) fand (1930) bei laugenvergifteten Menschen neben Azetonurie ebenfalls Verminderung der Alkalireserve. Neben unseren Ergebnissen zeigen demnach auch die Angaben des Schrifttums, daß bei NaOH-Vergiftung ebenso Azidose entsteht wie bei der Vergiftung mit NH_4OH bzw. im allgemeinen auf die Einwirkung gewisser Salze.

Berichte, die über den Gehalt des Blutes an anorganischem P, Ca, Cl bzw. Na bei NaOH-Vergiftung Aufschluß geben können, waren in dem uns zur Verfügung stehenden Schrifttum nicht zu finden, obwohl die Kenntnis des Verhaltens dieser Ionen besonders im Sinne des Säurebasengleichgewichts nicht unwichtig ist. Wie allgemein bekannt, zeigen diese Bestandteile des Blutes unter normalen Bedingungen stets gleichbleibende Werte und spielen bei der Erhaltung des Säurebasengleichgewichts eine hervorragende Rolle. Jede Änderung des Verhältnisses dieser Bestandteile zueinander hat eine Störung des Säurebasengleichgewichts zur Folge.

Im Schrifttum finden sich zahlreiche Angaben, aus denen hervorgeht, daß bei der durch verschiedene Säuren bzw. Salze hervorgerufenen Azidose im Blutchemismus ähnliche Veränderungen entstehen, wie wir sie einerseits bei der früher mitgeteilten NH_4OH-, andererseits bei der hier beschriebenen NaOH-Vergiftung antreffen konnten. So entsteht z. B. bei der peroralen und intravenösen Einverleibung verschiedener Mineral- oder organischer

Säuren Hyperglykämie [Elias (9), Lesser (31)]. Nach der Verabreichung von Milchsäure wurde bei Hunden die Verminderung des Serumcalciums [Floris (15)] sowie des Blutchlors [Van Caulaert, Pétrequin und Bauer (6)] beschrieben. Auch nach Harnsäureinjektionen sinkt bei Hunden der Chlorgehalt des Blutes [Capani (5)]. Nach der intravenösen Injektion von Mono- und Trinatriumphosphat [Zamorani (47)] sowie Natriumglycerophosphat [Boyd, Hines und Stearns (3)] konnte bei Hunden und Kaninchen die Vermehrung des P sowie die Verminderung des Ca im Serum beobachtet werden. Bei Sublimatvergiftungen wurde bei Menschen [E. Muntwyler, Ch. T. Way und E. Pomerene (34)] und Hunden [Trusler, Fisher und Richardson (42)], ferner auch bei der Vergiftung mit Hydrargyrum oxycyanatum [Laudan, Glass und Beiles (29)] die Senkung der Alkalireserve und des Chlorgehalts des Blutes nachgewiesen.

Das Wesen der durch NH_4Cl bewirkten Azidose erklären Pohl und Münzer (36), ferner Haldane (19) damit, daß im Blute aus NH_4Cl und CO_2 Salzsäure gebildet wird $2NH_4Cl + CO_2 = CO(NH_2)_2 + 2HCl + H_2O$; durch diesen Vorgang kommt die Verschiebung in der sauren Richtung zustande. Diese Erklärung scheint bei der durch Cl-haltige Salze und Säuren hervorgerufenen Azidose angebracht, genügt aber nicht, um auch die bei der NH_4OH- und NaOH-Vergiftung erzeugte Azidose zu erklären, da in den letzteren Fällen dem Organismus kein Chlor zugeführt wird. Man könnte sich allerdings vorstellen, daß hier das zur Salzsäurebildung notwendige Chlor dem Blute oder anderen Organen (z. B. der Haut) entstamme, dann wäre aber eine Steigerung des Serumchlors zu erwarten. Wie jedoch oben mehrfach erwähnt, kommt es bei der NaOH-Vergiftung zur Abnahme des Serumchlorgehalts; in diesen Fällen muß also für die Entstehung der Azidose eine andere Erklärung gesucht werden. Wie schon oben angeführt, werden von anderen Forschern für die Entstehung der Azidose bei der NaOH-Vergiftung vor allem Eiweißzerfallprodukte (Gaizler, Sekoulitch) bzw. die infolge der schlechten CO_2-Versorgung in größeren Mengen gebildeten organischen Säuren (Suzuki) und sogar auch Vorgänge von seiten des Nervensystems verantwortlich gemacht. Obwohl diese Auffassungen gegebenenfalls berechtigt erscheinen mögen, glauben wir dennoch auf Grund unserer Ergebnisse annehmen zu dürfen, daß bei der durch die NaOH-Vergiftung erzeugten Azidose auch die Störung im Gleichgewicht des Phosphor-, Calcium-, Chlor- und Natriumhaushalts eine erstrangige Rolle spiele; an erster Stelle sind hier offenbar die sauren Phosphate zu nennen. Hierfür spricht die bedeutende Vermehrung des anorganischen Blutphosphors, der zum größten Teil höchstwahrscheinlich den roten Blutkörpern entstammt. An der Stelle der lokalen Laugenwirkung (Magen) gehen bekanntlich ziemlich viel rote Blutkörper zugrunde und die auf dem Wege der Resorption in die Blutbahn gelangende Lauge übt ebenfalls eine hämolytische Wirkung aus. Auf diese Weise werden die Phosphate

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der roten Blutkörper frei und tragen in hohem Grade zur Verschiebung des Blutchemismus in saurer Richtung bei. Daneben ist natürlicherweise die Rolle der Schädigung auch anderer Organe (Leber, Nieren usw.) nicht zu vernachlässigen.

Nach der gemeinsamen Beurteilung unserer Versuchsergebnisse und der Angaben des Schrifttums gelangt man demnach zu dem Schluß, daß man bei der Laugenvergiftung nicht bloß mit der örtlichen, ätzenden Wirkung der Lauge zu rechnen hat, sondern auch mit schweren Veränderungen von seiten des Chemismus des Organismus. Diese Veränderungen führen auf dem Wege schwerer Störungen des Zuckerstoffwechsels sowie des Ionengleichgewichts zur Entstehung der Azidose, die in den verschiedenen Organen irreparable Schädigungen und somit den Tod verursachen kann.

Zusammenfassung.

Bei der experimentellen NaOH-Vergiftung der Kaninchen ließen sich folgende Änderungen des Blutchemismus feststellen:

1. Störung des Zuckerstoffwechsels in der Form der Hyperglykämie, die bei Tieren, welche die Vergiftung um mehr als 1 Tag überlebt hatten, mitunter in Hyperglykämie (Hunger!) überging.
2. Mächtige Vermehrung des anorganischen Phosphors im Gesamtblut.
3. Abnahme des Serumcalciums.
4. Mäßige Abnahme des Serumchlors.
5. Geringe Vermehrung des Serumnatriums.
6. Deutliche Azidose in der Form der Verminderung der Serumalkalireserve (CO_2-Kapazität).

Aus diesen Ergebnissen ist zu sehen, daß man bei Laugenvergiftungen nicht bloß mit der örtlichen, ätzenden Wirkung der Lauge, sondern auch mit den erwähnten schweren Schädigungen im Chemismus des Organismus zu rechnen habe.

Schrifttum.

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GERMAN TRANSLATION

Fat Embolisms in Experimental Sodium Hydroxide and Ammonia Poisoning

Dr. I. Gy Fazekas Instructor. (From the Legal Medicine Institute of the Royal Hungarian Franz Joseph University in Szegod, Hungary. Director: Prof. Dr. J. V. Balo)

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Our histological studies, performed both on human corpses (2) and experimental animals (3), have shown that quite severe fat embolisms can arise in various organs (brain, lungs, kidneys, etc.) during acute ammonia poisoning. We believe that the cause of fat embolism can be found in the lipemia (excess fat in the blood) resulting from the poisoning, which could be detected in animal tests by chemical methods (4). In our investigation of further details of this problem we found that the effect of ammonia poisoning leads to hyperglucemia, hyperphosphatemia, hypocalcemia and severe acidosis (5). It was quite surprising that ammonia in spite of its marked alkaline nature, causes acidosis in the same manner as NH_4Cl (Pohl and Munzer (12)) and various acids. On the other hand, it is commonly known that lipemia and acidosis are intimately connected. On the basis of these experimental facts, the logical question to ask is whether the changes observed during ammonia poisoning can also be noted during poisoning with other alkaline compounds. Our tests on rabbits led to the conclusion that the same changes in blood chemistry take place during sodium hydroxide poisoning as those noted in ammonia poisoning, namely hyperglucemia, hyperphosphatemia, hypocalcemia and acidosis. We wish here to stress particularly the fact that acidosis also takes place during NaOH

poisoning. Results of these tests have been reported in detail in earlier publications (6), and we refer therefore in this connection to the appropriate passages.

Our next task consisted in searching for fat embolisms in the organs of NaOH-poisoned animals; however, it was necessary at first to carry out supplemental studies also in regard to fat embolism after ammonia poisoning.

Previous observations did not provide a satisfactory explanation of the exact conditions causing fat embolism during ammonia poisoning. It was important to find out the earliest time at which fat embolism can arise in this type of poisoning, how long is the pssibility (danger) of its formation, what extent (degree) it may reach, how it is distributed in individual organs and whether it may be an immediate cause of death.

To clarify these questions, tests on both NaOH and NH_4OH poisoning were carried out on animals (rabbits), after 1 day of fasting and by administration of the compound with a stomach probe (intragastric). Group I included 34 rabbits, receiving a 1, 2, 3, 5 and 10% NaOH solution; Group II included 32 rabbits, receiving a 1, 2 and 10% NH_4OH solution. Animal organs were fixed immediately after death in 4 % formalin, and the frozen sections stained with Sudan III-hematoxylin. Results are shown in Tables I and II.

Table I (NaOH poisoning) shows the formation of fat embolisms in almost all animals (32 out of 34 = 94%). Clearly expressed fat embolisms were already visible in animals #1 and 2, which died 7 hours after poisoning, and could also be noted on the 9th and even 12th day after poisoning (#32 and 33). The organ most affected was the lung, where fat embolism was most severe. Fat embolisms were also found rather frequently in the plexus chorioideus of the brain (in 24 out of 34 animals = 70%), somewhat less frequently in the kidneys (11 animals = 32%), and only rarely in the spleen and liver (2-3 cases = 5-10%). No fat embolisms were detected in other organs.

The severity of fat embolisms in the lungs is indicated, according to its degree, by the symbols +, ++, +++, or ++++: + means that fat embolisms are present only in a few capillaries; ++ in numerous capillaries and in several precapillaires; +++ not only in capillaries and precapillaries, but also in some small arteries and veins; and ++++ that fat embolisms were found also in several smaller and medium-size arteries and veins.

TABLE I

Fat embolism in various organs during experimental NaOH poisoning.

1-Bodyweight
2-NaOH dose
3-Fat embolism
4-In lungs
5-In plexus chorioideus
6-In kidneys
7-In liver
8-In spleen
9-Duration of life after poisoning

Fat embolism of capillaries and precapillaries represents a homogenous mass, while in arteries and veins it consists mostly of large and smaller droplets. In the lungs of animals (#4, 5) which died 12 hours after poisoning a vacuole formation, considered already by Beneke (1), Wuttig (16) et al. as a sign of fat splitting, can be observed, as well as the formation of a jagged edge in individual fat globules. Similar phenomena are even more clearly expressed at a later date. In cases several days old however, the

majority of fat embolisms found do not exhibit any resorption properties. This indicates that, in cases of caustic alkali poisoning, fat embolism can already arise in a few hours, gradually expanding in the following days and finally reaching a maximum. In cases shown by the symbols +++ and ++++ fat embolism was already so severe that it alone could cause the death of the animals, while in cases marked + and ++ the consequences of such embolism was the factor which accelerated the death of the animals.

In the lungs, namely in the vicinity of fat embolisms, smaller or larger hemorrhages could mostly be found, which filled numerous alveoles. In such cases, fat drops were frequently found in the alveoles. Moreover, in cases a few hours old, an edema or inflammatory foci (pneumonia), consisting of mono-nuclear or lobed-nuclear white blood cells, had also been formed. This finding agrees with the observation made by Jankovich (8) that fat embolisms (of traumatic origin) are mostly likely found in the vicinity of compact pulmonary foci (interstitial pneumonia, bronchopneumonia, tuberculosis, etc.). The cause of this fact, as assumed by Jankovich, is that these areas have a poorer blood supply.

TABLE II

Fat embolism in various organs during experimental NH_4OH poisoning.

1-3-7, 9-same as in Table I
2-NH_4OH dose
8-In heart

In our cases (poisoning), however, we consider it more likely that fat embolism is the primary injury and that it leads to an extreme obstruction (stasis), by inhibiting blood circulation, and this again to a bursting of blood vessels (capillaries, precapillaries), and eventually even (per diapedesim) to the formation of edema and hemorrhages. In this manner, fat drops enter into the alveoles, and large numbers of white blood cells soon migrate into areas affected by obstruction (stasis) or hemorrhages. By the way, this has already been observed by T. Kojo (10) et al. on hand of their experimental fat embolisms in rabbits. We can therefore assume that the pneumonic foci formed during endogenous and exogenous poisoning or as a result of other injuries accompanied by fat embolism are due not so much to a deterioration of the heart activity or other causes, as much rather to the fat embolisms themselves and to the circulatory disturbances (bleeding, edema) caused by these embolisms. Confirmation of the correctness of this assumption also in regard to man would contribute to the clarification of etiological problems, on the one hand, and would also afford new therapeutic opportunities, on the other hand.

If we attempt to explain the formation of fat embolism, then we can conceive two possible ways of doing so; first, we must recall that fat is normally distributed (emulsified) in blood in the form of small, microscopically invisible, unstainable droplets. As a result of poisoning, a disruption of the chemical equilibrium (acidosis) in the organism takes place. This disruption could have the following effects: either fat conglomerates in the form of larger, now visible and stainable drops (somewhat like in the fat phanerosis of cells), and thus can be detected in organs, and primarily in the lungs, in the form of embolisms, or reserve fat is mobilized from fat stocks, thus causing a true lipemia. In the first case, we must

assume that the fat content of the blood remains unchanged or decreases due to the formation of fat embolisms, while in the second case the fat content of the blood should increase. A quantitative investigation of the blood fat content will show which of these two possibilities must be considered in caustic alkali poisoning; we are engaged in studies concerned with this matter. However, already now it seems to us likely that the second case takes place during alkali poisoning, namely that fat embolism must be attributed to the lipemia arising as a result of poisoning, as we have already proven in regard to ammonia poisoning.

From Table II we can see that, after experimental NH_4OH poisoning, fat embolisms are formed in the great majority of cases (about 97%). They are found most frequently and in the most severe form in the lungs (90%), rather often also in the plexus chorioideus of the brain (50%), much more seldom in the kidneys (about 20%) and only rarely in the liver or in the heart (about 3%).

We were able to detect a distinct fat embolism in the lungs already 1/2 hour after poisoning. Fat was always found in the pulmonary vessels of the animals which died after 3 days (#1-25). In isolated cases (#26, 29 and 31), no fat embolism could be detected 7, 15 and 17 days after poisoning while in other cases fat embolism was clearly expressed on the 12th, 14th, 17th and even 36th day after poisoning. In animal #26, a fat embolism was found on the 7th day in the plexus chorioideus of the brain, while lung findings were negative. Our findings are in complete agreement with our earlier observation that the higher fat content of blood can be quantitatively determined 6-8 days after ammonia poisoning. In the tests reported here, we were able to demonstrate that the lipemia arising after ammonia poisoning can remain in force for several more weeks after poisoning and can cause fat embolism. Formation of fat embolism of traumatic origin was observed by Melchior (11), further by

Jankovich (9), 14 days, and by Siegmund 18 days, after the occurence of trauma (bone fracture).

In the + cases, fat has also found here only in lung capillaries, in the ++ cases in the capillaries and precapillaries, in the +++ cases also in smaller arteries and veins, and in the ++++ cases also in medium-size and larger arteries and veins. The pulmonary fat embolisms designated by 2, 3, or 4 + signs were so severe that they alone could have caused the death of the animals. The next to severest fat embolisms were found in the plexus chorioideus of the brain, where vessels were often densely filled with fat. The vessels and capillaries of other organs contained only little fat.

Along with fat embolism, severe pulmonary edema and hemorrhages were noted (e.g. in animals #1 and 2) already 1/2 hour after poisoning, and 1-2 hours later also inflammatory foci, consisting mostly of mononuclear and lobed-nuclear white blood cells. In the areas where bleeding occurred, some alveoles contained isolated fat droplets. Fat embolism apparently played the main role in the onset of these pulmonary alterations, as was mentioned above in connection with NaOH poisoning.

The detection of fat embolism in the lungs and brain (plexus chorioideus) also constitutes per se an explanation of those sudden fatal cases occurring during both NaOH and NH_4OH poisoning sometimes within a few hours or days, without any more severe local changes (perforation, destruction of the larynx, laryngeal edema, etc.), which so far have mostly been considered as a shock effect.

We shall not discuss again here the significance and consequences of fat embolisms found in other organs, since these have already been

discussed in detail in my previous relevant articles. We shall only state here briefly that all statements made in regard to NH_4OH poisoning also apply to NaOH poisoning.

We shall also not discuss here in detail the extensive literature on fat embolism, since this would be far beyond the scope of this report. In this connection, attention is directed to the comprehensive survey published by T. Kojo and Seemann (14). The first author gives a detailed report on the older literature (up to 1922), while the second reviews the recent literature (up to 1931). It is worth mentioning that fat embolisms following certain types of poisoning have also been already observed by other authors, such as Puppe (13) in phosphorus poisoning and Winogradow (15) in potassium chloride poisoning. In regard to NaOH and NH_4OH poisoning, we were unable to find any such information in the pertinent literature.

Summary

1. On the basis of a histological study, it could be established with certainty that fat embolisms arise in the organs of experimental animals (rabbits) during NaOH poisoning in the great majority of cases (94%), in the same way as during NH_4OH poisoning (97%).

2. In NaOH (caustic) poisoning, fat embolisms could be detected at the earliest 7 hours and in NH_4OH poisoning already ½ hour after poisoning.

3. The latest time at which fat embolisms could still be detected was 9 and (or) 12 days after NaOH poisoning and 36 days after NH_4OH poisoning.

4. In both types of poisoning, fat embolisms occurred in the most severe form and most frequently (80-90%) in the lungs. In regard to frequency and severity, the sequence of occurrence was as follows:

plexus chorioideus of the brain (50-70%), kidneys (20%). Most seldom were fat embolisms found in the liver, spleen and heart (5-10%).

5. The severity of fat emoblism can reach such a high degree in both types of poisoning that it can cause per se the death of the animals (lung and brain fat embolisms). This fact explains the occasional sudden onset of death in such types of poisoning, which so far has been interpreted as a shock effect.

6. The fat embolism observed in NaOH poisoning must apparently be considered as a result of the lipemia occurring in response to the poisoning, in the same way as was established earlier on hand of chemical studies in the case of NH_4OH poisoning.

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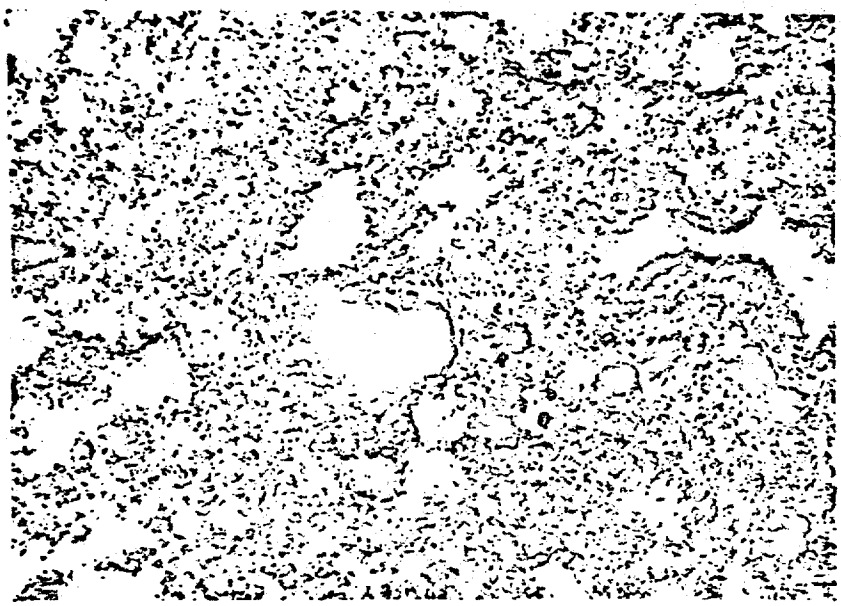
FIGURE 1



Fat embolism in the lung of a rabbit poisoned with 10% NaOH 10 hours and 15 min. after poisoning. Numerous fat globules filling the capillaries, precapillaries and lumen of a small vein. In the alveoles, serum (stasis) and numerous white blood cells. Frozen section, staining with Sudan III-hematoxylin. (Rabbit #3).

FIGURE 2

Fat embolism in the lung capillaries and precapillaries, and in some smaller veins, 33 hours after poisoning with 3% NaOH. In the vicinity of embolisms, the pulmonary tissue is completely atelectatic, alveoles are filled with numerous white blood cells, mixed with a few erythrocytes and serum (pneumonia). Frozen section, staining: Sudan III-hematoxylin (Rabbit #21).

FIGURE 3

Fat embolism in the lung, 37 hours after poisoning with 3% NaOH. The lumen of the medium-size artery is shifted (displaced) by the embolism consisting of numerous small, round fat globules. Frozen section; Sudan III-hematoxylin staining (Rabbit #23).

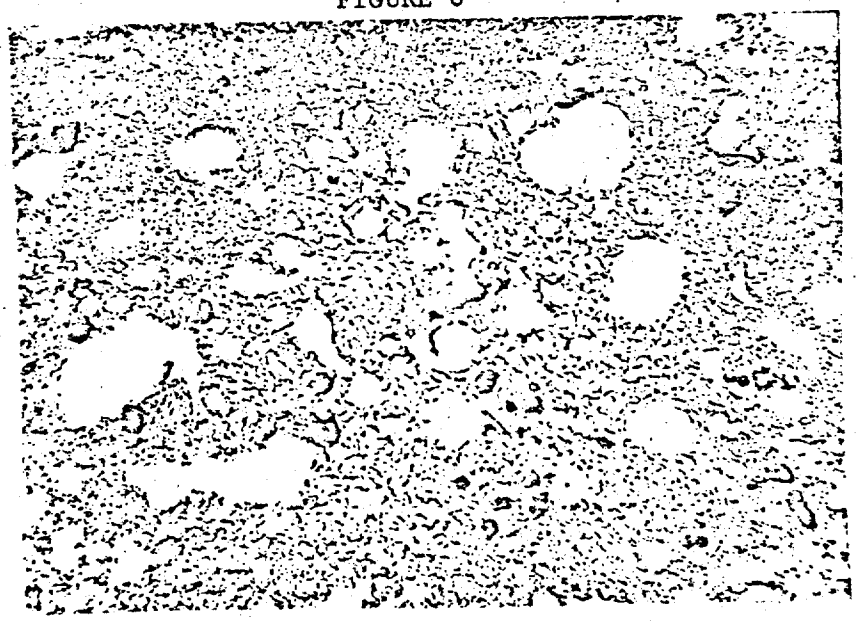
FIGURE 4

Fat embolism in the plexus chorioideus 17 hours after poisoning with 3% NaOH. Frozen section; Sudan III - hematoxylin staining (Rabbit #9).

FIGURE 5

Fat embolism in the plexus chorioideus 33 hours after poisoning with 3% NaOH. Frozen section, Sudan III - hematoxylin staining (Rabbit #21).

FIGURE 6

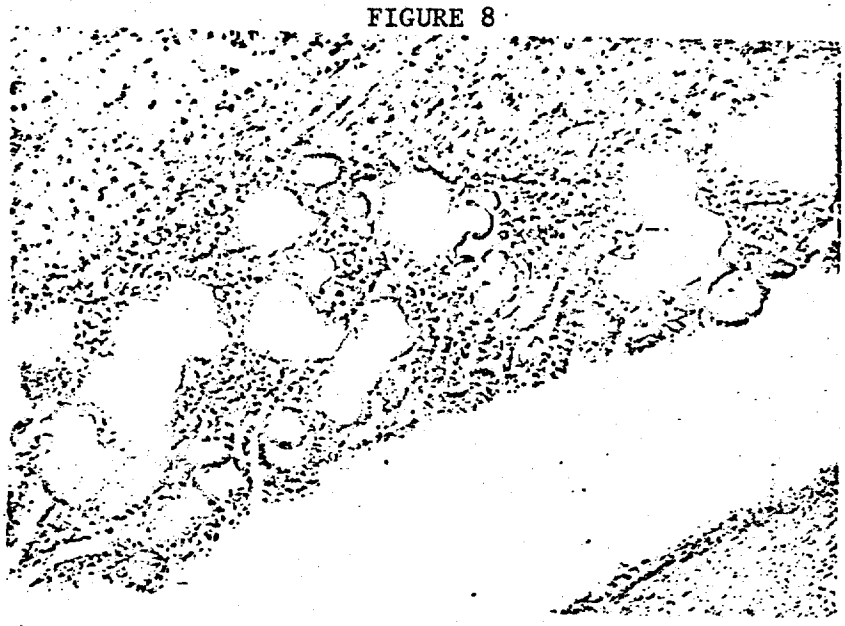


Fat embolism in the capillaries and precapillaries of the lung 1 hour and 30 min. after poisoning with 2% NH_4OH. Forzen section; Sudan III - hematoxylin staining (Rabbit #11).

FIGURE 7

Fat embolism in the lung 1 hour and 30 min. after poisoning with 2% NH_4OH. The lumen of the larger artery is filled with an embolism consisting of large fat drops. Scattered fat embolisms are also visible in several capillaries. Frozen section; Sudan III - hematoxylin staining (Rabbit #11).

FIGURE 8



Fat embolism in the plexus chorioideus 1 hour and 30 min. after poisoning with 2% NH_4OH. Fozen section; Sudan III - hematoxylin staining (Rabbit #11).

Aus dem Institut für Gerichtliche Medizin der königl. ung. Franz Joseph-Universität in Szeged (Ungarn). (Direktor Dr. J. v. Baló, o. ö. Prof.)

Fettembolien bei experimenteller Natriumhydroxyd- und Ammoniumhydroxyd-Vergiftung.

Von

Dr. I. Gy. Fazekas, Dozent.

Mit 8 Abbildungen und 2 Tabellen im Text.

Unsere — sowohl an menschlichen Leichen[2] wie auch an Versuchstieren[3] ausgeführten — histologischen Untersuchungen ergaben, dass bei der akuten Ammoniumhydroxydvergiftung in den verschiedensten Organen (Gehirn, Lungen, Nieren usw.) oft recht schwere Fettembolien entstehen können. Die Ursache der Fettembolie glaubten wir in der sich auf die Vergiftung einstellenden Lipämie gefunden zu haben, was sich im Tierversuch mit Hilfe chemischer Verfahren nachweisen liess[4]. Bei der Forschung nach näheren Einzelheiten in dieser Frage fanden wir, dass auf die Einwirkung der NH_4OH-Vergiftung Hyperglykämie, Hyperphosphatämie, Hypocalcämie und eine schwere Azidose[5] zustande komme. Es war recht auffallend, dass NH_4OH trotz seiner ausgeprägten alkalischen Natur ebenso zur Azidose führe wie etwa NH_4Cl (*Pohl* und *Münzer*[12]) bzw. die verschiedenen Säuren. Auf der anderen Seite ist allgemein bekannt, dass zwischen der Lipämie und der Azidose ein enger Zusammenhang herrscht. Nach diesen Erfahrungen musste man sich folgerichtig fragen, ob die bei der Ammoniumhydroxydvergiftung beobachteten Veränderungen nicht etwa auch bei Vergiftungen mit anderen alkalischen Stoffen entstehen können. Unsere an Kaninchen ausgeführten Versuche liessen darauf schliessen, dass nach der NaOH-Vergiftung im Blutchemismus dieselben Veränderungen vor sich gehen, wie sie bei der NH_4OH-Vergiftung nachzuweisen sind, also Hyperglykämie, Hyperphosphatämie, Hypocalcämie, Azidose. Besonders zu betonen ist hier, dass sich auch nach der NaOH-Vergiftung eine Azidose einstellt. Über die Ergebnisse dieser Versuche berichteten wir in früheren Mitteilungen[6] eingehend, wir verweisen daher diesbezüglich auf die angeführten Stellen.

Unsere nächste Aufgabe bestand nun darin, in den Organen der mit NaOH vergifteten Versuchstiere nach Fettembolien zu fahnden. Zunächst ergab sich aber die Notwendigkeit, unsere Untersuchungen auch in bezug auf die Fettembolie nach der NH_4OH-Vergiftung zu ergänzen.

Die bisherigen Feststellungen boten nämlich keinen genügenden Aufschluss über die näheren Entstehungsumstände der Fettembolie bei NH_4OH-Vergiftung. Es schien aber wichtig zu erfahren, wann sich die Fettembolie nach dieser Vergiftung frühestens einstellen könne, wie lange die Möglichkeit

(Gefahr) ihrer Entstehung anhalte, welchen Grad sie erreichen könne, wie sie sich auf die einzelnen Organe verteile und ob sie imstande sei, unmittelbar den Tod herbeizuführen.

Um diese Fragen zu klären, vergifteten wir versuchsweise Tiere sowohl mit NaOH wie auch mit NH_4OH. Die Versuchstiere (Kaninchen) liessen wir zunächst einen Tag lang hungern und führten dann das Gift durch die Magensonde ein.

Gruppe I umfasst 34 Kaninchen, die mit einer 1-2-3-5-10%igen NaOH-Lösung,

Gruppe II 32 Kaninchen, die mit einer 1-2-10%igen NH_4OH-Lösung vergiftet wurden.

Die Organe der Tiere wurden sofort nach dem Tode in 4% Formalin fixiert und die Gefrierschnitte mit Sudan III-Hämatoxylin gefärbt. Die Ergebnisse erhellen aus nachstehenden Tabellen (S. 526/27).

Aus Tabelle I (NaOH-Vergiftungen) geht hervor, dass fast bei allen — bei 32 von 34 Tieren = 94% — mit NaOH vergifteten Versuchstieren Fettembolien entstanden sind. Bei den 7 Stunden nach der Vergiftung verendeten Tieren (Nr. 1 und 2) waren schon deutliche Fettembolien zu sehen, diese waren aber auch noch am 9. und sogar am 12. Tage nach der Vergiftung (Nr. 32 und 33) nachzuweisen. Das von den Fettembolien bevorzugte Organ ist die Lunge, hier erreichten diese auch die schwerste Form. Ziemlich häufig fanden sich noch Fettembolien im Plexus chorioideus des Gehirns (unter 34 Tieren bei 24 = 70%), etwas seltener in den Nieren (11 = 32%) und bloss ausnahmsweise in der Milz und Leber (2 bis 3 Fälle = 5 bis 10%); in den anderen Organen waren keine Fettembolien zu finden.

Die Schwere der Fettembolien der Lungen wird je nach dem Grad mit +, ++, +++ oder ++++ bezeichnet: + bedeutet Fettembolien bloss in wenigen Capillaren; ++ = in zahlreichen Capillaren und in mehreren Präcapillaren; +++ = nicht nur in den Capillaren und Präcapillaren, sondern auch in einigen kleinen Arterien und Venen und ++++ schliesslich besagt, dass sich Fettembolien auch in mehreren kleineren und mittelgrossen Arterien und Venen vorfanden.

Die Fettembolie der Capillaren und Präcapillaren stellt eine homogene Masse dar, in den Arterien und Venen ist sie aber meist aus grösseren und kleineren Tropfen zusammengesetzt. Bei den 12 Stunden nach der Vergiftung verendeten Tieren (Nr. 4 und 5) ist in den Lungen schon die von *Beneke*[1], *Wuttig*[16] u. a. für ein Zeichen der Fettspaltung angesprochene Vakuolenbildung oder die Bildung eines gezackten Randes an den einzelnen Fettpfropfen zu beobachten. Ähnliche Erscheinungen sind später noch deutlicher zu sehen. Daneben finden sich jedoch auch in den mehrere Tage alten Fällen Fettembolien in der Mehrzahl, die keine Resorptionserscheinungen aufweisen. Dies spricht dafür, dass bei Lungenvergiftungen die Fettembolie *schon in wenigen*

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Tabelle I.
Fettembolie in den verschiedenen Organen bei experimenteller NaOH-Vergiftung.

Nr.	Körpergewicht in g	Dosis der NaOH ccm	%	g	Fettembolie der Lungen	des Plexus chorioideus	der Nieren	der Leber	der Milz	Lebensdauer nach der Vergiftung
1	2680	67	3	2,01	+	+	—	—	+	7 Stdn.
2	2700	75	3	2,25	++	+	—	—	—	7 „
3	2650	25	10	2,50	++++	++	—	—	—	10¼ „
4	2400	75	2	1,50	+	+	—	—	—	12 „
5	2600	65	3	1,95	++++	—	+	—	—	12 „
6	2900	73	3	2,12	+++	++	+	—	—	15 „
7	3100	78	3	2,34	+++	+	—	—	—	16 „
8	2800	72	3	2,26	+	—	—	—	—	17 „
9	2500	43	3	1,29	++	++	—	—	—	17 „
10	3200	80	3	2,40	++++	—	+	—	—	18 „
11	2100	25	5	1,25	+	+	—	—	—	22½ „
12	3000	45	5	2,25	+	++	—	—	—	23 „
13	2700	45	3	1,35	+	+	—	—	—	24 „
14	2980	74	3	2,23	+	—	—	—	—	24½ „
15	2600	75	2	1,50	++	+	+	—	+	26 „
16	2835	71	3	2,13	++	+	+	+	—	27½ „
17	3200	75	2	1,50	—	+	+	—	—	28 „
18	2470	75	3	2,25	++	++	+	—	—	28 „
19	2650	45	3	1,35	+	++	+	—	—	28 „
20	2700	46	3	1,38	+++	+	—	—	—	32 „
21	3170	79	3	2,37	+++	++	+	+	—	33 „
22	3600	100	2	2,00	+++	+	—	—	—	35 „
23	4000	75	3	2,25	+++	++	—	—	—	37 „
24	2200	25	5	1,25	—	+	—	—	—	37 „
25	3200	75	2	1,50	+	+++	—	+	—	40 „
26	4200	75	2	1,50	—	+++	—	—	—	40 „
27	3800	75	3	2,25	++	—	+	—	—	2½ Tage
28	3570	60	3	1,79	+	—	—	—	—	3½ „
29	2800	75	2	1,50	—	+	—	—	—	3½ „
30	2660	44	3	1,32	+	+	—	—	—	5 „
31	3160	75	1	0,75	—	—	—	—	—	5 „
32	2560	12,5	10	1,25	+++	—	—	—	—	9 „
33	3200	75	2	1,50	—	—	+	—	—	12 „
34	2550	12,5	10	1,25	—	—	—	—	—	14 „

Stunden entstehen kann, um sich in den folgenden Tagen allmählich auszubreiten und schliesslich ihren Höhepunkt zu erreichen. In den +++ und ++++-Fällen war die Fettembolie so schwer, dass sie auch an sich den Tod der Tiere herbeiführen konnte, in den + und ++-Fällen waren es ihre Folgen, die den Tod der Tiere beschleunigten.

In den Lungen konnten wir nämlich in der Umgebung der Fettembolien meist kleinere oder grössere Blutungen finden, die zahlreiche Alveolen ausfüllen. In derartigen Fällen waren die Fettropfen nicht selten auch in die

Tabelle II.
Fettembolie in den verschiedenen Organen bei experimenteller NH_4OH-Vergiftung.

Nr.	Körpergewicht in g	Dosis der NH_4OH ccm	%	g	Fettembolie der Lungen	des Plexus chorioideus	der Nieren	der Leber	des Herzens	Lebensdauer nach der Vergiftung
1	2450	75	2	1,5	++	+	—	—	—	½ Stde.
2	3000	75	2	1,5	++		—	—	—	½ „ u. 7′
3	2550	50	2	1,0	++	—	—	—	—	¾ „
4	2480	50	2	1,0	++	—	—	—	—	1 „
5	2375	50	2	1,0	+	+++	—	—	—	1 „
6	2400	75	2	1,5	+	+	—	—	—	1 „
7	2800	75	2	1,5	+++		—	—	—	1 „ u. 5′
8	3000	75	2	1,5	+++	++	+	+	—	1 „ u. 5′
9	3050	75	2	1,5	+++		+	—	—	1 „ u. 20′
10	2710	75	2	1,5	++	—	—	—	—	1 „ u. 25′
11	2800	50	2	1,0	++++	+++	+	—	—	1½ „
12	2800	50	2	1,0	+++	+	—	—	—	1½ „
13	2600	50	2	1,0	++	+	—	—	—	2 Stdn.
14	2450	75	2	1,5	++	—	—	—	—	2 „ u. 22′
15	2850	75	2	1,5	++	+	+	—	—	2½ „
16	2550	75	2	1,5	++	+	—	—	—	2½ „
17	2200	50	1	0,5	+	+	—	—	—	20 „
18	2755	50	2	1,0	+	—	—	—	—	25¼ „
19	2300	50	2	1,0	+++		—	—	—	25¼ „
20	2500	50	1	0,5	+		—	—	—	27 „
21	2450	50	2	1,0	+		+	—	—	28 „
22	2950	75	2	1,5	++	++	—	—	—	29½ „
23	2550	10	10	1,0	+	+	—	—	+	35 „
24	4000	75	2	1,5	+++	—	—	—	—	2½ Tage
25	2500	50	2	1,0	+	—	—	—	—	3 „
26	3200	50	1	0,5	—	+	—	—	—	7 „
27	2700	10	10	1,0	+	—	—	—	—	12 „
28	3200	50	2	1,0	++	+	—	—	—	14 „
29	2700	50	1	0,5	—	+	—	—	—	15 „
30	2520	10	10	1,0	++	—	—	—	—	17 „
31	2650	50	2	1,0	—	—	—	—	—	17 „
32	2350	50	2	1,0	+	—	—	—	—	36 „

Alveolen gelangt. Daneben hatten sich auch in den wenigen Stunden alten Fällen Ödem oder aus einkernigen und gelapptkernigen weissen Blutzellen bestehende, entzündliche Herde (Pneumonie) gebildet. Dieser Befund steht mit der Beobachtung von *Jankovich*[8] im Einklang, dass Fettembolien (traumatischen Ursprungs) am ehesten in der Umgebung der dichten Lungenherde (interstitielle Pneumonie, Bronchopneumonie, Tbc. usw.) zu finden sind. Als Ursache nahm *Jankovich* an, dass diese Gebiete schlechter mit Blut versorgt seien.

In unsren Fällen (Vergiftung) halten wir es jedoch für wahrscheinlicher, dass die Fettembolie die primäre Schädigung darstelle und durch die

Behinderung des Blutkreislaufes zur hochgradigen Stauung und diese wieder zum Bersten der Gefässe (Capillaren, Präcapillaren) fallsweise sogar — per diapedesim — zur Entstehung des Ödems und der Blutungen führe. Auf diese Weise gelangen die Fettropfen in die Alveolen, in die Gebiete aber, die durch Stauung oder Blutungen befallen sind, wandern alsbald weisse Blutzellen in grossen Mengen ein. Dies konnten übrigens auch schon *T. Kojo*[10] u. a. mit Hilfe ihrer an Kaninchen ausgeführten experimentellen Fettembolien nachweisen. Man darf demnach annehmen, dass die bei jenen endogenen und exogenen Vergiftungen oder anderen Schädigungen, die mit Fettembolien

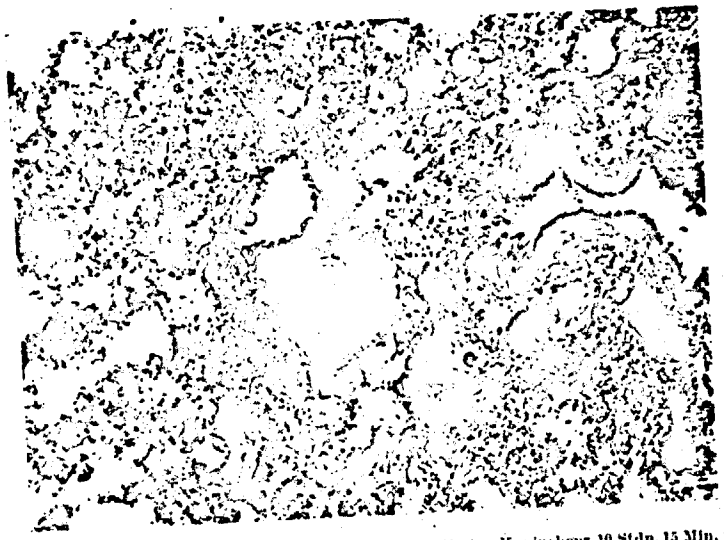

Abb. 1. *Fettembolie* in der *Lunge* eines mit 10% NaOH vergifteten Kaninchens 10 Stdn. 15 Min. nach der Vergiftung. Zahlreiche, die Capillaren, Präcapillaren und das Lumen einer kleineren Vene ausfüllende Fettpfropfe. In den Alveolen Serum (Stauung) und zahlreiche weisse Blutzellen. Gefrierschnitt. Färbung mit Sudan III-Hämatoxylin (Kaninchen Nr. 3).

einhergehen, entstandenen pneumonischen Herde ihr Zustandekommen nicht so sehr der Verschlechterung der Herztätigkeit bzw. einer anderen Ursache zu verdanken haben wie vielmehr den Fettembolien selbst bzw. den durch diese hervorgerufenen Kreislaufstörungen (Blutungen, Ödem). Der Nachweis der Richtigkeit dieser Annahme auch in bezug auf den Menschen würde einerseits zur Klärung ätiologischer Fragen beitragen, andererseits neue therapeutische Möglichkeiten bedeuten.

Will man versuchen, die Entstehung der Fettembolie zu erklären, dann kann man an zwei Wege denken: Zunächst sei daran erinnert, dass das Fett normalerweise im Blutplasma in feinen, mikroskopisch unsichtbaren, unfärbbaren Tröpfchen verteilt (emulgiert) ist. Infolge der Vergiftung kommt es nun zu einer Störung des chemischen Gleichgewichts (Azidose?) im Organismus.

Abb. 2. *Fettembolie* in den *Lungen*-Capillaren und Präcapillaren sowie in einigen kleineren Venen 33 Stdn. nach der Vergiftung mit 3% NaOH. In der Umgebung der Embolien ist das Lungengewebe vollkommen atelektatisch, die Alveolen sind mit zahlreichen weissen Blutzellen gefüllt, darunter vermengt finden sich wenig Erythrocyten und Serum (Pneumonie). Gefrierschnitt; Färbung: Sudan III-Hämatoxylin (Kaninchen Nr. 21).

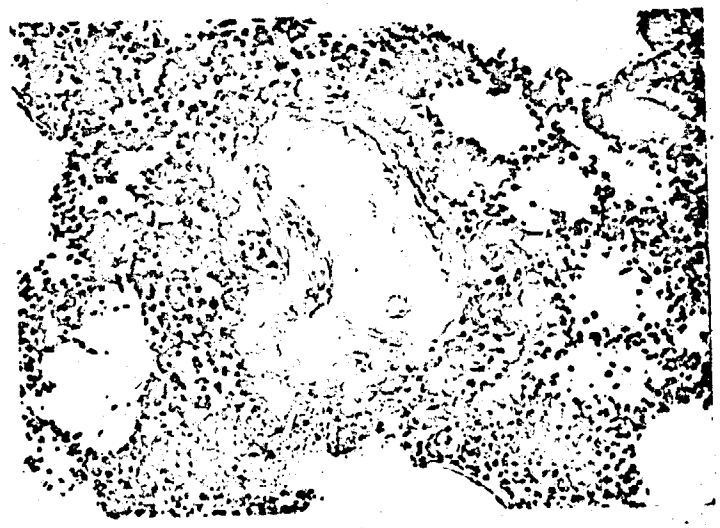

Abb. 3. *Fettembolie* in der *Lunge* 37 Stdn. nach der Vergiftung mit 3% NaOH. Das Lumen der mittelgrossen Arterie ist durch eine aus zahlreichen Fettkügelchen bestehenden Embolie verlegt. Gefrierschnitt; Sudan III-Hämatoxylin (Kaninchen Nr. 23).

Diese könnte nun bewirken, dass entweder das Fett sich zu grösseren, nunmehr sichtbaren und färbbaren Tropfen zusammenballt (etwa wie bei der Fettphanerose der Zellen) und dadurch in den Organen, in erster Linie in den Lungen, in der Form von Embolien nachweisbar wird, oder aber dass das Reservefett aus den Depots mobilisiert und somit eine echte Lipämie hervorgerufen wird. Im ersteren Fall ist anzunehmen, dass der Fettgehalt des Blutes unverändert bleibe oder durch die Bildung der Fettembolien abnehme, im zweiten Fall müsste der Fettgehalt des Blutes zunehmen. Mit welcher dieser beiden Möglichkeiten bei der Laugenvergiftung zu rechnen sei, wird die quantitative Untersuchung des Blut-Fettgehaltes zeigen; diesbezügliche Nach-

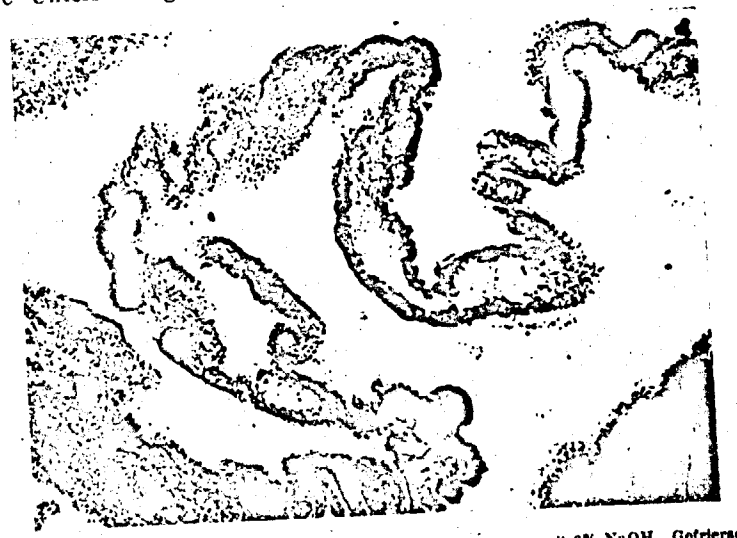

Abb. 4. *Fettembolie* im *Plexus chorioideus* 17 Stdn. nach der Vergiftung mit 3% NaOH. Gefrierschnitt; Sudan III-Hämatoxylin (Kaninchen Nr. 9).

forschungen sind bei uns im Gang. Es erscheint uns jedoch *schon jetzt wahrscheinlich, dass auch bei der Laugenvergiftung der zweite Fall vorliege, dass also die Fettembolie der infolge der Vergiftung entstandenen Lipämie zuzuschreiben sei, wie wir dies in bezug auf die NH_4OH-Vergiftung schon nachgewiesen haben.*

Aus Tabelle II ist zu ersehen, dass nach experimenteller NH_4OH-Vergiftung in der überwiegenden Mehrzahl der Fälle (etwa in 97%) Fettembolien entstehen. Am häufigsten und in der schwersten Form sind sie in den Lungen (90%) anzutreffen, ziemlich oft kommen sie auch im Plexus chorioideus des Gehirns (50%) vor, bedeutend seltener in den Nieren (etwa 20%) und bloss ausnahmsweise in der Leber oder im Herz (etwa 3%).

In der Lunge konnten wir schon ½ Stunde nach der Vergiftung eine ausgeprägte Fettembolie nachweisen. Bei den in 3 Tagen verendeten Tieren

Abb. 5. *Fettembolie* im *Plexus chorioideus* 33 Stdn. nach der Vergiftung mit 3% NaOH. Gefrierschnitt; Sudan III-Hämatoxylin (Kaninchen Nr. 21).

(Nr. 1 bis 25) war in den Lungengefässen ausnahmslos Fett zu finden. In vereinzelten Fällen (Nr. 26, 29 und 31) war zwar am 7., 15. bzw. 17. Tag nach der Vergiftung in der Lunge keine Fettembolie zu sehen, in anderen Fällen war

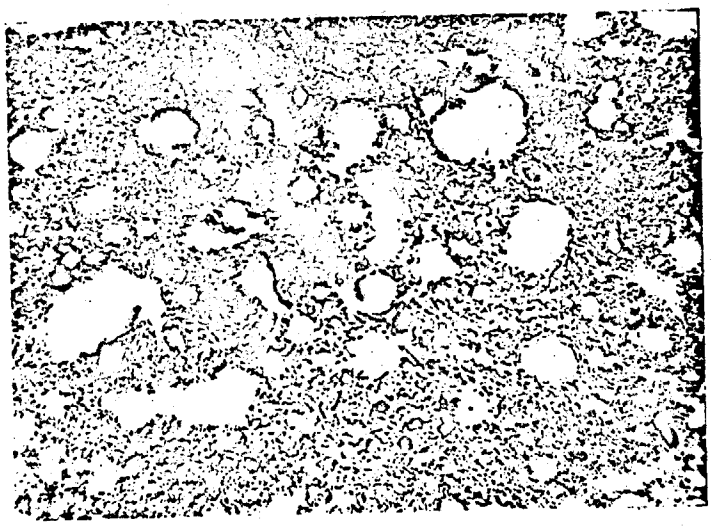

Abb. 6. *Fettembolie* in den Capillaren und Präcapillaren der *Lunge* 1 Stde. 30 Min. nach der Vergiftung mit 2% NH_4OH. Gefrierschnitt; Sudan III-Hämatoxylin (Kaninchen Nr. 11).

diese jedoch 12, 14, 17 und auch noch 36 Tage nach der Vergiftung deutlich wahrnehmbar. Im Fall Nr. 26 fand sich am 7. Tag bei negativem Lungenbefund eine Fettembolie im Plexus chorioideus des Gehirns. Unsere Befunde stimmen vollkommen mit unserer früheren Feststellung überein, wonach 6 bis 8 Tage nach der NH_4OH-Vergiftung der gesteigerte Fettgehalt des Blutes quantitativ nachzuweisen ist. Bei den nunmehr mitgeteilten Versuchen konnten wir zeigen, dass die sich nach der NH_4OH-Vergiftung einstellende Lipämie auch noch mehrere Wochen nach erfolgter Vergiftung (36 Tage!) bestehen und die Fettembolie hervorrufen kann. Das Zustandekommen der Fettembolie

Abb. 7. *Fettembolie* in der *Lunge* 1 Std. 30 Min. nach der Vergiftung mit 2% NH_4OH. Das Lumen der grösseren Arterie ist durch eine aus grossen Fettropfen bestehende Embolie erfüllt. Verstreut sind auch in mehreren Capillaren Fettembolien zu sehen. Gefrierschnitt; Sudan III-Hämatoxylin (Kaninchen Nr. 11).

traumatischen Ursprungs konnten *Melchior*[11], ferner *Jankovich*[9] 14 Tage, *Siegmund* 18 Tage nach erfolgtem Trauma (Knochenbruch) nachweisen.

In den +-Fällen fand sich Fett auch hier bloss in den Capillaren der Lunge, bei ++ in den Capillaren und Präcapillaren, bei +++ auch in kleineren Arterien und Venen und in den mit ++++ bezeichneten Fällen war Fett auch in den mittleren und grösseren Arterien und Venen anzutreffen. Die mit 2, 3 oder 4 + bezeichneten Lungen-Fettembolien waren so schwer, dass sie auch an sich den Tod der Tiere verursachen konnten. Die zweitschwersten Fettembolien fanden sich im Plexus chorioideus des Gehirns, hier waren die Gefässe oft mit Fett prall gefüllt. Die Gefässe bzw. Capillaren der anderen Organe enthielten bloss wenig Fett.

Neben der Fettembolie fanden sich (z. B. in den Fällen 1 und 2) schon ½ Stunde nach der Vergiftung schweres Lungenödem sowie schwere Blutungen,

1 bis 2 Stunden später ausserdem auch noch entzündliche Herde, die zum grossen Teil aus einkernigen und gelapptkernigen weissen Blutzellen bestehen. In den Gebieten mit Blutungen enthalten einige Alveolen vereinzelte Fetttropfen. Bei der Entstehung dieser Lungenveränderungen spielte offenbar auch die Fettembolie die Hauptrolle, wie dies weiter oben in bezug auf die NaOH-Vergiftung erörtert wurde.

Der Nachweis der Fettembolie in den Lungen und im Gehirn (Plexus chorioideus) stellt auch an sich eine Erklärung jener plötzlichen Todesfälle dar, die sowohl bei der NaOH- wie auch bei der NH_4OH-Vergiftung mitunter in

Abb. 8. *Fettembolie* im *Plexus chorioideus* 1 Std. 30 Min nach der Vergiftung mit 2% NH_4OH. Gefrierschnitt; Sudan III-Hämatoxylin (Kaninchen Nr. 11).

wenigen Stunden oder Tagen ohne schwerere lokale Veränderung (Perforation, Zerstörung des Kehlkopfes, Larynxödem oder dgl.) auftreten und die man bisher meist als Shockwirkung verbucht hatte.

Auf die Bedeutung und die Folgen der Fettembolien in den anderen Organen wollen wir hier nicht zurückkommen, da dies in meinen früheren einschlägigen Mitteilungen schon eingehend besprochen worden ist. Es sei hier bloss kurz erwähnt, dass sich das diesbezüglich bei der NH_4OH-Vergiftung gesagte auch auf die NaOH-Vergiftung bezieht.

Auch auf das fast unübersehbare Schrifttum der Fettembolie soll hier nicht näher eingegangen werden, da dies den Rahmen dieser Mitteilung weit überschreiten würde. Diesbezüglich sei auf die zusammenfassende Mitteilung von *T. Kojo* und *Seemann*[14] verwiesen. Der erstere dieser beiden Verfasser berichtet ausführlich über das ältere Schrifttum (bis 1922), der letztere über

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das neuere (bis 1931). Nicht unerwähnt soll bleiben, dass Fettembolien nach gewissen Vergiftungen auch schon von anderer Seite beobachtet worden sind: *Puppe*[13] bei Phosphor- und *Winogradow*[15] bei der Chlorkaliumvergiftung. In bezug auf die NaOH- bzw. NH_4OH-Vergiftung konnten wir derartige Angaben im einschlägigen Schrifttum nicht antreffen.

Zusammenfassung.

1. Auf Grund der histologischen Untersuchung liess sich an den Organen der Versuchstiere (Kaninchen) einwandfrei feststellen, dass es bei der NaOH-Vergiftung in der überwiegenden Mehrzahl (94%) der Fälle ebenso zur Bildung von Fettembolien komme wie bei der NH_4OH-Vergiftung (97%).

2. Die Fettembolien waren bei NaOH- (Laugen-) Vergiftung frühestens 7 Stunden, bei NH_4OH-Vergiftung schon in ½ Stunde nach der Vergiftung nachweisbar.

3. Spätestens waren Fettembolien noch vorzufinden: 9 bzw. 12 Tage nach der NaOH- und 36 Tage nach der NH_4OH-Vergiftung.

4. Bei beiden Vergiftungen traten die Fettembolien in der schwersten Form und am häufigsten (80 bis 90%) in den *Lungen* auf. Sowohl in bezug auf die Häufigkeit wie auch auf die Schwere folgen hierauf der Reihe nach: Plexus chorioideus des Gehirns (50 bis 70%), Nieren (20%). Am seltensten waren sie in der Leber, Milz und im Herz (5 bis 10%) anzutreffen.

5. Die Schwere der Fettembolie kann bei beiderlei Vergiftungen einen so hohen Grad erreichen, dass sie auch an sich zum Tode führen kann. (Lungen-, Gehirn-Fettembolien). Dieser Umstand erklärt das mitunter plötzliche Eintreten des Todes bei derartigen Vergiftungsfällen, das bisher als Shockwirkung angesprochen worden ist.

6. Die bei der NaOH-Vergiftung beobachtete Fettembolie ist offenbar ebenso als Folge der sich auf die Vergiftung einstellenden *Lipämie* aufzufassen, wie dies an der Hand chemischer Untersuchungen schon früher in bezug auf die NH_4OH-Vergiftung festzustellen war.

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Fiegl, Fritz, and Vinzenz Anger, 1972

Spot Tests in Inorganic Analysis, 6th English Edition

Elsevier Publishing Co., Amsterdam

FDA - GRAS Supplemental Info.

Company Code: [redacted]

Substance Reported: 0192 SODIUM HYDROXIDE

Supplemental Information: Boxes 53 and 55

To produce invert syrups, solutions of cane or beet sugar are acidified with hydrochloric acid to approximately pH 2, and heated until the desired degree of hydrolysis has taken place. The reaction is then stopped by neutralizing with an alkali such as sodium or calcium hydroxide.

The acid reacts with the alkali to form the appropriate neutral salt, either sodium or calcium chloride and water. At times invert syrups are shipped at a pH lower than neutral at the customer's request. The most extreme example is pH 5.0 at which level 15 ppm of free hydrochloric acid remains in a typical syrup. Attached is a description of the process which is taken from the following book:

MANUFACTURE AND REFINING OF RAW CANE SUGAR
Baikow, V. E.
Elseiver Publishing Co., 1967

Production of Liquid Sugar from Refined Crystallized Sugar

Granulated sugar dry or still wet after discharge from centrifugals can be melted at 20°C (68°F) in pure clean water to have a sugar solution at 67.5° Brix. This is sucrose syrup or liquid sucrose. However, if the granulated sugar is not absolutely white it will give a yellow cast to the solution. If this is undesirable, the dissolved refined sugar can be treated with decolorizing vegetable carbon and refiltered, producing a water-white liquid sucrose. If partially inverted liquid sugar is desired, one portion of sucrose liquid can be inverted with HCl, or organic acid (citric) and blended with the uninverted portion. Since Brix of partially inverted liquid sugar should be about 77.0, additional dry sugar can be dissolved in partially invert liquor until desired % solids is reached. A common practice for production of neutralized invert syrup is to invert melted granulated sugar at 180–185°F (82–85°C) with chemically pure hydrochloric acid. The liquid sugar adjusted to about pH 2 will invert almost completely within half an hour. After it is blended with the non-inverted portion it may be necessary to raise the pH of the blend with sodium hydroxide, bicarbonate or carbonate to stop further inversion. The % inversion can be determined by the formula:

$$\text{Invert \% on Solids} = \frac{\text{Brix} - \text{Pol.}}{0.013 \times \text{Brix}}$$

Example: The Brix of liquid sugar is 77.0 and Pol. = 29.0

$$\text{\% inversion on dry basis} = \frac{77 - 29}{0.013 \times 77} = 48\%$$

Generally, three methods are used for preparation of invert liquid sugar from dry granulated[4]:

(1) To dissolve all necessary sugar in water, heat, invert with acid, and neutralize at the right moment.
(2) To dissolve only a part of the sugar to all the water, heat, invert completely and neutralize. Then, to add and dissolve dry sugar to the desired Brix.
(3) To produce completely inverted liquor, which can be added to sucrose liquor in necessary proportion to obtain final invert liquid sugar of desired Brix and invert content.

Food Protection Committee, National Research Council, Washington, D.C., 1972

Table 2

Food Protection Committee, National Research Council, Washington, D.C., 1972

Table 13A

Pages 127-128

Food Protection Committee, National Research Council, Washington, D.C., 1972

Table 3

Food Protection Committee, National Research Council, Washington, D.C., 1972

Table IIB

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Chemicals Used in Food Processing

National Academy of Sciences - National Research Council, Washington, D.C.

Pages 45-46

Furia, Thomas E., 1972
CRC Handbook of Food Additives, 2nd Edition
Chemical Rubber Company, Cleveland, Ohio
Pages 925; 942

Detoxification and Deallergenization of Castor Beans[1]

J. Am. Oil Chemists' Soc. 37: 142-8 (1960)

H. K. GARDNER, JR., E. L. D'AQUIN, S. P. KOLTUN, E. J. McCOURTNEY, H. L. E. VIX, and E. A. GASTROCK, Southern Regional Research Laboratory,[2] New Orleans, Louisiana

DURING THE PAST FOUR YEARS acreage planted to castor beans has increased almost five-fold, from 5.1 to 24.0 thousand acres, and the annual domestic production has increased almost fifteen-fold, from 3.4 to 49.9 million pounds (1). This is attributed to the development of high-yielding bean varieties and improved harvesting machines. These improvements have made production of this crop more attractive to farmers as a replacement for crops in surplus supply or with acreage restrictions.

A very important economic factor affecting the further development of castor beans is that the meal

[1] Presented at the fall meeting, American Oil Chemists' Society, Los Angeles, Calif., September 28–30, 1959.

[2] One of the laboratories of the Southern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, New Orleans, Louisiana.

or pomace, produced by present commercial extraction processes, has value only as a fertilizer. It can be used neither as animal feed nor in the preparation of industrial products. The pomace contains three undesirable constituents (12): a violently poisonous heat-labile protein called ricin; a toxic alkaloid component called ricinine; and a powerful and very stable allergen, known as CB-1A (11). The ricin, which is present in relatively large amounts, can be detoxified in normal practice by treating or cooking the beans or pomace with steam (2, 3). The ricinine is present in small amounts and, because it is not seriously toxic, it is not considered particularly detrimental (3). The allergen, a powerful, nontoxic, protein polysaccharidic fraction, is the most troublesome constituent. CB-1A represents about 12.5% of the weight of the pomace, as determined by the antigen-dilution precipitin test.

With the anticipated increase in castor production and the appearance of larger amounts of the pomace in the fertilizer market, growers, handlers, processors, and public health officials have expressed deep concern over the risk that farmers and farm stock may become seriously sensitized to the potent castor bean allergen. This also applies to operating personnel in castor bean-extraction plants and to residents in the vicinity of such installations (2).

It is the consensus among castor bean processors that the industry needs a practical and inexpensive method for detoxifying the ricin and completely destroying or inactivating the allergenic factor without affecting the quality of the oil product and, if possible, without seriously degrading the meal product for industrial uses.

In work at this laboratory which demonstrated that castor beans can be directly solvent-extracted successfully on a bench-scale by the filtration-extraction process (4), the addition of sodium hydroxide in the moist cooking of flaked castor bean meats had been explored as a possible means of improving the color and reducing the fatty acid content of the oil product. Assays of the pomaces so produced indicated that the ricin could be completely detoxified and that the allergen content could be reduced by as much as 98% as a direct result of the the alkali treatment. It was on the basis of these promising results that the present research was undertaken.

This paper will report the results of probing experiments in which were investigated a number of types of treatments for the detoxification and deallergenization of castor beans and/or pomaces, with the anticipation that one or more selected conditions would point up potential avenues for further research. The treatments were mainly of the practical type, which could be readily integrated or combined with conventional processes of solvent extraction. They included cooking of the castor bean meats and pomaces under various conditions of moisture and temperature; dry-heat treatment at elevated temperatures; treatments with chemicals such as sodium hydroxide, hydrochloric acid, anhydrous ammonia, sodium hypochlorite, sodium chloride, ammonium sulfate, potassium permanganate, formaldehyde, urea, and combinations of the above; and biological treatments, including aerobic fermentation and enzymatic digestion. The results indicate that all of the treatments were effective in destroying or inactivating the allergenic factor to varying degrees. Some yielded pomaces of practically zero allergen content, as measured by the assay procedures described herein for the determination of allergen content.

Raw Materials and Equipment

The castor beans used in this investigation were obtained from commercial processors and were representative of current oil mill receipts. Of the four lots of beans, three were domestically produced, and one was of Brazilian origin. Varietal information was not obtainable. Each lot had been thoroughly cleaned and partially decorticated. Each was well mixed to insure uniformity and stored under refrigeration at 40°F. while awaiting processing. Pertinent data on the lots of beans are tabulated below:

Lot No.	A	B	C	D
Source	Domestic	Foreign	Domestic	Domestic
Moisture, %	4.4	4.8	4.4	4.5
Hull, coat, %	5	8	16	2

Flaking was carried out with a set of 12-in.-in-diam., one-pair-high smooth rolls. For cooking the prepared flakes, the following bench- or pilot-plant units were employed: a 15-lb. capacity vessel (5) equipped with agitator, reflux condenser, and spray injector for addition of steam, water, or chemical solutions; a 1-lb. capacity vessel (6), similarly equipped; and a 12-qt. household type of pressure cooker. The ammoniation experiments were conducted in an electrically-heated pilot-plant-size pressure reactor. For heat-treatment studies a forced-draft, electrically-heated cabinet dryer was used. The mixing and slurrying operations were conducted with a Waring Blendor or a 3-speed Hobart mixer equipped with a steam-jacketed bowl.

Solvent extraction of the treated materials was carried out with the 5¼-in.-in-diam. (500-g. capacity) bench-scale filter test unit used for evaluation of oil-bearing materials for filtration-extraction, previously described (7); or with a 1½-in.-in-diam. (120-g. capacity) unit.

Experimental Treatments

The treatments reported have been classified into Groups I through IV and are itemized in Tables I through IV, respectively. Groups I and IV comprised chemical and biochemical treatments of flaked castor bean meats and/or pomaces; Group II, treatments of pomaces with ammonia at elevated pressures; and Group III, heat treatment of pomaces at elevated temperatures.

In order to facilitate efficient extraction of the oil and to insure thorough detoxification of the ricin, it was decided at the outset to employ as a standard procedure, in most of the experiments, the initial steps, as described below, of moist-cooking the flaked raw meats, followed by hexane-extraction to recover the pomace.

The moist-cooking operation comprised the following sequence of steps: preheating the flaked castor bean meats, of 0.010-in. thickness, to about 170°F.; adjustment of moisture content to about 16% by the addition of water with or without chemicals; heating to 212–215°F.; maintaining this temperature by continuous heating under reflux conditions over a 12- to 15-min. period; and drying the cooked flakes to 7–9% moisture content in 10–20 min.

Solvent-extraction of the cooked flaked meats was carried out with the standard bench-scale filter test

TABLE I

Allergen Reduction by Alkaline and Nonalkaline Treatments of Castor Bean Meats and Pomaces

Experiment Group I

Exp. No.	Bean	Treatment				CB-1A in Pomaces		
		Meats			Pomace, alkali	Ring test	Precipitin test	Schultz-Dale test
		Moist cooking	Alkali	Solvent-extraction				
			%		%	%	%	%
1a	C	No	No	Isopropyl alcohol	No	12.5	12.5	.....
b	C	Yes	No	Isopropyl alcohol	No	12.5	6.3	.....
c	C	No	No	Hexane	No	12.5	12.5	.....
d	C	Yes	No	Hexane	No	6.3	3.1	.....
e	C	Yes	NaOH(1%)	Hexane	No	0.05	0.4	.....
f	C	Yes	NaOH(1%)[a]	Hexane	No	0.05	0.4	.....
2a	B	Yes	No	Hexane	No	1.6	6.3	.....
b	B	Yes	NaOH(1%)	Hexane	No	0.025	0.2	.....
3a	A	Yes	No	Hexane	No	3.1	3.1	.....
b	A	Yes	NaOH(1%)	Hexane	No	0.05	0.4	.....
4a	D	Yes	No	Hexane	NaOH(2%)[b]	0.0	1.6	.....
b	D	Yes	No	Hexane	NaOH(2%)[c]	1.6	6.3	.....
c	D	Yes	NaOH(2%)[d]	Hexane	No	0.0	0.0	.....
d	D	Yes	KOH(2%)[d]	Hexane	No	0.0[e]	0.0[e]	.....[f]
e	D	Yes	$Ca(OH)_2$(2%)[d]	Hexane	No	0.8	3.1	.....
						0.4	6.3	.....

[a] Extended cooking period (60 min.). [b] Solvent-damp pomace moistened to 20% H_2O and autoclaved with NaOH at 20 psig. [c] Solvent-damp pomace moistened to 20% H_2O and heated with NaOH at 0 psig. [d] Meats autoclaved at 20 psig. [e] Duplicated assay by Allergens Laboratory. [f] Not determinable. Doses reactive with sensitized tissue were of the same order as doses with the nonsensitized tissue.

unit, using hot hexane (130°F.) washes, and a solvent-meats ratio of 2:1 unless otherwise specified. The solvent-damp extracted pomace was then air-desolventized.

In the treatments of raw meats the chemicals were added during the cooking operation. In other experiments the pomaces so produced were subjected to various further treatments. In all cases the extent of detoxification and deallergenization achieved was determined by assay of the final pomaces by the test methods described herein for percentage of ricin and percentage of allergen (CB-1A).

The various experimental treatments are described below and in Tables I through IV.

GROUP I. ALKALINE AND NONALKALINE TREATMENTS OF CASTOR MEATS AND POMACES

Table I gives the conditions for alkaline treatments employing NaOH, KOH, or $Ca(OH)_2$ in the moist-cooking operation and for heat treatments of the pomace with NaOH. Alkaline and nonalkaline (control) treatments of flaked meats from four different lots of beans were studied.

GROUP II. AMMONIATION OF POMACES PRODUCED BY HEXANE-EXTRACTION OF ALKALI-COOKED CASTOR MEATS

Pomaces were ammoniated in the pressure reactor in a series of eight tests, based on statistical design (Latin Square technique). The experiments were conducted by using two selected conditions for each of the important variables, *i.e.*, moisture content, temperature, ammonia pressure, and treatment time (Table II).

GROUP III. HEAT TREATMENT OF POMACES PRODUCED BY HEXANE-EXTRACTION OF ALKALI- AND NONALKALI-COOKED CASTOR MEATS

Table III gives the conditions for the heat treatment of pomaces prepared by both nonalkaline and alkaline treatments of flaked meats from two lots of beans. For the second lot (D) the effect of time-temperature for the heat treatments was studied. The pomaces were placed in covered aluminum containers equipped with thermocouples and were heated in a dryer capable of developing temperatures up to 500°F.

TABLE II

Allergen Reduction by Ammoniation of Pomace Produced by Hexane-Extraction of Alkali-Cooked Castor Meats[a]

Experiment Group II

Experiment No.	Bean	Treatment				CB-1A in Pomace	
		Moisture	Temp. °F.	Ammonia pressure psig.	Time in min.	Ring test	Precipitin test
		%				%	%
1a[b]	B	8.5	.....	.....	.....	0.4	0.8
b	B	16.9	90	40	45	0.1	0.8
c	B	16.9	90	100	15	0.05	0.8
d	B	16.9	175	100	45	0.025	1.6
						0.05[c]	0.2[c]
e	B	16.9	175	40	15	0.1	1.6
f	B	6.4	90	100	45	0.05	0.8
g	B	6.4	90	40	15	0.05	0.8
						0.10[c]	0.4[c]
h	B	6.4	175	40	45	0.05	0.8
i	B	6.4	175	100	15	0.2	1.6

[a] Flaked meats moist-cooked with 1% NaOH. [b] Control. [c] Sample retested after 1½ months' storage with residual ammonia in a sealed container.

GROUP IV. CHEMICAL AND BIOLOGICAL TREATMENTS OF CASTOR MEATS AND POMACES

Table IV lists the conditions for these exploratory treatments. Pertinent data not included in the table are as follows.

Expt. 1a (NaOCl). Flaked meats were agitated in a Hobart mixer at 170°F. with 16.7% by weight of a 5.25% solution of NaOCl[3] for 15 min. This caused oil flow and agglomeration of the mixture (pH 6.3) into small ball-like masses. After pressure-cooking the mixture was washed with hexane, pulverized, and extracted with hexane.

Expt. 1b (NaOCl). Pomace was agitated in the Hobart mixer at 170°F. with 21% by weight of a 5.25% solution of NaOCl for 15 min. Treated mixture was brought to dryness on a steam bath.

Expt. 1c (NaOCl). Same as 1b except that the flaked meats had been cooked with 1% added NaOH.

Expt. 2a (NaOH, HCHO). Flaked meats were preheated to 170°F. in the small laboratory cooker with 1% by weight of NaOH dissolved in 10% by weight of water. After mixing for 2 min., 8% by weight of formalin (37.4% HCHO, 12.3% methanol, and 50.3% H_2O) was added, and mixing was continued for 2 min.

Expt. 2b (HCl, HCHO). Same procedure as 2a

[3] Clorox is manufactured by the Clorox Company, Oakland, Calif.

TABLE III

Allergen Reduction by Heat Treatment of Pomaces Produced by Hexane-Extraction of Alkali- and Nonalkali-Cooked Castor Bean Meats

Experiment Group III

Experiment No.	Bean	Meats, NaOH	Pomace		CB-1A in Pomace		
			Temp. reached, °F.	Time elapsed, min.	Ring test	Precipitin test	Schultz-Dale test
		%			%	%	%
1a	B	1.0	Control	Control	0.024	0.20	....
b	B	1.0	401 [b]	95	0.0	0.006	0
2a	B	0	Control	Control	1.6	6.2	....
b	B	0	401 [b]	95	0.0	0.006	0
3a	D	1.0	Control	Control	0.4	1.6	....
b	D	1.0	257	52	0.4	1.6	....
c	D	1.0	307	58	0.025	0.8	....
d	D	1.0	349 [a]	85	0.006	0.8	....
e	D	1.0	405 [b]	113	0.0	0.0	....
4a	D	0	Control	Control	3.1	6.2	....
b	D	0	264	52	1.6	6.2	....
c	D	0	302	58	3.1	6.2	....
d	D	0	347 [a]	90	1.6	3.1	....
e	D	0	405 [b]	125	0.0	0.0	....

[a] Pomaces had light-brown, scorched appearance. [b] Final pomaces had dark-brown, charred appearance.

except that 2.5% hydrochloric acid (36.9% HCl) was used in place of NaOH.

Expt. 2c (NaOH, HCHO). The alkaline pomace was agitated in the Hobart mixer for 15 min. at 170°F. with 27% by weight of formalin and 25% by weight of additional water. This mixture was dried to 6–8%.

Expt. 2d (NaOH, HCHO, NaOCl). The alkaline pomace was agitated in the Hobart mixer at 170°F. with 27% by weight of formalin for 5 min. Then 4% by weight of a 5.25% solution of NaOCl was added, and mixing was continued for 10 min. Mixture was dried to 6–8% on steam bath.

Expt. 4 (Urea). Pomace was mixed in a Hobart mixer at 170°F. for 15 min. with 5% by weight of urea dissolved in 180% by weight of water. Treated mixture was dried on a hot plate to 8–10% moisture.

Expt. 5 ($KMnO_4$). Pomace was mixed in a Hobart mixer for 45 min. with 10% by weight of potassium permanganate dissolved in 370% by weight of water. Slurry was heated to 175°F. to accelerate oxidation and was agitated at maximum mixer speed for 3 hrs. After standing over-night, the partially oxidized pomace (black in color) was dried on a hot plate to 5–6% moisture content.

Expt. 6 (Aerobic Fermentation). Pomace was mixed with 200% of tap water and allowed to stand uncovered for 72 hrs. at approximately 80°F. Then the slurry was incubated at 98.6°F. for 100 hrs. Considerable mold formation was observed along with a strong, sweetish odor. Pomace was recovered by drying (hot plate) to 5–6% moisture content.

Expt. 7 (Trypsin). Pomace (pH 8.3) was agitated in a Hobart mixer at 100°F. with 50% by weight of water and 6% by weight of trypsin, an amount equivalent to about 10% by weight of the protein in the pomace. The mixture was stirred vigorously, allowed to stand for 60 min. at an adjusted pH of 8.3, and incubated at 98.6°F. for 90 min. Then it was oven-heated at 212°F. for 90 min. to arrest enzymatic digestion and further dried to 5–6% moisture content.

Analytical Test Procedures

The product pomaces, obtained from the various physical, chemical, and biochemical treatments of the castor beans and pomaces, were assayed for allergen (CB-1A) content by a qualitative antigen-dilution precipitin method with modifications suggested by E. J. Coulson of the Allergens Laboratory, Eastern Utilization Research and Development Division (9), and by the Schultz-Dale test (10). Some of the pomaces were assayed for comparative ricin content by a blood corpuscle agglutination test recommended by the Baker Castor Oil Company (8). Brief descriptions of the test procedures are as follows.

BLOOD CORPUSCLE AGGLUTINATION TEST FOR RICIN

The red blood corpuscle solution was prepared by removing the plasma from citrated whole human blood (citrated guinea pig blood may be substituted) by centrifugation and pipetting, washing the corpuscles three times with a physiological saline solution (0.85% sodium chloride in demineralized water), and then diluting them to 10 times their volume with saline solution. Pomace to be tested is placed in solution by mixing a ground 0.5-g. sample in 49.5 g. of physiological saline solution, allowing it to stand 1 hr. and filtering. It is then stored under refrigeration until used.

The test procedure is as follows. Test tubes are placed in a rack, and 0.9 ml. of saline solution is pipetted into the first tube and 0.5 ml. in each of

TABLE IV

Allergen Reduction by Moist-Cooking of Castor Bean Meats with Chemicals and by Chemical and Biochemical Treatments of Pomaces

Experiment Group IV

Experiment No.	Bean	Treatment		CB-1A in Pomace		
		Meats,[a] chemical	Pomace, chemical or biochemical	Ring test	Precipitin test	Schultz-Dale test
		%	%	%	%	%
.....	D	NaOCl(0.9%)[b]	No	6.3	6.3	.....
.....	D	No	NaOCl(1%)	6.3	6.3	.....
.....	D	NaOH(1%)	NaOCl(1%)	0.8	0.8	.....
.....	D	NaOH(1%), HCHO(3%)	No	0.0	0.0	0.03
.....	D	HCl(0.9%), HCHO(3%)	No	0.0	0.0	0.003
.....	D	NaOH(2%)	HCHO(10%)	0.0	0.0	<0.003
.....	D	NaOH(2%)	HCHO(10%), NaOCl(1.5%)	0.0	0.1	.....
.....	D	NaCl(4%)	No	1.6	6.3	.....
.....	D	$(NH_4)_2SO_4$(3%)	No	6.3	6.3	.....
.....	D	No	Urea(5%)	1.6	6.3	.....
.....	D	No	$KMnO_4$(10%)	1.6	6.3	.....
.....	D	No	Aerobic fermentation	1.6	3.1	.....
.....	D	NaOH(0.7%)	Trypsin(6%)	0.0	0.05	0.15

[a] All treated meats defatted by hexane-extraction. [b] Autoclaved at 20 psig. pressure.

the remaining tubes. To the first tube 0.1 ml. of the pomace solution is added and mixed, using a small glass rod. One-half or 0.5 ml. of this solution is transferred to the second tube and mixed; a like amount is transferred to the third tube, etc., down to tube No. 10. Then, to each tube, 0.5 ml. of red blood corpuscle solution is added and thoroughly mixed. Thus the dilutions of the pomace solutions in the 10 tubes are 1:10, 1:20, 1:40, etc., and 1:5120, respectively. After standing for 5 min., the tubes are centrifuged exactly 2 min. Observations are then made relative to the appearance and characteristics of the red blood agglutination. The tubes are then shaken gently to redisperse the corpuscles, and the following ratings are made:

- 4+ complete agglutination with no dispersion upon shaking
- 3+ complete agglutination with some breaking up of blood upon shaking
- 2+ agglutination, but complete breaking up of blood in the agglutinated particles
- 1+ agglutination, easily dispersed to visible agglutinated particles
- ± upon redispersion, a magnifying glass is required to see the agglutinated particles

The highest final dilution showing a 1+ is called the titre. The titre values are in direct proportion to the amounts of ricin contained in the pomace sample.

ANTIGEN-DILUTION TEST FOR CASTOR BEAN ALLERGEN (CB-1A)

Chemical and Biological Reagents

Buffered Saline Solution. 0.50 g. $NaH_2PO_4 \cdot H_2O$, 2.60 g. $Na_2HPO_4 \cdot 12H_2O$, 8.8 g. NaCl (biological), and 0.1 g. merthiolate are dissolved in distilled water and diluted to one liter. This solution is then stored at refrigerator temperature.

Anti-CB-1A and Normal Rabbit Sera. These sera are stored at −4°F. Before use they are thawed and centrifuged clear at 36–41°F. They are not to be refrozen. The thawed sera are stable for 4–6 weeks at refrigerator temperature. Both were furnished and shipped under dry ice by the Allergens Laboratory.

CB-1A Stock Solution. To 5 mg. of powdered CB-1A (furnished by the Allergens Laboratory) were added 6.40 ml. of buffered saline. This is a 1:1280 dilution of CB-1A, equivalent to No. 8 serial solution.

Preparation of Extracts. The pomace samples to be tested are ground to pass a 40-mesh sieve. Extracts of the samples are prepared in the proportion of 2.0 g. of sample to 20 ml. of buffered saline (1:10 dilution). The extraction period is three days in a refrigerator at 43°F. with frequent shaking of the suspension. The suspension is then centrifuged in a refrigerated centrifuge at 36–41°F. The supernatant solution is clarified by filtration through a No. 50 Whatman filter paper. On storage in the refrigerator some of the castor extracts become cloudy. These should be clarified before use, by recentrifugation at 36–41°F.

Preparation of 2-Fold Serial Dilutions. Buffered saline is used for preparing the 2-fold serial dilutions of the extracts. For convenience the dilutions are designated by number. For example, the original 1:10 extract is placed in a 20-ml. tube labelled No. 1. For preparation of Dilution No. 2, 2 ml. of Dilution No. 1 are transferred into Tube No. 2, containing 2 ml. of buffered saline, giving a dilution of 1:20, etc.

For setting up the CB-1A reference standard, serial Dilutions Nos. 11 to 18, inclusive, should be prepared. It is not necessary to use the normal rabbit serum control with the CB-1A dilutions.

For raw castor meal and regular-cook castor meal, Dilutions Nos. 5 through 16 should be prepared for use with the antiserum. Dilutions Nos. 5 through 8 usually suffice for control tests with normal rabbit serum. Dilutions required for treated castor meal should include Dilutions Nos. 1 through 12. Reading of the "ring" test (see below) will indicate whether or not higher dilutions are needed. Control tests should be set up with normal rabbit serum in Dilutions Nos. 1 through 5.

Procedure. A series of new 45 mm. × 5 mm. precipitin tubes, each containing 0.15 ml. of CB-1A antiserum, is set up in a precipitation-tube rack. The required number of precipitin tubes containing 0.15 ml. of normal rabbit serum for control tests are also set up. Beginning with the highest dilution, 0.15 ml. of the castor extract and of the CB-1A stock solution are then carefully layered over the antiserum and the appropriate control serum, respectively. The tubes are then incubated 30 min. at 98.6°F. The reaction ("ring" test) at the interface of the two liquids in each tube is then observed and recorded. (If the "ring" test with the highest dilution of extract is markedly positive, additional dilutions of antiserum may be set up at this time.) The contents of each tube are then mixed with a small stirring rod, beginning with the most dilute and ending with the most concentrated. The tubes are again incubated 30–60 min., then stored in the refrigerator for 48 hrs. The precipitates formed are then observed and recorded.

Estimation of CB-1A Content in Pomace. To estimate the CB-1A content of a pomace by the "ring" or the "precipitin" test, the tube containing the highest dilution of extract that yields a barely perceptible precipitate is compared with the highest dilution of stock Cb-1A solution that yields a barely perceptible precipitate. To illustrate, if the stock CB-1A solution yielded a perceptible precipitate with the antiserum with dilution No. 16 (equivalent to 0.00305 mg. CB-1A) and if the sample castor extract yielded a perceptible precipitate with Dilution No. 8 (equivalent to 0.781 mg. CB-1A), it can be calculated that the pomace would contain 0.00305/0.781 times 100 or 0.39% of CB-1A. The method has been found to give reproducible results, particularly in the range of low contents of CB-1A. However it must be pointed out that because it utilizes serial dilutions, an error of one tube in a reading is equivalent to a 100% error.

SCHULTZ-DALE TEST

The Schultz-Dale technique (10) for determination of percentage of castor bean allergen (CB-1A) consists of measuring the degree of contraction of uterine muscles of a sensitized virgin female guinea pig immersed in a bath of oxygenated tyrode solution. The Schultz-Dale procedure was employed in this study for further assay (by the Allergens Laboratory) of certain of the treated pomace products which tested exceptionally low in CB-1A content by the precipitin test. No final tests were made on naturally sensitized human subjects.

Discussions of Results

Tables I through IV list for each of the experiments in Groups I through IV, respectively, the est-

mated percentages by weight of castor bean allergen (CB-1A) contained in the resulting pomaces, as determined by the modified antigen-dilution screening test and by the semiquantitative anaphylactic method (Schultz-Dale technique).

The over-all results indicate, in general, that the different treatments of meats and/or pomace were effective in reducing the content of allergen to varying degrees from 0 to 100% as discussed below.

Group I. Alkaline Treatments. Table I shows that for the three lots of beans, A, B, and C, moist-cooking with 1% added NaOH (Expts. 1e, 2b, and 3b) was effective in reducing the CB-1A content to 0.2–0.4%, a reduction of 97 to 98.4%, compared to only 50 to 75% for the controls (Expts. 1d, 2a, and 3a). In Expt. 4c cooking with 2% NaOH at 20 psig. pressure yielded a completely deallergenized pomace, as measured by the precipitin test. However, when assayed by the Schultz-Dale method, the zero reading was not determinable, *i.e.*, it was difficult to conclude that the sample had absolutely no CB-1A since a limiting value for CB-1A could not be established because sample doses reacting with sensitized tissue were of the same order as doses reacting with nonsensitized tissue. Expts. 4d and 4e demonstrated that the use of KOH or $Ca(OH)_2$ in moist-cooking at 20 psig. pressure was less effective than NaOH (Expt. 4c) in reducing allergen content.

In carrying out the antigen-dilution tests, it was observed that, in general, extracts of all of the pomaces produced by alkaline or other chemical treatments gave atypical or aberrant precipitin reactions and were characterized by large differences in the results of the "ring" test and the "precipitin" test. It may be postulated that such meals contain relatively large proportions of partially altered allergens.

Extracts of the pomaces prepared by simple solvent extraction of raw castor bean flakes (1a, 1c) and of flakes cooked without added chemicals gave typical precipitin reactions. All contained high concentrations of CB-1A. Preparations 1a and 1c produced copious precipitates with normal rabbit serum, which is typical of raw uncooked castor meal and can be attributed to the fact that ricin precipitates serum protein. However typical precipitin reactions occurred with the antiserum in the range where no precipitates were produced with normal serum. Extracts of pomaces from all other treatments in this study, where moist-cooking without added chemicals was employed, did not yield any precipitates in the normal serum, regardless of CB-1A content. Cooking apparently inactivates the ricin components.

Treatments 4a, 4b, and 4c show that when castor bean pomace prepared from moist-cooked flakes was cooked at atmospheric or at 20 psig. pressure with 2% NaOH in the presence of added moisture, the CB-1A content was not reduced as completely as by autoclaving the raw flakes at 20 psig. with NaOH and added moisture.

In experiments not listed in the table, acid treatments were tried, in which flaked raw meats were intimately mixed for 20 min. at 160°F. with 2.0% of hydrochloric acid and aqueous acetone, followed by acetone-extraction. The resultant pomaces (pH 3.9) showed practically no reduction in allergen content.

Group II. Ammoniation Treatments. Table II lists the results of the precipitin tests made on the eight samples of pomace which had been subjected to ammoniation under the various conditions of moisture, temperature, ammonia pressure, and treatment time. The purpose was to explore the use of ammonia as a means of further reducing the CB-1A content of pomace which had already been partially deallergenized to about 0.8% CB-1A content by alkali-cooking of raw flakes, followed by hexane-extraction. Data show no apparent reduction over control; however a previous experiment had shown that the allergen content of ammoniated pomace decreases with extended storage. Accordingly the least and the most severely treated pomaces from Expts. 1d and 1g, respectively, were hermetically stored and were retested after 45 days to ascertain the effect of prolonged contact with residual ammonia. The results indicated reductions down to 0.2% CB-1A, a result which was sufficiently promising to warrant additional storage. However, upon retesting after 150 days, no further reductions were noted.

Group III. Heat Treatment. Results in Table III indicate that partially deallergenized pomaces produced from Beans B and D by hexane-extraction of alkali- or nonalkali-cooked flakes were completely deallergenized by simple heating to about 405°F. in an elapsed period of about 125 min. However the resulting meals had a dark brown, charred appearance, indicative of considerable heat damage. The meals produced from Bean B (Expts. 1b, 2b) tested 0% CB-1A by both the precipitin and the Schultz-Dale methods. It is significant to note in the data for Bean D that temperatures of heating up to 347°F. were ineffective in reducing the CB-1A content to very low levels. It can be postulated that temperatures higher than 347°F. are necessary to destroy or inactivate the allergen by the combined effects of accelerated oxidation and chemical breakdown.

Group IV. Chemical and Biochemical Treatments. In this series (Table IV) the best results were obtained by the treatments with formaldehyde (Expt. 2) and by trypsin digestion of the pomace (Expt. 7).

In the formaldehyde treatments the pomaces produced in Expts. 2a, 2b, and 2c tested 0% CB-1A by the precipitin method and practically 0% by the Schultz-Dale method; that produced from Expt. 2d tested 0.1% CB-1A. The treatments differed from each other as shown in Table IV. All employed moist-cooking of flaked meats with different chemicals as noted, followed by hexane-extraction to produce the pomaces. In Expt. 2a, NaOH and formaldehyde were used; in 2b, HCl and formaldehyde; and in 2c and 2d, NaOH only. In the latter two experiments the alkaline pomaces produced were treated with formaldehyde without and with added NaOCl, respectively. The results demonstrate that formaldehyde definitely reacts with the allergenic component in some unexplained way (possibly by polymerization or reduction) to render it undetectable by both test methods.

In Expt. 7 deallergenization to 0.2% CB-1A (98.4% reduction) was achieved by trypsin digestion in an aqueous alkaline medium of partially deallergenized pomace that had been prepared from alkali-cooked flaked meats.

In Expts. 1a and 1b moist-cooking of castor bean flakes with NaOCl and treatment with NaOCl of pomace prepared from moist-cooked flakes yielded reductions in CB-1A content of only 50%. In Expt. 1c treatment of alkaline pomace with NaOCl did not further reduce the allergen content.

All of the other treatments listed in Table IV were essentially ineffective in reducing the contents of CB-1A by more than 50 to 75%, which may be considered negligible.

Summary

Results are reported of a series of experimental treatments of flaked castor bean meats and pomaces directed toward total detoxification of the ricin and total destruction or inactivation of the allergenic property.

The treatments comprised cooking of the flaked raw meats or pomaces under various conditions of moisture and temperature, with chemicals such as sodium hydroxide, hydrochloric acid, sodium hypochlorite, formaldehyde, ammonia, ammonium sulfate, potassium permanganate, and urea, and with selected combinations of these. Also tried were aerobic fermentation, enzymatic digestion, and simple heating of pomaces at elevated temperatures.

The five most promising deallergenization treatments and the corresponding percentage reductions in allergen content as measured by the precipitin test were as follows: dry heating of pomace to 401°F., 100%; moist-cooking of flaked meats with 2% NaOH and 10% HCHO, possibly 100%; moist-cooking with 0.9% HCl and 3% HCHO, possibly 100%; moist-cooking with 2% NaOH at 20 psig. pressure, possibly 100%; moist-cooking with 1% NaOH, 98.4%. For the first four treatments the Schultz-Dale test indicated possible reductions of 100, 99.9, 99.9, and 100%, respectively. While the latter method is generally considered to be reasonably accurate, final tests would have to be conducted with naturally sensitive human subjects.

The data also showed that the ricin component can be completely detoxified by a mild moist-cooking of the flaked meats, either with or without added alkali.

Acknowledgments

Grateful appreciation is expressed to Henry Stevens and E. Jack Coulson, Allergens Laboratory, Eastern Utilization Research and Development Division, A.R.S., U.S.D.A., for furnishing the modified antigen-dilution precipitin test procedure, for conducting many of the biochemical assays, and for their many helpful suggestions in interpretation of test results; to Don S. Bolley, technical director, Baker Castor Oil Company, for furnishing the test procedure for ricin determination and for his valuable suggestions; to Baker Castor Oil Company, Bayonne, N. J., and Bunge Corporation, New York, for their cooperation in furnishing the castor beans for the experiments; and to H. J. Deobald and Joseph Pominski for their helpful assistance in various stages of the work.

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TASTE RESPONSES IN RUMINANTS. II. REACTIONS OF SHEEP TO ACIDS, QUININE, UREA AND SODIUM HYDROXIDE[1]

W. D. Goatcher and D. C. Church
Oregon Agricultural Experiment Station, Corvallis[2]

TASTE is believed to be involved in the motivation of food acceptance through at least two psychological processes: (1) by influencing drive state (activity associated with food seeking behavior), and (2) by contributing to the reinforcing value of a food. In animals having a normal urge to eat, sensory cues may be dispensed with, yet regulation of food intake compatible with life may still prevail. In many cases, however, it is necessary that domestic animals be "over fed" with respect to their caloric needs—in such cases motivation to eat becomes critical to the achievement of satisfactory production levels.

The sense of taste has been studied in many of the domestic animals. Goatcher and Church (1970) has reported on the taste responses of sheep to compounds stimulatory to the sweet and salty taste modalities. The study reported herein was designed to provide data on the reactions of sheep to stimulants that evoke the sour and bitter taste sensations in man.

Experimental Procedure

The method used to assess taste responses in this study has been described in a previous report (Goatcher and Church, 1970). Briefly, it consisted of a two-choice preference test where two containers were presented to each animal, one container holding tap water and the other the chemical solution (taste stimulant) being tested. The taste stimulants were generally presented in an ascending series of concentrations with each concentration being double the preceding one. In one test, butyric acid was offered in opposition to hydrochloric acid. A total of three groups of 10 sheep, each, were used during the research.

Because the pH of the tap water varied from day to day, pH's of samples of the acid and sodium hydroxide solutions were determined using a standard laboratory-type pH meter.

The upper discrimination threshold (UDT) and lower discrimination threshold (LDT) have been set at the 60 and 40% level of intake, respectively. The preference threshold (PRT) has been arbitrarily set at the 80% level of intake and the rejection threshold (RET) at the 20% level.

The data were analyzed using the linear regression procedure. Table 1 shows the relevant concentrations along with associated X values (the independent regression variable) which are related to the logarithms of the concentrations and constitute a linear scale.

Results

Acids. Figure 1 shows the responses of Group 3 sheep to various concentrations of acetic acid (HAc). The LDT was reached at a concentration of .014 ml/100 ml (.0024 M) and the RET was at a concentration of .158 ml/100 ml (.028 M). A summary of the linear regressions of sheep taste responses on ascending concentrations of HAc and of propionic (HPr), butyric (HBu), lactic (HLa) and hydrochloric (HCl) acids is presented in table 2.

Group 3 sheep were placed in a choice situation involving HBu (.03 ml/100 ml, pH of 4.1) and HCl (.03 ml/100 ml, pH of 2.6). Their responses are shown in figure 2. The preference for HBu in this situation was 71% and for HCl, 29%. Total fluid consumption, however, was reduced by 30.7%.

Quinine. Taste responses of Group 1 to concentrations of quinine hydrochloride (QHCl) are shown in figure 3. The LDT was at a concentration of 29.6 mg/100 ml (.00082 M) and the RET was at 157.6 mg/100 ml (.0044 M). A summary of the reactions to QHCl of all three groups is presented in table 3.

Urea and Sodium Hydroxide. The responses to urea and sodium hydroxide (NaOH) are shown in figures 4 and 5, re-

[1] Technical Paper No. 2709, Oregon Agricultural Experiment Station.
[2] Department of Animal Science.

TABLE 1. RELEVANT CONCENTRATIONS OF THE VARIOUS TASTE STIMULANTS AND THEIR RELATED X VALUES

Stimulant[a]	X value					
	1	2	3	4	5	6
HAc[b]	.04	.08	.16	.32	.63	1.25
HPr[b]	.01	.02	.04	.08	...	...
HBu[b]	.01	.02	.04	.08	.16	...
HLa[b]	.01	.02	.04	.08	...	...
HCl[c]	.01	.02	.04	.08	...	...
NaOH[d]	.04	.08	.16	.32	...	...
QHCl[e]						
Group 1	.016	.032	.063	.16	.32	.63
Group 2	.016	.032	.063	...	...	...
Group 3	.0063	.016	.032	.063	.16	.32

[a] The acids acetic, propionic, butyric, lactic and hydrochloric and sodium hydroxide and quinine hydrochloride, respectively.
[b] Concentration in ml of pure acid/100 ml of solution.
[c] Concentration in ml 37.5% HCl/100 ml of solution.
[d] Concentration in ml of 12 M NaOH/100 ml of solution.
[e] Concentration in mg of QHCl/100 ml of solution.

spectively. The reactions to urea were quite variable (R^2=.111) at the higher concentrations. Therefore, the linear regression analysis is not presented for this chemical. A summary of the responses to NaOH is displayed in table 2.

Discussion

Taste sensitivity in sheep is less for sugars than for salts (Goatcher and Church, 1970). The next group of taste stimulants in the order of stimulating effectiveness (least to most stimulatory) was the acids. On a molar basis, HAc was rejected at 7.3% of the concentration required to cause rejection of NaCl. The sensitivity series of the acids, based on molar concentration at the RET and in order of increasing sensitivity, was: HAc, HLa, HPr, HBu and HCl. When the LDT was considered, HAc was equal in concentration to HLa and the relative positions of HBu and HPr were reversed. The sensitivity series

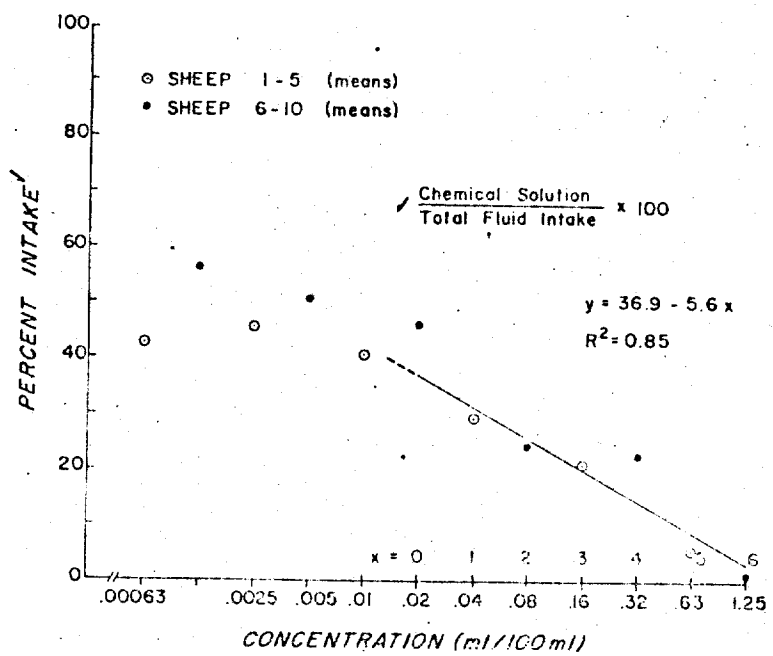

Figure 1. Taste responses of Group 3 to ascending concentrations of acetic acid.

TABLE 2. SUMMARY OF THE LINEAR REGRESSIONS OF SHEEP TASTE RESPONSES ON ASCENDING CONCENTRATIONS OF VARIOUS ACIDS AND SODIUM HYDROXIDE

Item	Chemical					
	HAc	HPr	HBu	HLa	HCl	NaOH
Threshold						
LDT[a] (y40%)						
Ml/100 ml	.014[n]	.004[n]	.002[n]	.017[n]	.003[j]	.031[k]
Molar conc.	.0024	.00054	.00022	.0024	.00037	.0036
pH	4.2	5.1	6.6	4.1	3.9	11.5
RET[n] (y20%)						
Ml/100 ml	.158[n]	.029[n]	.052[n]	.070[n]	.018[j]	.110[k]
Molar conc.	.028	.0039	.0057	.0097	.0022	.013
pH	3.4	4.2	4.7	3.0	2.9	12.0
Slope (b)[m]	−5.60[e]	−6.74[e]	−4.54[e]	−9.84[e]	−8.19[d]	−10.83[e]
Intercept (a)	36.9[f]	36.6[f]	35.1[h]	57.3[f]	35.1[g]	46.0[f]
R^2	.846	.688	.603	.582	.966	.845
S.E. of estimate	5.00	7.17	6.73	13.74	2.43	7.32
S.D. of $y_{50\%}$	5.32	7.95	7.46	16.88	2.56	8.12
S.D. of b	1.19	3.20	2.13	6.14	1.09	3.28

[e] Different from 0 ($P<.01$), [d] ($P<.025$), [c] ($P<.20$).
[f] Different from theoretical mean of 50% ($P<.005$), [g] ($P<.01$), [h] ($P<.025$), [j] Not significant.
[j] Specific gravity of 1.19, 37.5% HCl.
[k] Molarity of 12.
[m] Associated X values are related to the logarithms of the concentrations as per table 1.
[n] Rejection threshold.
[p] Lower discrimination threshold.
[q] Stock solutions adjusted to provide the indicated amount of pure acid.

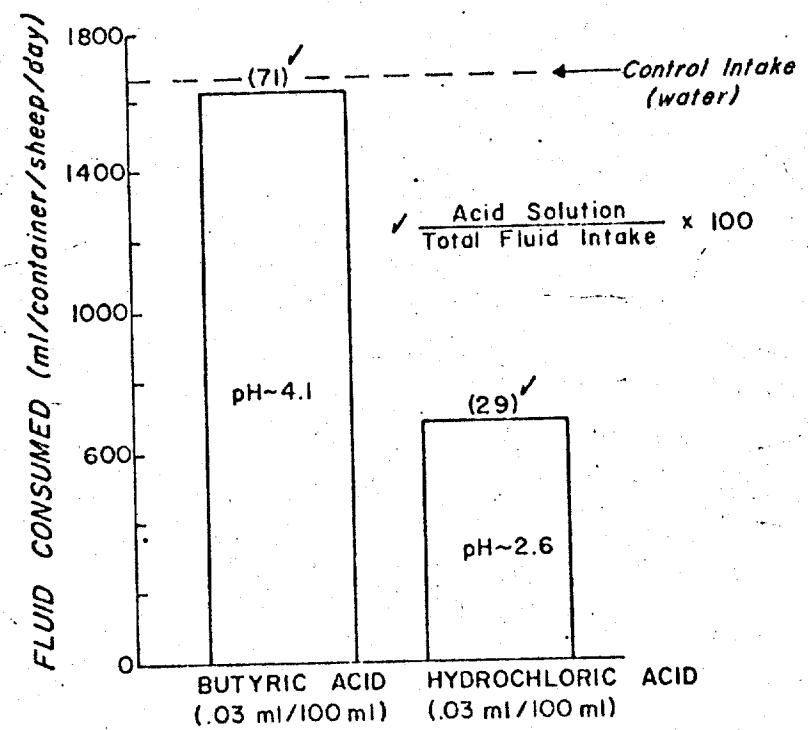

Figure 2. Taste reactions of Group 3 in a choice situation involving acids.

was changed when, at the rejection threshold, decreasing H^+ concentration (or increasing pH) was used as the criteria, the series then being: HCl, HLa, HAc, HPr and HBu.

The fact that HAc is rejected at lower H^+ concentrations (and higher molar concentrations) than HCl may be explained on the basis that the undissociated HAc molecules are entering into stimulatory activities. However, it is not clear why HPr and HBu were rejected at H^+ and molar concentrations much lower than for HAc. One possible explanation for the lower thresholds for HPr and HBu is that these acids are being responded to through the sense of smell; of course, this may also apply to HAc. It is a well known fact that, when a substance has the property of giving rise to sensations of both taste and smell, detection of the substance through the sense of smell is possible at concentrations far below that required for detection through the sense of taste.

TABLE 3. SUMMARY OF THE LINEAR REGRESSIONS OF THE TASTE RESPONSES OF GROUPS 1, 2 AND 3 SHEEP ON ASCENDING CONCENTRATIONS OF QUININE

Item	Group 1	Group 2	Group 3
Threshold			
LDT[i] ($y_{40\%}$)			
Mg/100 ml	29.60	3.92	5.4
Molar conc.	.00082	.00011	.00015
RET[h] ($y_{20\%}$)			
Mg/100 ml	157.60	18.10	108.80
Molar conc.	.0044	.0005	.0030
Slope (b)[g]	−8.99[e]	−9.65[e]	−5.16[d]
Intercept (a)	57.8[f]	33.6[f]	43.8[f]
R^2	.753	.789	.563
S.E. of estimate	10.77	7.07	9.52
S.D. of $y_{50\%}$	11.77	10.44	10.60
S.D. of b	2.57	4.95	2.27

[e] Different from 0 ($P<.05$), [d] ($P<.10$), [c] ($P<.40$).
[f] Not significantly different from a theoretical mean of 50%.
[g] Associated X values are related to the logarithms of the concentrations as per table 1.
[h] Rejection threshold.
[i] Lower discrimination threshold.

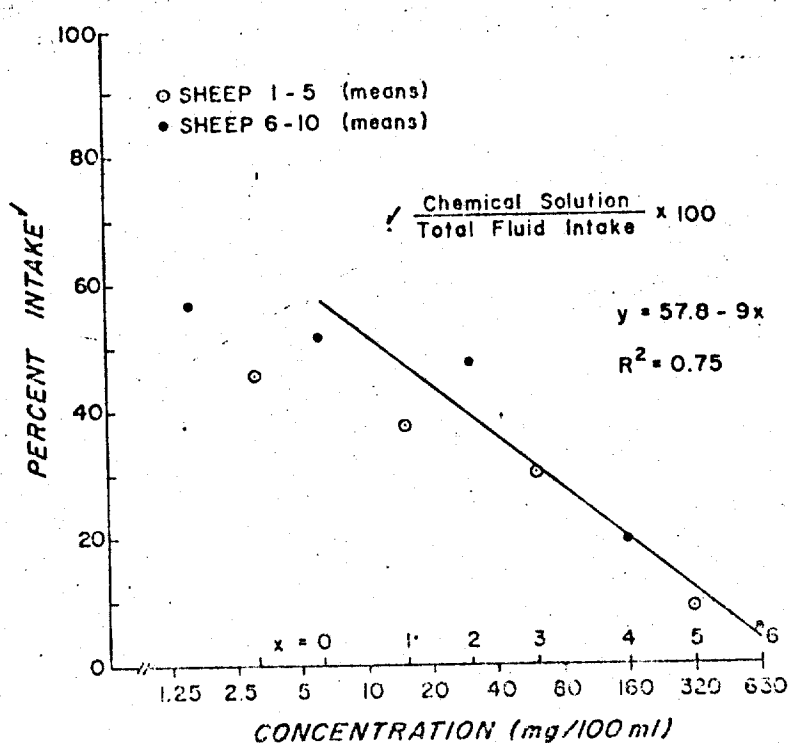

Figure 3. Taste responses of Group 1 to ascending concentrations of quinine hydrochloride.

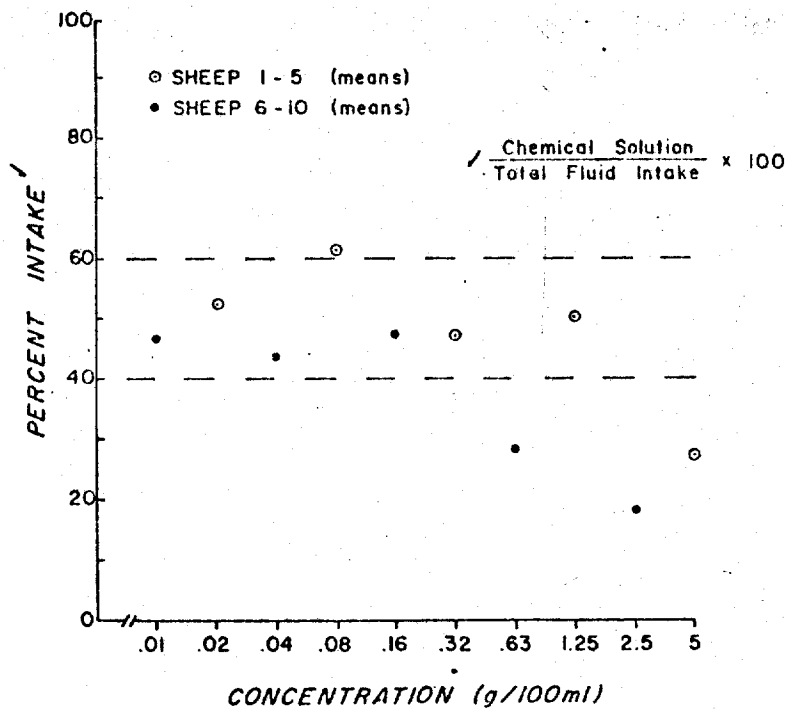

Figure 4. Taste reactions of Group 2 to ascending concentrations of urea.

The combined amount and molar proportions of HAc, HPr and HBu normally present in the rumen vary depending on the diet and time after feeding. The average combined amount of HAc, HPr and HBu present in the rumen of the sheep is about 100 mM and their molar proportions are about 60, 25 and 10, respectively (Church, 1969). From table 2 it can be seen that, at the LDT, HAc was at a concentration of 2.4 mM (or about 1/20 of the concentration normally present in the rumen), while HPr and HBu were at .5 and .2 mM, respectively, these latter two acids being responded to at only 1/50 of their average rumen concentration.

Bernard and Kare (1961), using calves, obtained electrophysiological responses to HAc at lower molar concentrations than to HPr—.001 for HAc as opposed to .01 for HPr. They suggested that the wide range of responses observed for these acids may indicate a role for chemoreception in the process of rumination. The data obtained in this study indicate that smell may be the basis for differential behavioral sensitivity to HAc, HPr and HBu.

When HBu and HCl were offered as the two choices, both at pH's somewhat below those of their RET's, a decided preference was exhibited for HBu (71% intake). However, despite the preference demonstrated for HBu, total fluid intake was reduced by 30.7% as compared to intake of controls. The reduction in total fluid intake was primarily the result of an aversion for HCl at the concentration tested, indicating that in this case pH was more influential than smell.

The responses to quinine were determined for Groups 1, 2 and 3. As can be seen in table 3, the threshold concentrations varied markedly between the groups, the greatest differences being between the LDT's. The relatively low quinine thresholds demonstrated by Group 2 may be explained, at least in part, on the basis of prior treatment. Group 2 was involved in assessing urea responses before they were placed on quinine. In figure 4 it can be seen that consistent rejection of the urea solution by the two units of sheep did not occur until the 2.5 and 5% concentrations were reached. Appreciable amounts of urea solution were ingested at the higher concentrations; so much, in fact, that one animal died and two others became noticeably ill. Presumably, others of the group experienced varying degrees of illness. It was

assumed that the illness associated with the bitter taste of urea may have influenced subsequent tests involving bitter stimulants such as quinine. On the basis of this assumption, only the threshold values for Groups 1 and 3 were used to determine the average threshold concentrations of quinine. These concentrations were, at the LDT, .00048 M, and at the RET, .0037 M.

The sheep of Group 3 commenced to discriminate between NaOH solution and water at a pH of 11.5, and at a pH of 12 the RET was reached. This represents a range of .5 pH units between the LDT and RET. For HCl the corresponding values at the LDT and RET and the range between the thresholds were 3.9, 2.9 and 1.0 pH units, respectively. Thus, the range between thresholds was somewhat narrower for NaOH and slightly greater tolerance was exhibited on the alkaline side of neutral. Kare (*personal communication*) has found the calf to be tolerant to pH, particularly on the alkaline side of neutral.

When the LDT's and RET's of glucose, NaCl (Goatcher and Church, 1970), HAc and QHCl are considered, the four primary taste groups can be arranged in the sensitivity series as presented in table 4. With respect to either the LDT or RET, sheep were least sensitive to the sweet taste and most sensitive to the bitter taste. There was greater sensitivity to saltiness than to sweetness and greater sensitivity to sourness than to saltiness. The taste-group thresholds within the LDT and RET sensitivity series progressed by about one order of magnitude and the two series differed within a single taste group, also, by about one order of magnitude. Bell and Kitchell (1966) obtained electrophysiological responses with sheep to glucose, NaCl, HAc and quinine dihydro-

TABLE 4. THE SENSITIVITY SERIES OF THE FOUR PRIMARY TASTE GROUPS BASED ON SHEEP TASTE RESPONSES

Taste group	Threshold: Lower discrimination	Threshold: Rejection
Sweet (glucose, M)	>.56	...
Salty (NaCl, M)	.043	.38
Sour (HAc, M)	.0024	.028
Bitter (QHCl, M)	.00048	.0037

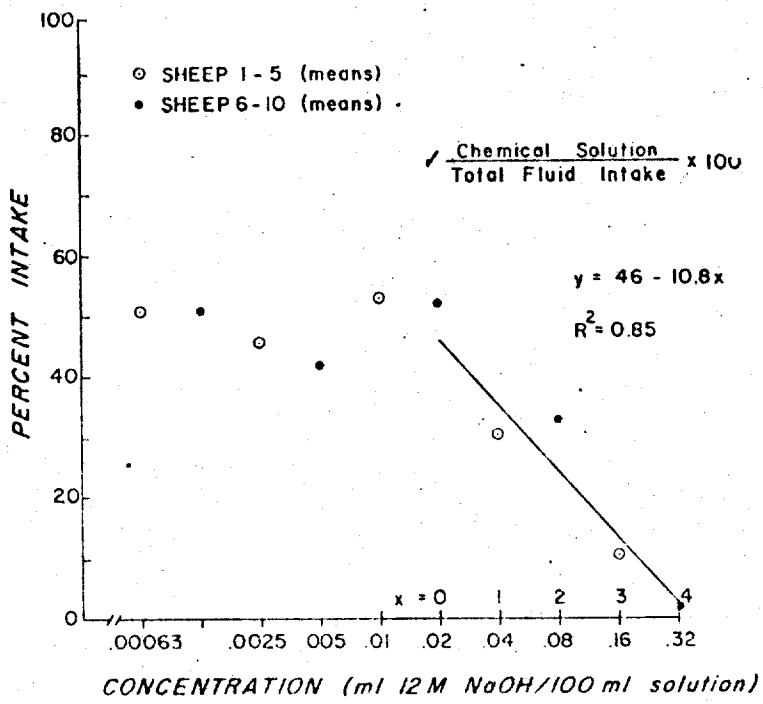

Figure 5. Taste responses of Group 3 to ascending concentrations of sodium hydroxide.

chloride at molar concentrations of .83, .005, .2 and .02, respectively. With the exception of NaCl appreciably greater concentrations were involved in eliciting electrophysiological responses than in inducing the preference behavior in this study. The opposite case was true for NaCl.

Bell (1959) used the two-choice preference test to determine the reactions of calves and goats to chemicals representing the four primary taste groups. He used glucose, NaCl, HAc and quinine dihydrochloride and offered the chemicals in descending concentrations. When the threshold values for the calf and the goat are compared with the values obtained for the sheep, marked species differences in taste sensitivities seem to exist. At the Oregon station, studies have recently been undertaken to compare (under similar environmental and nutritional conditions) taste responses in pygmy goats, normal goats, sheep and cattle. The data are to be presented in future reports.

Summary

This study involved the use of the two-choice preference test, where the choices were tap water and tap water-chemical solutions, to determine the responses of sheep to ascending concentrations of acetic (HAc), propionic (HPr), butyric (HBu), lactic (HLa) and hydrochloric (HCl) acids, urea, quinine hydrochloride (QHCl) and sodium hydroxide (NaOH).

Responses were expressed on the basis of percent intake of total fluids. The mean responses of units of five sheep were plotted graphically and analyzed by the linear regression technique when definite rejection trends were apparent. The chemical concentrations at the lower discrimination thresholds (LDT) and rejection thresholds (RET) —that is, where the test chemical comprised 40 and 20% of total fluid intake, respectively —were estimated from the regression line.

The threshold concentrations and pH's obtained for HAc were: LDT, .0024 M, pH of 4.2; RET, .028 M, pH of 3.4. The thresholds for HCl were at lower concentrations and lower pH's than for HAc. HPr and HBu were discriminated against at lower concentrations and higher pH's than was HAc—possibly due to smell. The concentrations at the LDT and RET for NaOH were, respectively, .0036 M, pH of 11.5 and .013 M, pH of 12.0. A comparison of these values with those for HCl indicates that sheep are more tolerant of high pH's than of low pH's.

QHCl elicited LDT and RET concentrations of .00048 and .0037 M, respectively. The responses to urea were highly variable, but appreciable intake was observed at concentrations of .42 and .83 M.

With respect to glucose, NaCl, HAc and QHCl, the sensitivity series for the four primary taste groups, in order of increasing sensitivity was: sweet, salty, sour and bitter.

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PREVENTS POTATO PEEL POLLUTION

Dry caustic peeling process reduces B.O.D. of plant effluents

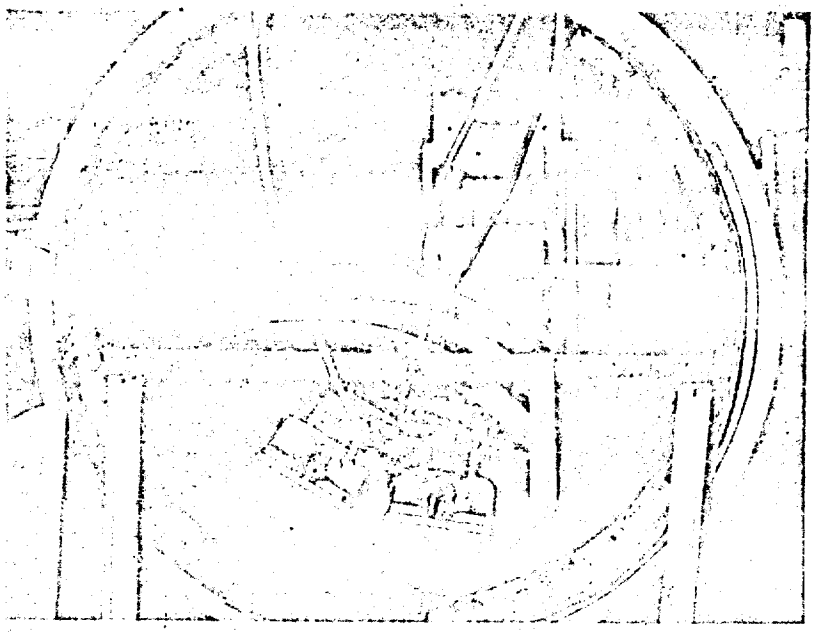
R. P. GRAHAM, C. C. HUXSOLL, M. R. HART,
M. L. WEAVER, and A. I. MORGAN, JR.
Western Utilization Research and Development Division,
ARS, USDA, Albany, Calif.

WHEN POTATOES ARE PEELED for food use, about 80% of the B.O.D. in the plant effluent results from the peeling process. Thus, any modification of the peeling process which prevents the peel residues from becoming mixed with plant effluents greatly reduces the B.O.D. of the effluent. Such a dry caustic peeling process, called WURPEEL, has been developed at the Western Utilization Research Laboratory in Albany, Calif.

In conventional caustic peeling of potatoes, the potatoes are washed, contacted with a hot lye solution, and held briefly at a temperature equivalent to that of the lye solution. Then, they pass through a barrel washer where high pressure water jets erode the softened peel and potato tissue from the surface. This is how the B.O.D. gets into the effluents. While the conventional lye process leads to a satisfactory peeling, it also results in peel residues and solutes being mixed with large quantities of water.

Dry Process

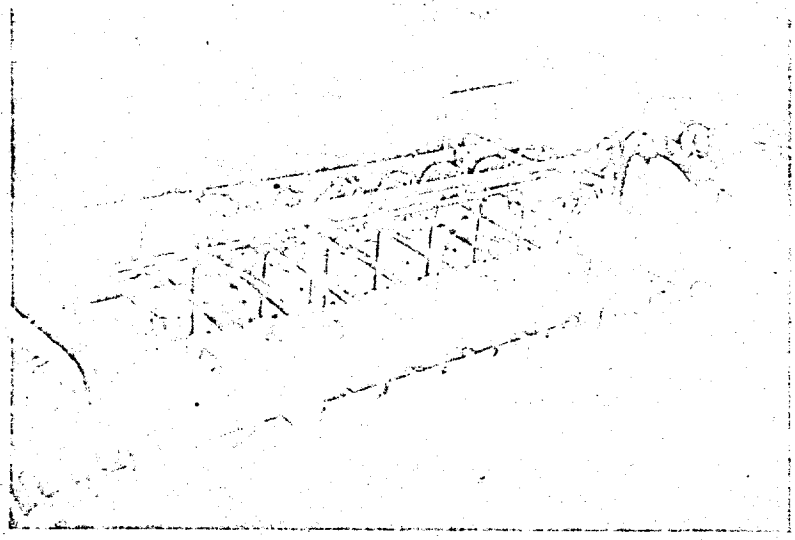
In WURPEEL, the potatoes are washed and contacted with a hot lye solution for 3 min. Then, they are held at room temperature for about 5 min. to allow the lye to penetrate and to permit the temperature in the potato to equilibrate. Following the holding period, the potatoes are surface-heated for 2-3 min. under gas-fired infrared burners. Infrared heating dries the surfaces of the potatoes and enhances the action of the lye. The potatoes are moved directly from the infrared heater to a mechanical peeling device. One such device has rollers covered with rubber projections to abrade the softened peel and potato tissue from the surface within a few seconds. Approximately 95% of the peel material is removed in the mechanical peeler. After peeling, the potatoes are immersed in water for cooling and to remove a small amount of residue from the surfaces of the potatoes. No appreciable



INFRARED HEATERS

TUMBLING BARREL has infrared heaters mounted about 5 in. above inside surface of barrel.

LIVE-ROLL CONVEYOR has infrared heaters mounted above it.



PREVENTS POTATO PEEL POLLUTION (CONTINUED)

amount of lye or dissolved peel is introduced into the water.

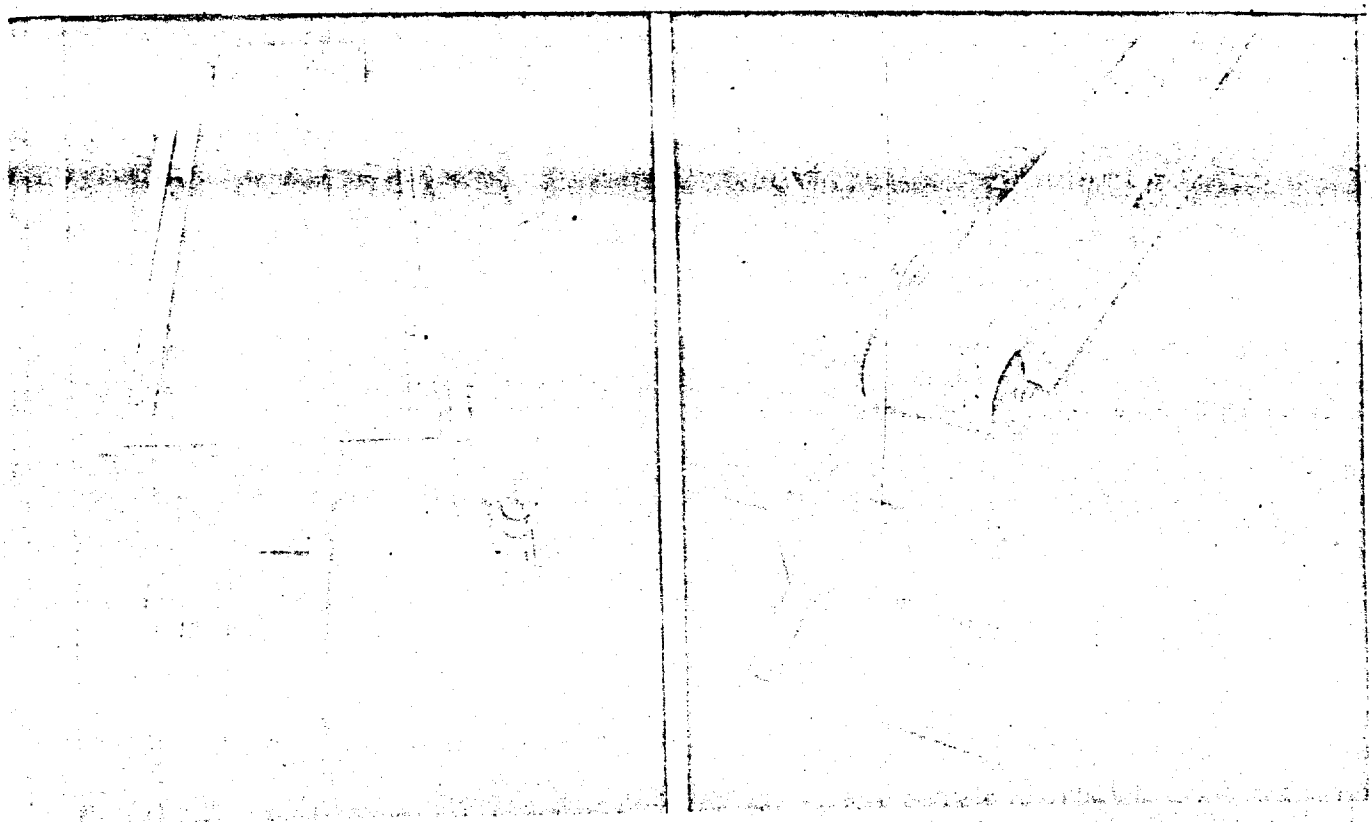
The peel residue from the mechanical peeler has a solids content of about 22 to 25% with a dry-basis ash content of about 20%. This material could be burned or buried for land disposal; or it could be fermented to reduce the pH from 10.5 to less than 7.0 and then used as animal feed.

Equipment

The equipment for washing and lye-treating the potatoes may be the same for both the conventional process and WURPEEL. The equipment for holding the potatoes between the lye treatment and further processing may also be similar.

As shown in the photos, two types of infrared heaters have been used. A tumbling barrel was used in the first pilot line. Two rows of gas burners are mounted so that the radiating surfaces are about 5 in. above the inside surface of the barrel.

The other infrared heater consists of a live-roll conveyor over which infrared heaters are mounted. The live rollers continually rotate the



MECHANICAL PEELERS

TROUGH-TYPE PEELER has 3 rollers that form the trough. Rollers are covered with a rubber scrubber matting having ½-inch rubber fingers.

CONVEYOR-TYPE PEELER has 8 rollers (out of sight inside the machine) covered with rubber scrubber matting. Potatoes travel perpendicular to axis of rollers.

potatoes under the burners whch uniformly heat the surfaces. The potatoes are exposed to the heaters for 2 to 4 min.

The gas-fired infrared heaters are the type commonly used for space heating. Combustion occurs over a ceramic mantle which radiates at 1600F.

Two types of mechanical peelers have also been tested. One of these consists of three parallel 8-inch diameter rollers arranged to form a trough. The rollers are covered with a rubber scrubber-matting with ½-inch rubber fingers. The lower roller turns at 560 rpm and the upper rollers turn at 450 rpm. All rollers turn in the same direction. The rotational speeds are high enough so the rollers are self cleaning.

The other mechanical peeler consists of eight 5½-in. rollers covered with the same rubber material. They all turn in the same direction, but adjacent rollers turn at different speeds. The movement of the potatoes in this peeler is perpendicular to the axis of the rollers.

The quality of peeling is very similar in both the conventional and WURPEEL processes. To use WURPEEL, only a gas-fired surface heater and a rubber roll peeler are needed. (End)

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Experimental Asbestosis

The Development of Lung Cancer in Rats With Pulmonary Deposits of Chrysotile Asbestos Dust

Paul Gross, MD; Robert T. P. deTreville, MD, DSc; Ethel B. Tolker, BS; Marianne Kaschak, BS; and Mary Ann Babyak, BS, Pittsburgh

DURING a study of experimental asbestosis involving guinea pigs, rats, and hamsters that had inhaled chrysotile dust, it was found that pulmonary cancers had developed in several rats. Spontaneous primary lung cancers, exclusive of lymphoblastomata, have been exceedingly rare in our rats (we have not seen a single spontaneous primary pulmonary carcinoma or fibrosarcoma in about 10,000 rats examined). Therefore, a cause-and-effect relationship between the pulmonary deposition of chrysotile dust and tumor development was suspected. Suspicion increased as the number of malignant lung tumors discovered in rats that died more than 16 months after initiation of the chrysotile exposure increased rapidly.

There has been increasing evidence, mostly epidemiologic, linking long-term exposure to asbestos dust with the development of pulmonary cancer in man.[1,2] Since the development due to the pulmonary deposition of chrysotile asbestos dust of primary lung cancer in an experimental animal has not been reported, the occurrence of malignant lung tumors in our asbestotic rats is being reported and a hypothesis explaining this phenomenon is offered.

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From the Industrial Hygiene Foundation, Mellon Institute, Pittsburgh.

Reprint requests to 4400 Fifth Ave, Pittsburgh 15213 (Dr. Gross).

Materials and Methods

Canadian chrysotile asbestos was ball-milled and subsequently hammer-milled, using essentially the method of Holt et al.[3] The principle of this technique is to reflux the comminuted chrysotile through the high-speed hammer-mill so that much of the dust is of ultramicroscopic dimensions (Fig 1). Within less than one year, it became necessary to replace the steel-alloy hammers with new ones because of excessive wear. Also, to maximize the dust concentration in the chamber, the output of a second hammer-mill was added to the inhalation chamber.

The chamber measured 8 × 8 × 8 ft. Initially, it had only one four-inch input opening connecting with the outflow duct of a hammer-mill. After 52 weeks, a second four-inch input for the second hammer-mill was installed. There were three exhaust openings in the chamber. Two of these were 1.5 inches in diameter each. Both led to electrostatic precipitators and then connected with a vacuum pump. The flow of exhaust air through one precipitator was approximately 125 liters per minute whereas the flow through the other precipitator was adjusted accurately to 8 liters per minute. The dust in the latter was carefully weighed at the end of each week to determine the average dust concentration in the chamber during that week. The third exhaust opening in the chamber, ten inches in diameter, led to a ten-inch furnace-type filter and then to an exhaust fan. The latter was used to exhaust the chamber rapidly at the end of each day's exposure. The chamber was actually under a slight, but definite, negative pressure during the dust exposure.

A total of 131 male white rats were exposed to chrysotile dust in the chamber six hours a day, five days a week for a maximum of 62 weeks. Of these 131 animals, 70 had had an intratracheal application of 0.05 ml of 5% aqueous sodium hydroxide, the purpose of which was to impair clearance from the lungs of inhaled dust. (The 70 rats were the survivors from a total of 81 animals that had received the caustic applications intratracheally.) The intratracheal caustic applications were accomplished with the animals under ether anesthesia and with the aid of a self-retaining illuminated speculum that allowed visualization of the vocal chords. A long 18-gauge needle attached to a microliter syringe was used. The end of the needle was plugged, rounded off, and two small openings made on opposite walls about 5 mm from the tip. After expelling the contents of the syringe onto the tracheal wall, the needle was withdrawn with a spiral motion to distribute the caustic as widely as possible.

The caustic-treated animals were introduced into the inhalation chamber at different stages of healing of the tracheal lesions. Some were exposed to the dust immediately; others, one, two, and three weeks after the caustic application. The 61 rats not treated with caustic were also exposed in the chamber to chrysotile dust and were assumed to have normal tracheas. Dust exposures were discontinued after 62 weeks. The survivors were then pastured for the remainder of their lives.

Dust concentrations in the chamber during the first year, with only one mill in operation, ranged from 42 to 106 mg/cu m. During the second year, with both mills in operation, the range was 67 to 146 mg/cu m. The overall average was 86 mg/cu m (based on weekly determinations).

Another group of 64 rats was given intratracheal injections of chrysotile dust obtained from the electrostatic precipitators. The same injection technique was used as that used for intratracheal caustic applications except that the 18-gauge needle had its opening in the tip, the edges of which were rounded off. The dose injected was 3.5 mg suspended in 1 ml water. Of the 64 rats, 14 animals received but one injection; 14 received two; 1 received three; 9, four; and 26, six.

In addition, 30 rats of the same shipment as that of the animals receiving exposure served as laboratory controls and were not treated in any manner. They were housed in a room, remote from the exposure chamber, and were pastured for the remainder of their lives. Another group of 15 rats served as controls for the caustic intratracheal applications. They had survived an intratracheal application of sodium hydroxide but had not been exposed to asbestos dust. Finally, a group of ten rats served as controls for the rats injected intratracheally with

Fig 1.—Electron photomicrograph of chrysotile dust obtained by thermal precipitation from the inhalation chamber.

Table 1.—*Overall Mortality and Lung Cancer Incidence**

Animal Group	No. of Rats	16-Month Survivors		Rats With Lung Cancer*	
		No.	% of Total	No.	% of Survivors
Chrysotile inhalation with intratracheal NaOH	71	31	44	15	48
Chrysotile inhalation without intratracheal NaOH	61	41	67	10	24
Chrysotile, intratracheal	64	19	30	3	16
Laboratory controls (no treatment)	40	25	63	0	0
NaOH controls (no chrysotile)	15	14	93	0	0

* Exclusive of lymphoblastomas (known to occur spontaneously in these rats).

asbestos dust. They had received intratracheal injections of water.

The survivors of the multiple intratracheal chrysotile injections were killed 21 months after the first injection.

The lungs of all rats were distended with 10% formalin solution under a pressure of 12 cm water. Sections were cut at 6μ from blocks of lung embedded in paraffin and stained with hematoxylin and eosin. After photomicrography the sections were decolorized and impregnated with silver according to the method of Gordon and Sweets. Some sections were also incinerated in order to obtain information on the amount and distribution of the chrysotile dust.

Results

The earliest lung cancer was found in a rat that had died 16 months after initiation of chrysotile dust exposure in the inhalation chamber. Calculations of the prevalence of lung tumors were based on the number of animals that live 16 months or longer after having been placed in the inhalation chamber (72 rats) or after they had received the first intratracheal injection (19 rats). Of the 72 surviving rats from the inhalation chamber, 31 had received an intratracheal application of sodium hydroxide and 41 had not been so treated. (These data are shown in Table 1.)

Many lung cancers were microscopic in size but some were visible grossly, the size ranging from 1 to 5 mm in diameter. All tumors were peripheral in location and none originated in a bronchus or bronchiole. The tumors were light gray-tan in color, bulged slightly above the surface, and were covered by a shiny, thin, transparent pleura. The consistency was firm, verging on hardness. On section, the tumor presented a dull, gray, dry, and compact appearance. The cut surface did not appear to retract or bulge. The edges of the tumor were smooth and stood out sharply from the surrounding retracted, moist, generally dark-red lung tissue. There was no evidence of a capsule. Distinguishing these tumors grossly from the lymphoblastomata that occurred spontaneously in our aging rats offered no difficulty. The latter tumors were located in the hilar regions, although some of the larger ones extended to the periphery. They were infiltrating, hence their edges were less sharply defined. The cut surface was glistening gray, tended to bulge slightly and, most important, it tended to be lobulated, each lobule having a tiny hole in the center. The hole was the lumen of either a vessel or a bronchus (or bronchiole), whereas the lobule represented adventitial and periadventitial tumor infiltration.

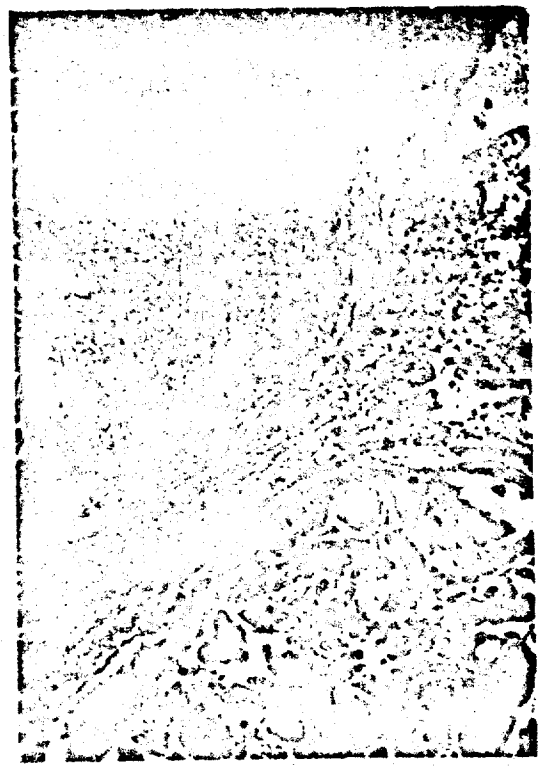

Fig 2.—Adenocarcinoma occupies almost the diagonal upper half of the field whereas the lower portion of the field is occupied by a large papillary adenoma. Neither tumor is encapsulated. Compressed alveolar tissue separates the two tumors. This is from a rat that was exposed to chrysotile dust for 62 weeks beginning immediately after the intratracheal caustic application. The animal died 12 months after removal from the exposure chamber (hematoxylin and eosin, × 40).

Altogether, there were 28 animals with primary malignant tumors of the lung, exclusive of spontaneously occurring pulmonary lymphoblastoma but inclusive of one malignant mesothelioma of the pleura and three questionable, but probable, adenocarcinomata of the lung. The overall prevalence was 31%, based on 91 animals surviving 16 months or longer. Of the 28 rats with malignant pulmonary tumors, 25 had inhaled chrysotile dust, giving this group a cancer prevalence of 35% (based on a co-

Fig 3.—Adventitial and periadventitial invasion by tumor glands about a pulmonary artery in a rat exposed to chrysotile dust for 62 weeks beginning three weeks after the intratracheal caustic application. It died 15½ months after removal from the exposure chamber (hematoxylin and eosin, × 150).

Fig 4.—Example of one of the many perplexing lesions found in the asbestotic rat lungs. The lesion is characterized by a collection of fairly large spaces that have occasional irregular evaginations, lined by deeply staining, hyperplastic cuboidal epithelium. The walls are relatively thick but not collagenized. The lesion resembles an adenoma, but more likely represents adenomatoid metaplasia and hyperplasia. From the same lung as in Fig 3 (hematoxylin and eosin, × 150).

hort of 72); three had been injected intratracheally with the dust, a prevalence of 16% (based on a cohort of 19 rats). Of the 25 animals with primary lung cancer including the mesothelioma in the inhalation group, 15 had received an intratracheal application of lye, giving a cancer prevalence of 48% (based on a cohort of 31 rats treated with lye and surviving 16 or more months). The remaining ten animals with lung cancer that had not had intratracheal lye treatment, represented a cancer prevalence of 24% based on a cohort of 41 rats.

There were 17 rats with certain adenocarcinomata and three animals with questionable but probable adenocarcinoma. Of seven rats with fibrosarcomatous lung tumors, three are also listed with the ones having adenocarcinoma. Of four rats with a pulmonary squamous cell cancer, one also is listed with the 20 having adenocarcinoma. A list of the animals with tumors, their exposure, survival time, and nature of the tumor is given in Table 2.

No lung cancer was seen in any control animal other than the spontaneously occurring lymphoblastoma.

The diagnosis of adenocarcinoma is usually not difficult since a large nodule composed of crowded, diminutive and atypical glands makes recognition of this malignant tumor relatively easy (Fig 2). Often, however, other foci of altered morphology in the same lung or even in the same section lead to less certain conclusions. The diagnosis of some pulmonary lesions is not easy and, in a few instances, was designated as "questionable but probably correct." A fairly common, but fascinating lesion involves regions of acellular, collagenous scarring affecting respiratory bronchioles, their adjoining alveolar ducts, and their evaginating alveoli. In a few

Table 2.—Rats With Lung Tumors and Their Survival Time

Rat No.	NaOH*	Dust Exposure: 62 Weeks	Dust Exposure: No. of 3.5-mg Doses	Survival (Mo)	Primary Lung Cancers†				
					Adeno	Sq Cell	Fibro	Meso	Adenoma
8804	—	+	—	16		+			
8847	—	+	—	16			+		
8742	1	+	—	16			+		
8752	0	+	—	16¼	+				
8761	1	+	—	16¼		+			
9068	0	+	—	23	+				
8747	3	+	—	25¾	+		+		
8781	2	+	—	26	+		+		+
8740	0	+	—	26½	+				+
8834	—	+	—	27¼			+		
8835	—	+	—	27¼	+				
8782	2	+	—	27½			+		
8863	—	+	—	27¾	+		+		+
8767	1	+	—	28				+	
8854	—	+	—	28¼	+	+			
8743	1	+	—	28½	+				
8826	—	+	—	28½	+				
8796	3	+	—	28¾	+				
8768	1	+	—	29½	+				
8811	—	+	—	30¼		+			
8840	—	+	—	31	+				
8783	2	+	—	31¼	+				
10566	3	+	—	32	+				+
10567	3	+	—	32¾	+				
11175	—	+	—	34	+				
9188	—	—	6	21 K‡	+				
9191	—	—	4	21 K	+				
9204	—	—	4	21 K	+				

* Applied intratracheally weeks prior to inhalation exposure. Animals exposed to dust immediately after the caustic application are designated by 0. Those that received no caustic application are designated by —.

† Exclusive of lymphoblastoma, which is a common spontaneous tumor in these aging animals. Adeno, adenocarcinoma; Sq, squamous; Fibro, fibrosarcoma; Meso, mesothelioma.

‡ Killed. All other animals were found dead in their cages.

scarred foci in many lungs, the air spaces are lined by darkly staining cuboidal to columnar epithelium. The combination of distortion of the air spaces with abnormal epithelial lining give the impression of neoplasia. Because the lining epithelium is uniformly regular, well oriented, and devoid of excessive mitotic activity, these foci, if considered as tumors, should be designated as benign, ie, adenomata. But if they are indeed to be considered as adenomata, they are quite different from the spontaneous pulmonary adenomata seen commonly in mice (and rarely in rats and guinea pigs). Although the usual pulmonary adenoma is not circumscribed when small, the larger it grows, the more it tends to compress the surrounding lung tissue and thus develop a pseudocapsule of compressed lung tissue. The adenoma-like foci in the lungs of asbestotic rats are irregular in shape and although of moderate

Table 3.—Trace-Metal Content of Chrysotile Dust and of Worn Part From Hammer Mill

Element	Chrysotile: Prior to Hammer Milling, µg/gm Sample	Chrysotile: After Hammer Milling, µg/mg Sample	Chrysotile: After Hammer Milling, Increase, %	Worn Hammer From Mill, µg/gm Sample
Nickel	134.8	245.5	82	2,338
Cobalt	8.8	21.6	145	185
Chromium	114.6	153.3	34	...
Lead	None	37.9	...	3.5

Fig 5.—To the right of this large adenocarcinomatous mass there are two small foci of peribronchiolar adenomatosis. To the right and below center of the field is a smaller nodule of adenocarcinoma in which glandular epithelium is darker than in larger mass. This is from a rat that received six intratracheal injections, each of 3.5 mg chrysotile dust. It was killed 21 months from time of the first injection (hematoxylin and eosin, × 150).

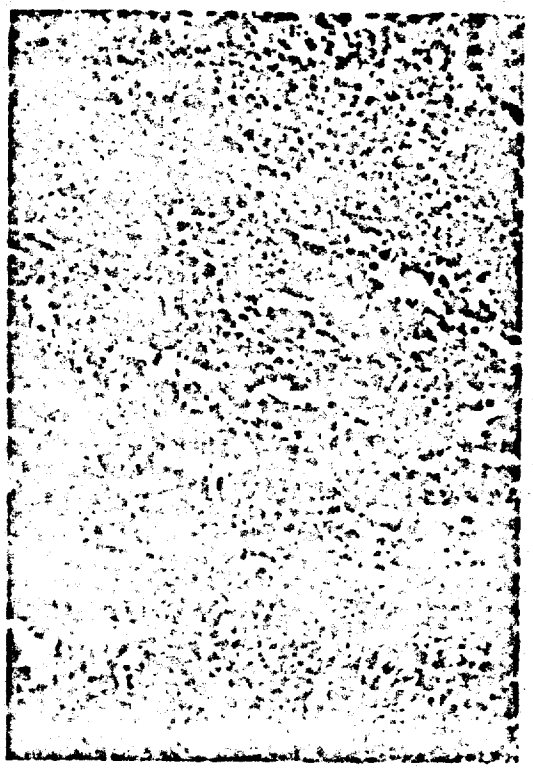

Fig 6.—Small pleomorphic, darkly-staining tumor cells, many of them more or less spindle-shaped, lining the thick walls of slit-like spaces. With silver impregnation the thick walls are recognizable as septal walls. The pleomorphic cells are associated with fine argyrophilic fibers distinct from the thicker septal fibers. This is from a rat exposed 62 weeks to chrysotile dust without an intratracheal caustic application. It died about 11 months after removal from the inhalation chamber (hematoxylin and eosin, × 150).

size, lack even a suggestion of circumscription. Other significant points of difference between adenoma-like foci in asbestotic rat lungs and the usual spontaneous pulmonary adenoma of small laboratory animals are the presence in the latter of crowded glands with minimal intervening stroma, tall columnar epithelial cells with abundant pale cytoplasm, and papillae with delicate stromal stalks. None of these features are found in the adenoma-like lesions of asbestotic rat lungs.

Yet, there are similar foci in which the lining epithelium of the spaces is so crowded as to suggest growth activity beyond that consistent with mere hyperplasia; the epithelium is larger with more abundant cytoplasm; intra-acinar papillae are present, and smaller oval or tubular glandular structures are found within expanses of acellular collagenous stroma. These foci are manifestly neoplastic. Malignancy is evidenced by the presence of numerous irregular small glands and cell nests within broad, densely collagenous scars, forming a picture reminiscent of a scirrhous breast cancer. In other lungs, foci of malignant neoplastic glands may be characterized by cellular atypia such as piling up of nuclei with poorly defined cell boundaries and loss of cellular polarity. Occasionally the diagnosis of malignancy is facilitated by demonstration of epithelial tissue in the adventitia or the periadventitial stroma of larger vessels (Fig 3). Diagnostic problems arise when lesions intermediate between those manifestly neoplastic and those considered as atypical metaplasia are encountered. Such intermediate lesions are fairly common (Fig 4).

In a number of rats, multiple foci of ade-

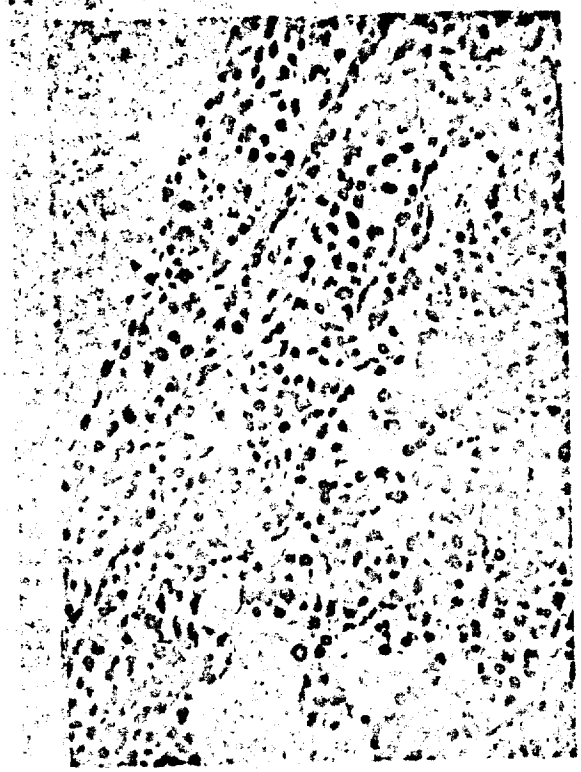

Fig 7.—This field represents an undifferentiated, large cell sarcoma involving subpleural parenchyma and also extending over the pleural surface. The silver preparation indicates an intimate association of argyrophilic fibers with these cells. From a rat exposed 62 weeks to chrysotile dust, beginning two weeks after an intratracheal caustic application. The animal died 11½ months after removal from the inhalation chamber (hematoxylin and eosin, × 300).

Fig 8.—Combination of adenocarcinoma and fibrosarcoma of the lung. Both tissue elements are well differentiated. Glandular cells secrete mucin whereas stromal cells are spindleshaped and resemble those of endometrium. This field is taken from the lung of the same rat as in Fig 7 (hematoxylin and eosin, × 150).

nocarcinoma are present in the same section, indicating the multicentric nature of the tumor (Fig 5).

In several animals, a cellular fibrosarcomatous tumor was found in the lungs (Fig 6). On the periphery of such tumors, the air spaces are narrowed, their walls thickened and composed of pleomorphic cells with hyperchromatic nuclei and scanty cytoplasm. Coherent masses of these cells partially fill the air spaces. They are associated with a delicate, argyrophilic supporting stroma. The more substantial stroma of the alveolar septa remains intact. Although superficially resembling the lymphoblastoma that occurs spontaneously in aging rats, these fibrosarcomas differ from the latter by the pleomorphism of their cells, their tendency to respect the alveolar architecture at their periphery, and the presence there of a delicate argyrophilic stroma. In contrast, the spontaneous lymphoblastoma completely destroys the alveolar architecture whether it is of the lymphocyte or the reticulum cell type. A second form of fibrosarcoma was encountered in a lung, with an adenocarcinoma in which the stroma is composed of crowded, fairly uniform vesicular ovoid nuclei and an unusually dense argyrophilic network. In one animal there were foci in which sarcomatous cells resemble decidual cells (Fig 7). Interesting combinations of adenocarcinoma and fibrosarcoma were found in several animals (Fig 8). The fibrosarcoma may be pure or may contain epithelial components (Fig 9).

Perhaps the most interesting of all, because of its rarity, was a pleural mesothelioma in one rat that covered portions of both lungs and the pericardium like a cuirass. The tumor consists of straight and tortuous as well as branching tubular glands seen in longitudinal and cross sections, associated

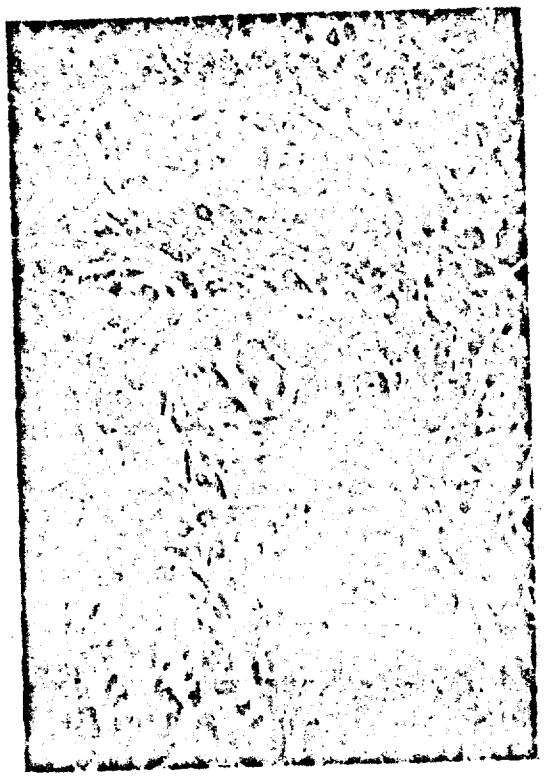

Fig 9.—Another combination of adenocarcinoma and fibrosarcoma of the lung showing a small gland in the center surrounded by well differentiated fibrosarcomatous tissue. This is from a rat exposed to chrysotile dust for 62 weeks and died 13 months after removal from the exposure chamber. It had received no intratracheal caustic application (hematoxylin and eosin, × 300).

Fig 10.—Portion of thick, pleural mesothelial tumor composed of irregular glands embedded in a dense collagenous tissue. Except for a few small superficial foci, the tumor does not invade lung tissue. This tumor was found in a rat exposed to chrysotile dust for 62 weeks beginning one week after an intratracheal caustic application. The animal died 13½ months after removal from the inhalation chamber (PAS stain, × 40).

with a moderately cellular but relatively dense stroma (Fig 10). The glands are lined by sharply delimited, cuboidal to low columnar epithelium with round, uniform-appearing nuclei (Fig 11). The lumen of some of the glands contains PAS-positive material, yet the cytoplasm of the lining cells is PAS-negative. The density of the stroma is best seen in the silver-impregnated section where crisscrossing thick collagen bundles seem to enclose glandular elements. The stromal cells are ovoid in shape and their nuclei of a uniform, moderately dense character. Over the greater portion of the pleural surface, the tumor is sharply confined to the pleura. However, in several foci there is superficial invasion of alveolar tissue. Several mesothelial tumor nodules appear as isolated foci upon an otherwise normal pleura. Whether these are metastases or multicentric primaries is difficult to say. The smallest consists of stroma only and forms a lentiform pleural thickening.

Although the squamous cell carcinomata are not difficult to diagnose (Fig 12), occasional foci of intra-alveolar atypical squamous cell production have some malignant aspects (Fig 13). These have been categorized as probable atypical metaplasia.

Another interesting tumor is a mucinous adenocarcinoma, forming a subpleural mass about 4 mm in diameter. This tumor, also termed "colloid" carcinoma, consists of irregular, mucin-filled cystic spaces that have destroyed and replaced the alveolar architecture (Fig 14). Very few viable tumor cells lining these spaces can be identified (Fig 15). This type of cancer is more commonly seen as a primary tumor in the gastrointestinal tract.

With the exception of one squamous cell carcinoma that metastasized to a satellite

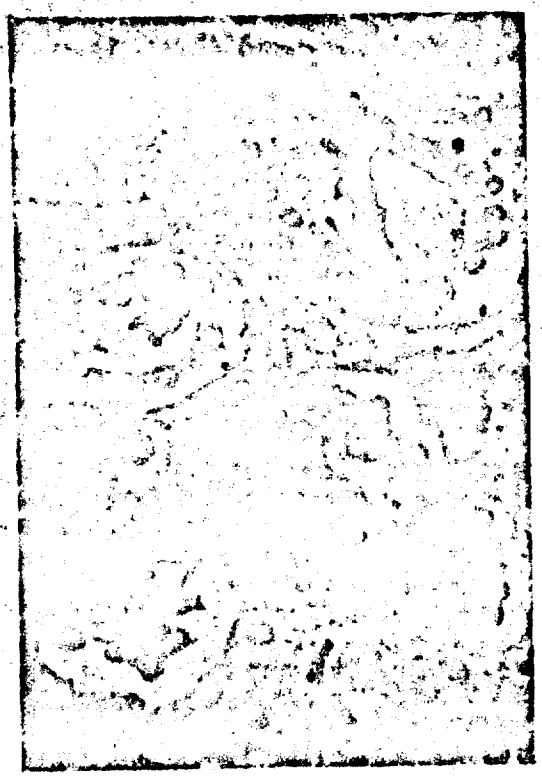

Fig 11.—Higher magnification of several tubules of the mesothelioma. Cytoplasm of the lining epithelium is PAS-negative although PAS-positive material is present in the lumen (× 300).

Fig 12.—Irregular cords and nests of well-differentiated squamous cells show no keratin formation. Extensive keratinization is present in other fields of this pulmonary squamous cell carcinoma. From a rat exposed to chrysotile asbestos for 62 weeks without caustic treatment. Animal died 14 months after removal from exposure chamber (hematoxylin and eosin, × 150).

lymph node and the possibility that some of the discrete pleural foci of the mesothelioma represent secondary nodules, no evidence of metastasis has been observed in satellite nodes or elsewhere. Examination of other organs in animals with lung cancer failed to demonstrate the presence of a tumor that could have produced a pulmonary metastasis.

These lung cancers have no apparent relation to the degree of asbestotic involvement. Not one tumor-bearing rat had diffuse asbestosis. In all animals the asbestotic involvement was multifocal, with abundant normal, or near-normal alveolar tissue between such foci. Lesions were centered in the proximal portions of the primary lobule.

Comment

The development of lung cancer in rats caused by the pulmonary deposition of chrysotile dust confirms experimentally the cause-and-effect relationship between chrysotile dust exposure and lung cancer. Until this study, such a cause-and-effect relationship was only surmised epidemiologically by a study of workers exposed to chrysotile dust in the British asbestos textile industry[1] (largely prior to 1933). In the United States an epidemiologic study of members of an asbestos workers' union also indicated increased prevalence of lung cancer, but the nature of the dusts to which the workers had been exposed was uncertain.[2]

The present study, with its 31% prevalence of pulmonary cancer, is in striking contrast to results reported by Wagner et al[4] who encountered no lung cancer. It seems unlikely that a difference in susceptibility to lung cancer in the strains of rats used could account for the differences in results. However, there is an excellent probability that a particular quality of chrysotile dust

Fig 13.—Nests of transitional or partially differentiated squamous epithelial cells derived by metaplasia from alveolar lining epithelium. Although there is loss of cellular polarity and nuclei are piled up on one another, latter are uniform in appearance and show no mitotic activity. Squamous cells are confined within limits of alveolar spaces. Lesions such as these are not classified as neoplastic. This is from a rat that had an adenocarcinoma in another portion of lung. This animal had been exposed to chrysotile dust for 62 weeks and had died 16½ months after removal from exposure chamber. There had been no intratracheal caustic application (hematoxylin and eosin, × 150).

Fig 14.—Typical mucinous adenocarcinoma, most commonly primary in gastrointestinal tract but found in lung of rat exposed for 62 weeks to chrysotile dust without a prior intratracheal caustic application. Peripheral (subpleural) nodule composed of irregular mucin-filled spaces that have destroyed and completely replaced alveolar tissue. Rat died 19½ months after removal from inhalation chamber (hematoxylin and eosin, × 40).

deposited in the lungs of our rats was significant in producing lung tumors. The prolonged hammer-milling used to prepare the chrysotile dust in our investigation was not employed in other long-term studies. Wagner et al[4] did not further comminute the dust they used for animal exposures but reported obtaining material directly from industrial dust collecting apparatus. We, therefore, presume, although we are not certain, that the tracemetal content of the latter dust may not have been as high as in the dust to which our animals were exposed (Table 3). The probability exists that factors other than the crystalline hydrated magnesium silicate in chrysotile asbestos dust may account for the severe pathologic response of lung and pleural tissues to this dust. These factors are as follows:

1. The chrysotile dust used in this investigation has a high trace-metal content, considerably higher than it was prior to the hammer-mill treatment (Table 3). The increase in content of trace metals after milling was nickel, 82%; cobalt, 145%; chrome, 34%. Aanlysis of the worn hammer of the hammer mill revealed a nickel content of 2,338μg/gm of sample.

2. There is a disparity in the published prevalence of lung cancer among those who worked in the British asbestos textile industry prior to 1933[1] and that among Canadian chrysotile asbestos miners who worked from 1950 to 1956.[5] Although the workers in the British asbestos textile industry had a tenfold greater risk of developing lung cancer than had the general population,[1] this in-

Fig 15.—Higher magnification of several of the mucin-filled cysts seen in Fig 14, shows the loss of lining epithelium of these cysts, a feature commonly present in this type of cancer (hematoxylin and eosin, X 150).

creased risk of lung cancer was not found among workers in the Canadian chrysotile industry.[5] Aside from differences in climatic conditions, ethnic backgrounds, density of dust exposure, and possibly also in smoking habits, it appears probable that differences in the dust itself to which these two population groups had been exposed may account for the disparity. For example, at the mines, chrysotile mineral is subjected to considerable crushing contact with high-strength steel alloys to "open" the fibers and remove adherent nonfibrous mineral components prior to shipment to manufacturers. However, considerably more extensive contact with nickel- and chrome-steel alloys, involving more and more of the most finely divided fibers, takes place during this process and during carding as well as spinning, essential processes in the fabrication of asbestos textiles. This, in a manner similar to the hammer-milled asbestos, may cause chrysotile asbestos dust to acquire a higher content of potentially fibrogenic and carcinogenic trace-metals[6] than is present in chrysotile dust at the mine.

3. Preliminary investigations in this laboratory, using synthetic chrysotile prepared by W. T. Granquist, PhD, of Mellon Institute suggest it is biologically "inert" (Gross, P.; deTreville, R. T. P.; and Granquist, W. T. unpublished data). Something other than pure chrysotile crystals may be responsible for the lesions found in human and animal lungs which contain asbestos.

4. There is a uniqueness not only in the association of asbestos dust exposure with the occurrence of mesothelioma but also in the association with lung cancer insofar as there is no relationship between severity of involvement and occurrence of neoplasia. It has not been possible to demonstrate convincingly the presence of the presumed inciting agent at the site of neoplasia. It is very difficult to demonstrate asbestos fibers or asbestos bodies even in non-neoplastic thickened pleurae. If the association of asbestos-dust exposure and neoplasia represents a cause-and-effect relationship—and there is little reason to doubt it—then a logical explanation might be that something accompanying asbestos dust adversely affects tissues located at considerable distances from the site of dust deposition. This could be one or several trace metals that are added to the mineral dust from machinery and become eluted after pulmonary deposition.

Of the trace-metals associated with the asbestos dust used in our study, only nickel has been shown to be capable of producing lung cancer experimentally. Sunderman et al[6] found that rats exposed to nickel carbonyl developed pulmonary squamous cell carcinoma.

The many different types of primary malignant lung tumors in our animals with pulmonary deposits of chrysotile dust and the different types of cells in these tumors bring to mind a concept originated by Waddell[7] and defended by work in this laboratory.[8] This theory holds that the parent cell of the alveolar membrane is multipotential, capable of differentiating either into epithelial or connective tissue. Except for the mesothelioma, all the lung tumors described in this paper are believed to have originated from cells of the alveolar membrane, a uni-

port the unitarian concept of tumor origin from alveolar cells. Despite extreme exposure conditions, diffuse asbestosis was not seen. Fibrosis observed was multifocal, centered in the proximal portion of primary lobules with normal appearing tissue intervening.

Cancer production with chrysotile in this study appears to involve trace metals introduced during hammer milling which increased nickel, chrome, and cobalt. Previous investigators have not reported subjecting their dusts to hammer milling. The increase in content of trace metals after milling was as follows: nickel, 82%; cobalt, 145%; chrome, 34%. Analysis of the worn hammer of the hammer mill revealed a nickel content of 2,338μg/gm of sample. This finding, if validated by further studies, should provide an important aid to guide industrial hygiene controls of potential health hazards.

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James M. McNerney participated in the early stages of this investigation.

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SALINE AND ALKALINE DRINKING WATERS

By Victor G. Heller

(From the Department of Agricultural Chemistry Research, Oklahoma Agricultural and Mechanical College, Stillwater)

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A NUMBER of years ago a preliminary report (1) was made of the effect of saline drinking waters upon growth and reproduction of animals. Since that time the problem has been continued and has increased in interest as well as in scope and practical application. As stated in the previous article, of the several nutriments needed by man and animals, drinking water is undoubtedly the greatest variable in this day of rapid travel and the one most difficult of correction in many regions of the United States. There are large areas of the earth's surface where deep wells cannot be used due to the extreme alkalinity or salinity of waters procured. Many streams flow through salt beds on the surface or through areas where they become contaminated with the waters from oil wells, factories or mines. When the flow of water is limited, due to dry seasons, the dissolved salts make the use of these waters questionable. In other sections alkali beds of great area become leached by rains or underground waters, causing the water to be even more deleterious for birds or aquatic life.

A search of the literature, as previously stated, reveals little to answer the question of a possible standard for drinking purposes and the physiological effects of an excess of salts. The U. S. Public Health Service (2) has set up an arbitrary standard for interstate carriers, which may be an ideal to be sought but one not possible to obtain in many regions. The pharmacologic literature is very complete in regard to the effect of minimum lethal doses of specific salts but is of little use in postulating the effect of a continued use of these salts in drinking waters. Volumes have been written by developers of mineral springs lauding their merits, especially in the literature of a generation ago; but as stated by Van Noorden (3), "The truth is that so little is known of the bearing of mineral waters on biological processes that most of the statements in biological literature may be stigmatized as idle make-believe." There are many articles having some bearing on the problem, but they are of no direct importance in the solution of our difficulty. In the medical literature we find references to the effect of certain waters in the stimulating of intestinal secretions, their effect upon digestion, and upon hydrogen ion concentration of the body fluids. Chase (4) makes a report of the apparent health of people living in

hard water sections of Ohio compared to the health of those living in soft water regions. Roberts (5) states that young fish can stand Cl up to 5,750 parts per million; while Young (6) believes that a shift in the osmotic effect to about six atmospheres, due to total salt, is fatal. Shaw (7) in his duck disease studies of salt areas makes record of the amount of soluble salts found necessary to injure and kill migrating ducks.

A few articles having more direct bearing upon the subject have appeared in the literature since the publication of our first article. These pertain to deaths thought to have been caused by saline waters in the British Colonies. Legg (8) describes the death of sheep that accidentally drank salt solutions while being shipped. Ramsay (9) states that cattle in cases can live on water containing 17,120 p.p.m. of total salt and thrive on 11,400 p.p.m. Horses thrive on waters containing up to 5,720 p.p.m. and can be sustained on 9,140 p.p.m., if not worked too hard; while sheep are similar to cattle in this respect. Scott (10) gives a rather detailed description of the clinical symptoms of cattle suffering from the effects of salty waters.

Clough (11) and Mitchell, Carl and Carman (12) refer to deaths of poultry from the excessive consumption of ordinary table salt. We have observed in many cases deaths among hogs and cattle that had been overfed fine salt while in a salt-hungry condition. Hogs, especially, seem to be particularly susceptible. In most of these cases, however, we are dealing with the effect of single large doses of salts in salt solution or with unknown mixtures. In our problem it is necessary to know the effect of continuous use of such solutions over long periods of time. Likewise, it is necessary to know whether there is possibly some physiological change whereby the body can adjust itself to these altered conditions. Such a change has been noted (13) in freezing points of blood of eels after immersing them in saline water, indicating that some salts are evidently absorbed and retained in the blood. The writer has often found both man and animal sustaining no apparent injury from continuously using waters that caused very serious discomfort and in some cases death to animals not accustomed to such concentrations.

The purpose of this investigation has been to demonstrate what concentrations of various commonly occurring salts and alkalies produced death, stunted growth, inhibited reproduction and disturbed lactation; to determine by mixture of salts if there is an antagonistic reaction between salt ions as has been proved to be effective for plants; to ascertain if there is some physiological readjustment by which animals can become accustomed to saline drinking waters; and lastly, to make a study of the deleterious condition produced and mechanisms by which physiological conditions are altered.

Method of Procedure

In all related studies undertaken by other workers, it has been the custom when deaths occurred to obtain a sample of the water which produced the effect and to make an analysis of it. In these cases it was then necessary to postulate what was the effect of the mixture. It has seemed advisable in our case to proceed by a method previously used in plant nutrition studies, that is, to use distilled water and add varying amounts of the salt under consideration until the deleterious levels were determined. Rats were used as experimental animals for all preliminary tests, because of convenience of control, the possibility of using very large numbers, and comparatively short reproductive periods. The animals were housed in cylindrical wire cages commonly used for nutrition tests, and fed a well-balanced ration (consisting of yellow corn, whole wheat, milk powder, alfalfa, cottonseed meal, and tankage) regularly used for our breeding colony. One per cent of sodium chloride and 1 per cent of calcium carbonate were added for mineral supplements. The drinking water was furnished from glass bottles fitted with glass syphons. The usual precautions in regard to measuring the amount of water and feed consumed, the recording of weights of all animals, and the practice of sanitary measures familiar to nutrition workers were observed.

The first studies were made with sodium chloride, that being the most widely distributed salt in most regions. Ten solutions containing sodium chloride were prepared as follows: 0.5, 1.0, 1.25, 1.5, 1.75, 2.0, 2.25, 2.5, 3.0, and 4.0 per cent. Ten cages containing 4 or more rats each, previously selected so as to be as nearly comparable as possible, were started and given the various solutions as a sole source of drinking water. Within a few days deaths occurred among the higher levels, and within 10 days no rats receiving 2.5, or more per cent salt were alive. It was interesting to note that at lower levels the amount of water consumed became greater with an increasing amount of salt until a concentration was reached which they refused to drink at all, refraining from drinking until thirst finally compelled them to drink a large quantity at one time causing death in a short time. Farmers often give a similar description of cattle dying apparently from salty waters. It was observed that waters containing 1.5 per cent were often fatal to young rats. Growth stopped, the animals presented an emaciated appearance, the coats became rough, eyes were often sore, presenting a condition resembling ophthalmia, and diarrhea was always

present. An autopsy showed the intestines to be inflamed and, in severe cases, bloody. In many cases, spots were found on liver and lungs. The sites of infection, we believe, are due to a secondary cause brought about by the general weakened condition. It was later demonstrated that when the animals on levels up to 1.5 per cent salt survived the preliminary stunted period, they resumed growth and in many cases reproduced and reared their young in a manner somewhat comparable to normal rats. A few survived at higher levels, although the ability to nourish young was very limited. Occasionally, normal young were born, but they often died seemingly due to a lack of action of the mammary glands. Therefore, it is quite apparent that there is some physiological readjustment which should explain how man and animals reared in certain sections thrive on waters that kill or injure those accustomed to the drinking of soft waters only. It was also observed in the course of the investigation that old animals were more resistant to these changes than young. Rats four weeks of age and weighing 50 grams, when placed on 1.5 per cent salt nearly always died. The ones surviving passed through a stunted period, in time started growing, and in some cases grew normally. Full grown animals lost weight but rarely died. We believe this explains why a herd of cattle shipped into these regions will die or be seriously injured, while other animals accustomed to these conditions will live in apparent good health. Likewise, aquatic fowls will rear young on saline lakes, while migrating birds from clear water regions will die by the hundreds in such lakes. The writer's attention has been called to fish living in salt water wastes of oil wells, this water containing over 1 per cent of sodium chloride alone as well as other salts. Yet, fish from surrounding streams would have died if placed in such concentrations.

In working with plant nutritive solutions, it has been found that at a certain level sodium chloride becomes toxic. To this concentration calcium salts could be added, and despite the increased concentration the plant could live. This is explained on the basis of antagonistic action of ions. In order to determine whether this would be true for animals, other salts were added to the dangerous levels of sodium chloride. The additional salt made the previous toxic salt more toxic. Evidently, either antagonistic ion action is not effective for animals, or some other factor predominates in the final results. Animals are also different in that they can use much greater concentrations of salts than plants. All of our observations lead us to believe that there is an osmotic rather than a toxic reaction of salts. The accompanying table shows the quantities of the various salts used, the number of test animals, the effect upon reproduction, the number of

TABLE I

RESULTS OBTAINED BY USE OF SALINE WATERS AS A SOLE SOURCE OF DRINKING SUPPLY FOR RATS

Salt	Per cent	Males	Females	Litters	Young born	Young lived	Young died	Remarks
NaCl	0.5	2	3	3	24	20	4	Growth normal.
	1.0	1	1	1	7	4	3	Growth normal.
	1.5	3	5	5	39	31	8	Growth subnormal. Some die.
	2.0	2	3	3	20	17	3	Young die.
	2.2	1	2	0	0	0	0	Old and young die.
	2.5	4	6	0	0	0	0	Old and young die.
	3.0	2	2	0	0	0	0	Sudden loss in wt., diarrhea, rough hair, and death.
	3.5	2	2	0	0	0	0	Young and mature die soon.
	4.5	4	2	0	0	0	0	Die at once.
NaCl+ KCl	1.5							
	0.7	2	3	3	39	0	39	Young die within 15 days.
	1.7							
	0.6	2	2	0	0	0	0	Old ones die.
	2.5							
	1.0	2	4	0	0	0	0	Old and young die.
	3.0							
	1.5	2	4	0	0	0	0	All die within a week.
NaCl+ $CaCl_2$	1.5							
	0.7	5	4	1	7	0	7	Hair thin and rough. No gain.
	3.0							
	1.5	4	4	0	0	0	0	All die at once.
$CaCl_2$	1.0	2	2	2	13	10	3	General condition good.
	1.5	3	6	7	77	62	15	Growth satisfactory. Condition good.
	2.0	1	2	5	30	6	24	Interferes with lactation.
	2.5	1	1	0	0	0	0	Old ones die.
$MgSO_4$	0.5	2	2	2	10	10	0	Growth and reproduction.
	1.0	1	3	6	51	24	27	Young poor; old emaciated.
	1.5	3	3	3	20	13	7	Growth subnormal; diarrhea, rough coat.
$MgCl_2$	1.0	2	4	2	15	1	14	Condition of old not normal.
	1.5	1	2	0	0	0	0	Growth below normal.
$MgCl_2$+ $CaCl_2$	1.0							
	0.5	2	2	1	7	0	7	Growth of old normal. Lactation poor.

TABLE I (*Continued*)

Salt	Per cent	Males	Females	Litters	Young born	Young lived	Young died	Remarks
$Mg(Ac)_2$	1.5	2	2	2	17	8	9	Growth satisfactory. Reproduction.
Na_2CO_3	1.0	3	7	7	45	31	24	Growth of offspring unsatisfactory.
	1.5	1	7	2	19	0	19	Young die; old rough coated, red eyes, diarrhea.
$NaHCO_3$	1.5	2	4	7	55	48	7	Old somewhat undersize. Growth of offspring impeded.
	2.0	1	2	2	14	7	7	Growth and appearance unsatisfactory.
NaOH	0.5	2	4	0	0	0	0	Practically normal growth.
	1.0	3	4	0	0	0	0	Little growth, animals dirty, very nervous, diarrhea, sore eyes.
$Ca(OH)_2$	Sat.	4	4	4	30	19	11	Some interference with growth during lactation. 3 generations produce.
$CaSO_4$	Sat.	3	8	7	33	28	5	Satisfactory growth.
Na_2SO_4	1.5	1	2	1	11	6	5	Appearance of adults satisfactory.
NaF	1.0	1	2	0	0	0	0	Growth poor. Animals emaciated.
KI	1.0	1	2	0	0	0	0	Growth fair. No reproduction.

young reared, and the general condition of test animals. It also illustrates the results of introducing a mixture of salts into drinking waters. It should be noted at this point that these results have been checked and rechecked over a period of four years. Whenever reproduction occurred, the young were continued on their mother's diet through five or six generations.

The effect of seasonal variation was also given consideration. As one might predict, the hot dry days of summer seemed to be most fatal to animals drinking saline waters, a result undoubtedly due to a greater thirst and the [illegible] greater salt intake.

In streams contaminated by oil well wastes, calcium chloride and

magnesium chloride are often found in large quantities. Examination of these wastes entering streams often shows a salt content of over 20.0 per cent. In many sections near the gypsum beds, waters saturated with calcium sulfate are common; while sodium carbonate and bicarbonate frequently occur in several western states. For these reasons these salts were studied separately and in combinations, as is shown in the table. Calcium chloride is not normally found in most waters, but it is one of the common salts in oil well wastes. Streams and pools in the neighborhood of oil fields are often heavily saturated with this salt. A series of experiments as conducted with sodium chloride were carried out, using calcium chloride as dissolved salt. It was expected that more deleterious effects would be observed, but the contrary was established. An inspection of the table will reveal that at 1.5 per cent, rats, after the first rather difficult adjustment period, grew normally, reproduced their young, and were able to suckle them through the lactating period. In fact, the general appearance, coat, eyes, and build of the rats seemed superior to those of the breeding colony. All of our tests indicated that calcium chloride might prove to be a more satisfactory source of calcium in mineral mixtures than calcium carbonate. Even at 2.0 per cent, growth after the introductory period was normal, but lactation was hindered. At higher levels, results similar to those with sodium chloride were encountered.

Waters saturated with $CaSO_4 \cdot 2H_2O$ are common about gypsum deposits. However, this salt is only soluble to the extent of 0.24 per cent. Waters of this type have not proved to interfere with normal growth, and reproduction.

Magnesium, the third ion quite commonly found in salt well wastes in the form of magnesium chloride, and in many well waters near sulfate deposits as magnesium sulfate, was used in the form of magnesium chloride, sulfate, and acetate. An examination of the tables will demonstrate that magnesium is not so satisfactorily used as either sodium or calcium, but the true condition can only be appreciated by observation of the living animals. When the animals were first given these solutions there was a rapid loss of weight and severe diarrhea; and even in cases where the animals recovered, they did not have smooth coats and a general emaciated condition was presented. It is evident that magnesium in company with the sulfate radical is more detrimental than magnesium chloride and much more so than the acetate.

In many sections of the west, alkali waters containing Na_2CO_3, $NaHCO_3$ and even NaOH are to be found. We have had occasion to analyze samples which contain large quantities of $Ca(OH)_2$. The latter is only soluble to

the extent of 0.18 per cent, but it was rather surprising to find that this hydroxide could be used as a sole source of drinking water through four generations without any evident deleterious results with the exception of a somewhat greater mortality of the young. Sodium hydroxide to the extent of 0.5 per cent permitted life and growth, but 1.0 per cent allowed no growth; the animals became thin and very highly nervous and irritable, the eyes became sore and diarrhea followed. A similar condition was likewise produced by 1.5 per cent of Na_2CO_3; 1.0 per cent of the latter did permit growth and reproduction, but there was a high mortality of young. Sodium hydrogen carbonate, 1.5 per cent, permitted growth, reproduction, and rearing of young. These young, as well as the adult rats, were somewhat undersized. At 2.0 per cent the general appearance became unsatisfactory. With the sodium hydroxide and carbonate we found indications of factors other than osmotic pressure producing their effect; however, it was rather surprising to note the amount of alkali that could be utilized. A number of other salts have been used to answer certain specific cases called to our attention, but as they are not of general interest no comment need be added to the facts presented in the table.

The question has been raised whether or not data obtained from rats would be applicable to other animals. We have made sufficient isolated tests to furnish evidence that they are, at least for cattle, sheep, and rabbits. Other tests now under way will determine this conclusively and should it be proved that larger animals are exceptions to the rule, that fact will be reported at a later date.

Several series of very definite chemical analyses and measurements are also being run to answer definitely certain questions postulated in this article, namely, in regard to the change in osmotic effect, changes in pH of blood stream, and the effect on the organ size. A detailed description of the methods used and results will be forthcoming.

Conclusions

1. Waters containing large amounts of salts in solution are deleterious for drinking purposes.

2. The effect produced seems to be more osmotic in reaction than due to any specific ion.

3. From 1.5 to 1.7 per cent seems to be the maximum amount of soluble salts that can be used with safety.

4. Animals seem much less susceptible to salt solutions than plants, and antagonistic effect of ions is lacking or is a secondary factor.

5. Chloride salts are less injurious than sulfates, and organic salts are less injurious than inorganic.

6. Alkalies are more deleterious than normal salts; evidently the osmotic effect is coupled to a harmful effect of the changed pH.

7. An interference in lactation and reproduction is noticeable even before the level producing stunted growth or death is reached.

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THE DISINFECTANT ACTIVITY OF CAUSTIC SODA

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By BETTY C. HOBBS, Ph.D., B.Sc., Dip.Bact. and G. S. WILSON, M.D., F.R.C.P., D.P.H., *London School of Hygiene and Tropical Medicine*

(With 2 Figures in the Text)

In connexion with an investigation made for the Ministry of Health into the cleaning and sterilization of milk bottles, the general results of which it is hoped to publish in a future issue of this *Journal*, it proved necessary to obtain exact information on the bactericidal activity of caustic soda. In particular, the effect of varying the concentration and the temperature of the solution on the destruction of sporing and non-sporing organisms had to be determined before it was possible to lay down the strengths at which caustic soda should be used under varying conditions of plant operation in order to bring about a given degree of sterility. Careful quantitative observations were therefore made in the laboratory.

1. Observations on *Bact. coli*

Technique

After some preliminary work, the following technique was adopted.

A non-clumping, relatively heat-resistant strain of *Bact. coli* was selected and used throughout. It was grown for 21 hr. at 37° C. on heart extract agar slopes. The growth was washed off with sterile glass-distilled water and standardized by opacity to 3000×10^6 per ml., using a standard opacity tube made by counting a suspension of *Bact. coli* from a 24 hr. agar culture in a Helber chamber adapted for dark-ground illumination (Wilson, 1922, 1926).

Four ml. of the 3000×10^6 suspension were added to 36 ml. of a sterile 1/5000 dilution in glass-distilled water of a full-cream dried milk powder, approximately equivalent to a 1/500 dilution of ordinary liquid milk. The total count of *Bact. coli* in this suspension was 300×10^6 per ml.

Five ml. of this bacterial suspension in milk were added to 5 ml. of an accurately titrated solution of caustic soda contained in 30 ml. alkali-free glass bottles fitted with metal caps. The temperature both of the bacterial suspension and of the caustic soda solution was brought to the required level before mixing by immersion of their respective containers in a water-bath.

The temperature of the water in the bath was maintained approximately constant throughout the experiment by the addition of minute pieces of ice if the temperature required was 20° C., or by the use of a bimetallic regulator if the required temperature was 30° C. or over. It was the whole-time duty of one assistant to supervise these baths during the course of an experiment, and to keep the suspensions well mixed by frequent shaking.

Viable counts were made by the roll-tube method (Wilson, 1922; Wilson, Twigg, Wright, Hendry, Cowell & Maier, 1935) on the original suspension in milk immediately the experiment was begun. As the dropping pipette technique would have been difficult with fluids at so many different temperatures, specially made straight-sided pipettes

able of delivering 0·1 and 0·2 ml. quantities were used instead of inoculating roll tubes and for making certain dilutions. The viable count on the milk suspensions was usually about 100×10^6 per ml., making the viable count on the disinfectant mixture about 50×10^6 per ml.

Counts were made at intervals, regulated by a stop-watch, of the disinfectant mixtures. The quantity withdrawn, 0·1 or 1·0 ml., was inoculated into $N/4$ Ringer solution containing an amount of HCl just sufficient to neutralize the caustic soda. Further dilutions were made in ordinary $N/4$ Ringer solution. *Counts were made only on dilutions*, since experience showed that if the disinfectant mixture was added directly to the agar, even if this contained the requisite amount of HCl to neutralize the caustic soda, the results were very irregular. Usually two 0·1 ml. and two 0·2 ml. quantities were put up for each dilution, so that the results were based on the mean values of two to four tubes, depending on the number that were countable. All roll tubes were incubated at 37° C. and counted by one observer after 3 days.

In working out the constants of the disinfectant at different concentrations and temperatures the formulae proposed by Phelps (1911) were used. For the reaction velocity k, the formula was

$$k = \frac{1}{t} \log \frac{B}{b},$$

where t = time in minutes, B = number of viable organisms at the start, and b = number of viable organisms at the end of time t.

For the concentration coefficient n, the formula was

$$n = \log \frac{k_2}{k_1} \div \log \frac{C_2}{C_1},$$

where k_1 = reaction velocity at concentration C_1, and k_2 = reaction velocity at concentration C_2.

For the temperature coefficient θ, the formula was

$$\theta^{10° \text{C.}} = \frac{k_1}{k_2},$$

where k_1 = reaction velocity at higher temperature and k_2 = reaction velocity at lower temperature.

Results. Mean values of k, n, and θ

Reaction velocity k

The reaction-velocity constants for different strengths of caustic soda at different temperatures were worked out for each interval of time, and the mean value estimated by averaging the values for equal intervals. This yielded results which were, of course, practically identical with those obtained by taking the single value for the whole period. The figures are set out in Tables 1–6. There was some variation in the values of k between different experiments at any one concentration and temperature, probably due to imperfect standardization of all the various factors concerned. To obtain reliable averages three to seven experiments had to be carried out under each set of conditions.

The rate of disinfection of *Bact. coli* by 0·05% sodium hydroxide at 20° C. was comparatively slow, while at 40° C. it was about seven times as rapid, and approximately equal to that of 0·1% NaOH at 20° C. Fig. 1 shows the great differences in the values of k produced by relatively small changes in concentration and temperature.

Table 1. *Mean values of reaction velocity constant k for* 0·05% NaOH *at* 20° C. *with* Bact. coli

Date of exp.	Time in min.	Log of no. of surviving organisms at Start	Log of no. of surviving organisms at End	Mean *k*
12. x. 38	0–40	7·7198	5·4575	0·057
11. x. 38	0–40	7·8016	5·1750	0·066
25. xi. 38	0–40	7·4771	5·5323	0·049
13. xii. 38	0–60	7·7853	4·3617	0·057
11. i. 39	0–60	7·7364	5·1790	0·043
17. i. 39	0–60	9·0021 6·9751	6·5260 3·0682	0·053
Total	300	38·5202	25·7055	0·325
Arithmetic mean	50	7·7040	5·1411	0·054

Table 2. *Mean values of reaction velocity constant k for* 0·1% NaOH *at* 20° C. *with* Bact. coli

Date of exp.	Time in min.	Log of no. of surviving organisms at Start	Log of no. of surviving organisms at End	Mean *k*
25. xi. 38	0–5	7·4771	5·2014	0·455
13. xii. 38	0–10	7·7853	4·0162	0·377
11. i. 39	0–12	7·7364	4·0759	0·305
20. i. 39	0–9	7·8435	4·8663	0·331
29. iii. 39	0–6	7·7023	5·2430	0·410
30. iii. 39	0–8	7·7924	4·9345	0·357
31. iii. 39	0–8	7·7216	5·0635	0·333
Total	58	54·0586	33·4008	2·568
Arithmetic mean	8·3	7·7227	4·7715	0·367

Table 3. *Mean values of reaction velocity constant k for* 0·05% NaOH *at* 30° C. *with* Bact. coli

Date of exp.	Time in min.	Log of no. of surviving organisms at Start	Log of no. of surviving organisms at End	Mean *k*
20. i. 39	0–40	7·8435	3·4983	0·109
24. i. 39	0–40	7·6628	3·9644	0·092
27. i. 39	0–40	7·1501*	2·2430	0·123
31. i. 39	0–30	7·7764	4·3324	0·115
7. ii. 39	0–30	7·7559	3·4713	0·143
Total	180	38·1887	17·5094	0·582
Arithmetic mean	36	7·6377	3·5019	0·116

* Suspension diluted three times more than usual by mistake.

Table 4. *Mean values of reaction velocity constant k for* 0·1% NaOH *at* 30° C. *with* Bact. coli

Date of exp.	Time in min.	Log of no. of surviving organisms at Start	Log of no. of surviving organisms at End	Mean *k*
20. i. 39	0–6	7·8435	2·9230	0·820
14. ii. 39	0–6	7·7924	4·0112	0·630
17. ii. 39	0–6	7·7076	3·7941	0·652
Total	18	23·3435	10·7283	2·102
Arithmetic mean	6	7·7812	3·5761	0·701

Table 5. *Mean values of reaction velocity constant k for* 0·025% NaOH *at* 40° C. *with* Bact. coli

Date of exp.	Time in min.	Log of no. of surviving organisms at Start	Log of no. of surviving organisms at End	Mean *k*
3. ii. 39	0–10	7·5139	6·1903	0·132
7. ii. 39	0–20	7·7559	2·7959	0·248
17. ii. 39	0–10	7·7076	5·8382	0·187
21. ii. 39	0–20	7·7889	2·4955	0·265
29. iii. 39	0–12	7·7023	3·2337	0·372
31. iii. 39	0–8	7·7216	6·0483	0·209
Total	80	46·1902	26·6019	1·413
Arithmetic mean	13·3	7·6984	4·4337	0·236

Table 6. *Mean values of reaction velocity constant k for* 0·05% NaOH *at* 40° C. *with* Bact. coli

Date of exp.	Time in min.	Log of no. of surviving organisms at Start	Log of no. of surviving organisms at End	Mean *k*
31. i. 39	0–10	7·7764	3·6604	0·412
3. ii. 39	0–10	7·5139	3·4830	0·403
7. ii. 39	0–10	7·7559	4·2148	0·354
14. ii. 39	0–10	7·7924	3·9112	0·388
Total	40	30·8386	15·2694	1·557
Arithmetic mean	10	7·7097	3·8174	0·389

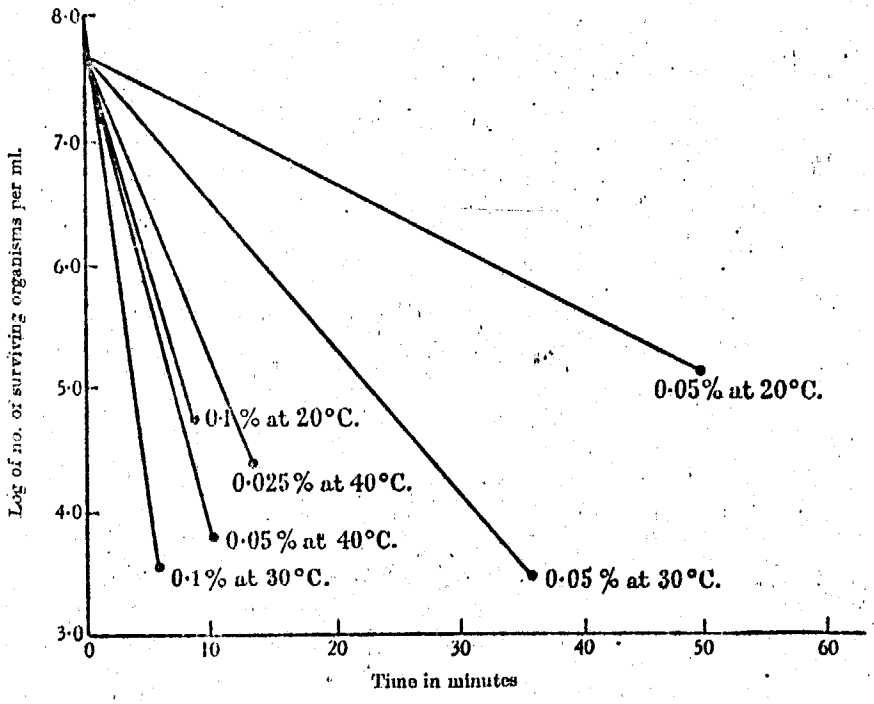

Fig. 1. Rate of death of *Bact. coli* when submitted to different concentrations of sodium hydroxide at different temperatures.

Comparison of the reaction velocity rates at successive intervals of time under a given set of conditions showed that, with the exception of 0·05% NaOH at 40° C. in which the results tended to be irregular, the value of k was always slower at the beginning of disinfection than later (Table 7). In other words, the rate of disinfection increased throughout the experiment. It is possible that if observations could have been continued till the sterility point was nearly reached, the value of k might have diminished again, giving a

Table 7. *Mean values of reaction velocity constants at different stages of the disinfection curve* NaOH *with* Bact. coli

Concentration, temperature and no. of experiments (in brackets)	Above: Interval in min. Below: Mean k			
0·05% NaOH at 20° C. (1)	0–10 0·031	10–20 0·015	20–30 0·070	30–40 0·054
0·1% NaOH at 20° C. (3)	0–4 0·286	4–8 0·389		
0·05% NaOH at 30° C. (3)	0–10 0·076	10–20 0·124	20–30 0·174	
0·1% NaOH at 30° C. (3)	0–3 0·493	3–6 0·632		
0·025% NaOH at 40° C. (2)	0–10 0·247	10–20 0·266		
0·05% NaOH at 40° C. (3)	0–5 0·491	5–10 0·311		

sigmoid type of curve instead of a straight line when the logarithms of the surviving organisms were plotted against time. There was a suggestion of this in the experiments with 0·05% NaOH at 20° C., when the average value of k rose from 0·031 in the first 10 min. to reach a maximum of 0·070 between 20 and 30 min., and then fell again to 0·054 in the 30–40 min. period. Unfortunately, owing to the technical difficulties referred to above of making satisfactory observations on the undiluted disinfectant mixture, it proved impossible with the higher concentrations and temperatures to follow the reactions far enough to determine whether the diminution in the value of k in the later stages of disinfection was a general phenomenon or not.

One experiment was carried out to see if the rate of disinfection was appreciably affected by the number of organisms in the suspension. Using 0·05% NaOH at 20° C. counts were made at 20 min. intervals on two suspensions of *Bact. coli*, one containing 10 million and the other 1000 million per ml. viable bacilli at the start. The results, which are given in Table 8, show that destruction occurred rather more rapidly with the weaker than with the stronger suspension.

Table 8. *Mean values of reaction velocity constants for* 0·05% NaOH at 20° C. *with different numbers of* Bact. coli

Size of inoculum	k			Mean k
	0–20 min.	20–40 min.	40–60 min.	
10×10^6 per ml.	0·019	0·075	0·072	0·065
1000×10^6 per ml.	0·029	0·026	0·019	0·011

A few experiments were also performed to find out whether the presence of 1/500 milk in the suspension protected the organisms and delayed the rate at which they were destroyed. In each experiment one suspension was made up with glass-distilled water

alone, the other with glass-distilled water containing a 1/5000 concentration of full-cream dried milk, which was approximately equivalent to a 1/500 concentration of ordinary liquid milk. The findings are recorded in Table 9. There was some variation in the values

Table 9. *Comparison of velocity constants for* NaOH *between suspensions of* Bact. coli *in distilled water and in* 1/500 *milk*

Date of exp.	Concentration and temperature	Time in min.	Mean value of k: Distilled water	Mean value of k: 1/500 milk
29. iii. 39	0·1% NaOH at 20° C.	0–6	0·445	0·410
30. iii. 39	" "	0–8	0·266	0·357
31. iii. 39	" "	0–8	0·319	0·333
Arithmetic mean	" "		0·353	0·367
29. iii. 39	0·025% NaOH at 40° C.	0–6	0·381	0·182
30. iii. 39	" "	0–4	0·088	0·100
31. iii. 39	" "	0–8	0·191	0·209
3. iv. 39	" "	0–6	0·076	0·060
Arithmetic mean	" "		0·184	0·138

of k in different experiments, but on the whole the averages were fairly similar. With 0·1% NaOH at 20° C. the average reaction velocity was slightly higher in the milk suspension, while with 0·025% NaOH at 40° C. it was rather higher in the distilled water suspension. It may be concluded that the presence of milk in a *final* concentration of 1/1000 has little effect on the germicidal power of caustic soda. Had one of the phenol group of disinfectants been used, which coagulate albumin, there is little doubt that the presence of the milk would have slowed the reaction considerably.

Concentration coefficient n

In Table 10 the mean values have been worked out for the concentration coefficient n.

Table 10. *Mean values of concentration coefficient n for destruction of* Bact. coli *by* NaOH

Concentration and temperature	No. of exps.	Mean k	Mean n
0·05% NaOH at 20° C.	6	0·054	2·77
0·1% " "	7	0·367	
0·05% NaOH at 30° C.	5	0·116	2·60
0·1% " "	3	0·701	
0·025% NaOH at 40° C.	6	0·236	(0·72)
0·05% " "	4	0·389	
Arithmetic mean			2·69

The values of k at 40° C. were very irregular, and it is probable that the figure of 0·236 obtained with 0·025% NaOH was too high. Omitting therefore these results, and taking only the findings at 20 and 30° C., it is seen that the average value of n was about 2·7. This means in effect that doubling the concentration of NaOH increases the reaction velocity by $2^{2\cdot7}$, or approximately 6·5 times. Halving it, on the other hand, decreases it by the same amount. The importance of using as high a concentration as possible of caustic soda when the time factor is concerned is thus clearly demonstrated.

Temperature coefficient θ

In Table 11 the mean values of the temperature coefficient $\theta^{10°\,C.}$ are recorded. There was some variation under different sets of conditions. The value for the comparison between 30 and 40° C. is probably too high. The reason for this is discussed on p. 444. The average

value may be taken as about 2. In practice this means that raising the temperature 10° C. doubles the rate of destruction. Though this effect is not as great as that of doubling the concentration of disinfectant, it is nevertheless sufficient to justify the use of as high a temperature as possible during the actual disinfectant stage of bottle washing.

Table 11. *Mean values of temperature coefficient $\theta^{10°\,C.}$ for destruction of* Bact. coli *by* NaOH

Concentration and temperature	No. of exps.	Mean k	Mean θ^{10}
0·05% NaOH at 20° C.	6	0·054	2·15
" " 30° C.	5	0·116	
0·05% NaOH at 30° C.	5	0·116	(3·35)
" " 40° C.	4	0·389	
0·1% NaOH at 20° C.	7	0·367	1·91
" " 30° C.	3	0·701	
Arithmetic mean			2·03

2. Observations on spores of *B. subtilis*

Technique

A spore-bearing organism isolated from milk, which for the sake of convenience will be referred to as *B. subtilis* though its exact identity was never worked out, was selected because it was found possible to prepare from it a homogeneously distributed suspension of spores.

The growth from twelve 4-day agar slope cultures at 37° C. of this organism was washed off with glass-distilled water and treated for an hour in the mechanical shaker. The suspension was centrifuged at about 2500 r.p.m. for 7 min. to throw down aggregated particles. The supernatant fluid was withdrawn and heated to 60° C. for 90 min. A viable count was made by the roll-tube method, and the suspension was stored in an amber bottle in the ice chest.

Counts were made every few days. The number of viable organisms fell fairly rapidly for the first few days, after which it remained almost constant, at about $10–15 \times 10^6$ per ml., during the next 3 months. The suspension was not used for disinfection experiments till it was over a fortnight old.

This suspension lasted for nearly the whole series of our observations. A fresh suspension, however, had to be made up for the last three experiments; it proved to be slightly stronger than the first.

The experiments with caustic soda were carried out in the same way as with *Bact. coli*. The suspension of spores was mixed with a 1/5000 dilution of full cream dried milk in the proportion of 1/9, and this suspension was added to an equal quantity of the caustic soda solution under test. Counts were made by the roll-tube method as before. The average initial number of organisms exposed to the disinfectant was about 6×10^6 per ml., instead of about 50×10^6 per ml. with *Bact. coli*. This meant that observations could be made over a rather smaller range of the disinfection curve.

Results. Mean value of k, n, and θ

Reaction velocity k

The mean values of the reaction-velocity constants for different concentrations of caustic soda at different temperatures are recorded in Tables 12–17. It will be noted that some variation occurred in the values between different experiments carried out under

Table 12. *Mean values of reaction velocity constant k for* 5% NaOH *at* 30° C. *with* B. subtilis *spores*

Date of exp.	Time in min.	Log of no. of surviving organisms at Start	End	Mean k
28. ii. 39	0–60	5·8639	4·8814	0·016
3. iii. 39	0–60	5·7118	4·5353	0·020
7. iii. 39	0–60	5·7453	4·5575	0·020
10. iii. 39	0–60	5·7364	4·6101	0·019
Total	240	23·0574	18·5843	0·075
Arithmetic mean	60	5·7644	4·6461	0·019

Table 13. *Mean values of reaction velocity constant k for* 10% NaOH *at* 30° C. *with* B. subtilis *spores*

Date of exp.	Time in min.	Log of no. of surviving organisms at Start	End	Mean k
28. ii. 39	0–30	5·8639	4·0315	0·061
3. iii. 39	0–30	5·7118	3·9045	0·060
10. iii. 39	0–30	5·7364	3·7559	0·066
Total	90	17·3121	11·6919	0·187
Arithmetic mean	30	5·7707	3·8973	0·062

Table 14. *Mean values of reaction velocity constant k for* 2% NaOH *at* 50° C. *with* B. subtilis *spores*

Date of exp.	Time in min.	Log of no. of surviving organisms at Start	End	Mean k
24. ii. 39	0–30	5·6663	3·7745	0·063
28. ii. 39	0–30	5·8639	3·6258	0·075
3. iii. 39	0–30	5·7118	3·0253	0·090
7. iii. 39	0–20	5·7453	4·2644	0·074
Total	110	22·9873	14·6900	0·302
Arithmetic mean	27·5	5·7468	3·6725	0·076

Table 15. *Mean values of reaction velocity constant k for* 5% NaOH *at* 50° C. *with* B. subtilis *spores*

Date of exp.	Time in min.	Log of no. of surviving organisms at Start	End	Mean k
28. ii. 39	0–5	5·8639	4·3617	0·300
3. iii. 39	0–6	5·7118	3·2480	0·411
10. iii. 39	0–4	5·7364	3·8639	0·468
Total	15	17·3121	11·4736	1·179
Arithmetic mean	5	5·7707	3·8245	0·393

Table 16. *Mean values of reaction velocity constant k for* 1% NaOH *at* 70° C. *with* B. subtilis *spores*

Date of exp.	Time in min.	Log of no. of surviving organisms at Start	Log of no. of surviving organisms at End	Mean *k*
21. iii. 39	0–4	5·8349	4·6184	0·297
22. iii. 39	0–6	5·6322	2·9491	0·447
23. iii. 39	0–4	5·7973	4·4410	0·338
24. iii. 39	0–5	5·6385	3·6902	0·390
27. iii. 39	0–4	5·7284	4·4713	0·314
28. iii. 39	0–4	5·7578	5·1055	0·163
30. iii. 39	0–4	6·1461*	5·5267	0·155
31. iii. 39	0–4	6·1075*	5·2863	0·205
Total	35	46·6427	36·1218	2·309
Arithmetic mean	4·4	5·8303	4·5152	0·289

* New spore suspension used.

Table 17. *Mean values of reaction velocity constant k for* 2% NaOH *at* 70° C. *with* B. subtilis *spores*

Date of exp.	Time in min.	Log of no. of surviving organisms at Start	Log of no. of surviving organisms at End	Mean *k*
27. iii. 39	0–1	5·7284	4·8488	0·880
28. iii. 39	0–1	5·7578	5·2467	0·511
30. iii. 39	0–1	6·1461*	5·6271	0·519
31. iii. 39	0–2	6·1075*	4·6258	0·741
3. iv. 39	0–2	6·3174*	5·3118	0·518
Total	7	30·0872	25·6602	3·169
Arithmetic mean	1·4	6·0174	5·1320	0·634

* New spore suspension used.

designedly the same conditions. As with *Bact. coli*, this variation tended to be greater at the higher temperatures—40° C. with *Bact. coli* and 70° C. with *B. subtilis* spores. It is probable that at these temperatures the level was being approached at which the heat by itself was lethal, and that the rate of disinfection was therefore determined by the coagulative action of the heat as well as by the germicidal power of the disinfectant. At such a critical level, minute changes in temperature may well have brought about a result incommensurate with that caused by similar changes at lower levels, and so led to greater variation in the value of *k* than was expected.

This explanation is to some extent borne out by a study of Tables 11 (p. 442) and 21 (p. 447). It will be noticed that the value of $\theta^{10^\circ C.}$ is considerably higher when the comparison is made between 30 and 40° C. with *Bact. coli* and between 50 and 70° C. with *B. subtilis* spores than at lower temperatures. Presumably some additional factor came into operation at the higher temperature to augment the germicidal action of the caustic soda.

The rates of disinfection under different conditions are represented graphically in Fig. 2. Comparison with Fig. 1 (p. 439) shows how much stronger concentrations of caustic soda were required at any given temperature to destroy *B. subtilis* spores than *Bact. coli*.

Table 18 confirms the findings with *Bact. coli*, recorded in Table 7, namely, that destruction of bacteria proceeded less rapidly at first than later.

With both organisms a definite lag was observable in the early stages of disinfection, after which the rate of destruction increased to an appreciable, and often considerable extent. Whether it would have fallen off again in the later stages it is impossible to say,

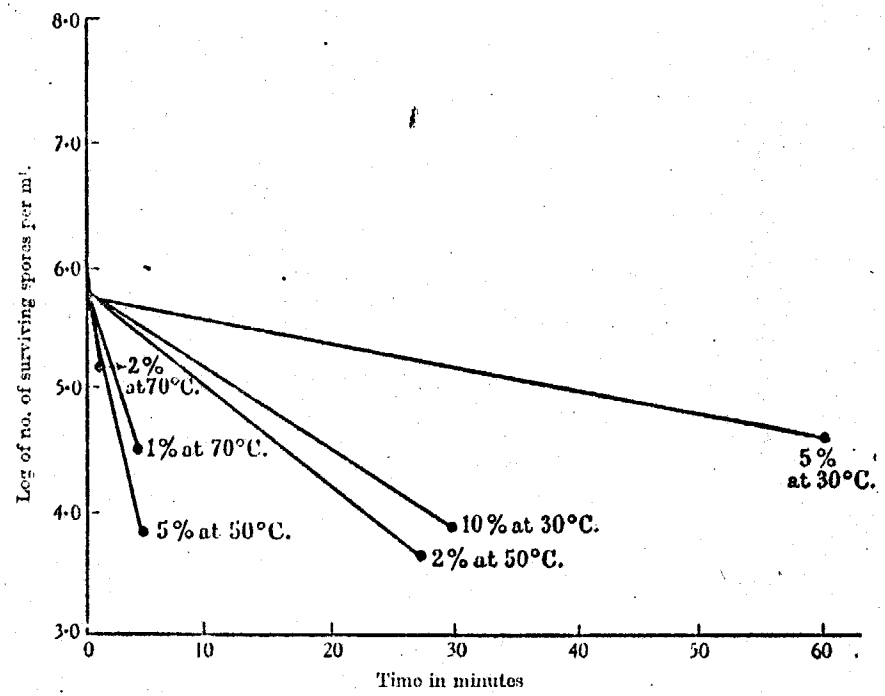

Fig. 2. Rate of death of spores of *B. subtilis* when submitted to different concentrations of sodium hydroxide at different temperatures.

Table 18. *Mean values of reaction velocity constants at different stages of the disinfection curve.* NaOH *with* B. subtilis *spores*

Concentration, temperature and no. of experiments (in brackets)	Above: Interval in min. Below: Mean *k*	
5% NaOH at 30° C. (4)	0–30 0·016	30–60 0·021
10% NaOH at 30° C. (3)	0–15 0·018	15–30 0·074
2% NaOH at 50° C. (3)	0–15 0·050	15–30 0·102
5% NaOH at 50° C. (2)	0–2 or 3 0·337	2 or 3–4 or 6 0·542
1% NaOH at 70° C. (6)	0–2 0·242	2–4 0·249
2% NaOH at 70° C. (2)	0–1 0·601	1–2 0·658

since technical difficulties prevented observations being carried out close to the sterility point.

A few experiments were carried out to see if the 1/500 concentration of milk in the bacterial suspension lowered the rate of disinfection by protecting the organisms against the caustic soda (Table 19).

Table 19. *Comparison of velocity constants for* NaOH *between suspensions of* B. subtilis *spores in distilled water and in* 1/500 *milk*

Date of exp.	Concentration and temperature	Time in min.	Mean value of k: Distilled water	Mean value of k: 1/500 milk
24. iii. 39	1% NaOH at 70° C.	0–5	0·218	0·390
31. iii. 39	" "	0–4	0·097	0·206
Arithmetic mean	" "		0·158	0·298
3. iv. 39	2% NaOH at 70° C.	0–2	0·556	0·518

With 2% NaOH at 70° C. the reaction velocity was rather greater in the distilled water than in the diluted milk suspension, but with 1% NaOH the reverse was observed. It is unfortunate that these comparisons were made at the high temperature of 70° C., since, as has already been pointed out, results tended to be more variable at 70° C. than at lower levels. If the results are taken in conjunction, however, with those obtained with *Bact. coli* (Table 9), it may be concluded that the presence of milk in a *final* concentration of 1/1000 does not have any appreciable protective action against caustic soda.

Concentration coefficient n

In Table 20 the mean values of the concentration coefficient n are recorded. The average value tends to be rather lower with *B. subtilis* spores than with *Bact. coli* (see

Table 20. *Mean values of concentration coefficient n for destruction of* B. subtilis *spores by* NaOH

Concentration and temperature	No. of exps.	Mean k	Mean n
5% NaOH at 30° C.	4	0·019	1·71
10% " "	3	0·062	
2% NaOH at 50° C.	4	0·076	1·79
5% " "	3	0·393	
1% NaOH at 70° C.	8	0·289	(1·13)
2% " "	5	0·634	
Arithmetic mean			1·75

Table 10). It will be noticed, however, that in each instance the values at the highest temperatures observed—70° C. with *B. subtilis* spores and 40° C. with *Bact. coli*—were less than at the lower temperatures. This suggests that at the higher temperatures some factor other than the mere difference in concentration was playing a part, and adds further support to the opinion already expressed that the heat by itself was beginning to exert a coagulative effect on the protein of the bacteria. If the figures at the higher temperatures are neglected, then the mean value of n for *B. subtilis* spores works out at 1·75. This means, in practice, that doubling the concentration of caustic soda increases the reaction velocity by $2^{1\cdot75}$, or 3·4 times.

The mean value of n for *Bact. coli* and *B. subtilis* spores is 2·2. Doubling the concentration of caustic soda would therefore increase the reaction velocity by $2^{2\cdot2}$, or 4·6 times. In other words, if with a given concentration and temperature of caustic soda sterility was reached in 9 min., then doubling the concentration would reduce the time to 2 min., while halving the concentration would increase it to about 40 min.

Temperature coefficient θ

Table 21 sets out the values of the temperature coefficient $\theta^{10°\,C.}$. For the reason already suggested, the value for $\theta^{10°\,C.}$ calculated from a comparison of the reaction velocities at 50 and 70° C. was probably too high. Insufficient figures are available to estimate an

Table 21. *Mean values of temperature coefficient* $\theta^{10°\,C.}$ *for destruction of* B. subtilis *spores by* NaOH

Concentration and temperature	No. of exps.	Mean k	Mean θ
5% NaOH at 30° C.	4	0·019	1·44
" " 50° C.	3	0·394	
2% NaOH at 50° C.	4	0·076	2·89
" " 70° C.	5	0·634	
Arithmetic mean			2·17

average value at lower temperatures, but it is probable that the real value was somewhere about 1·5. This is rather lower than that of 2·03 calculated for *Bact. coli* (see p. 442). It will be observed, in fact, that the effect both of increasing the concentration of disinfectant and of raising the temperature was less with *B. subtilis* spores than with *Bact. coli*. The explanation of this is not clear.

Comparison of k for Bact. coli *and spores of* B. subtilis

In Table 22 comparative values of the reaction velocity k for *Bact. coli* and for *B. subtilis* spores are recorded. It will be seen that at 30° C. the reaction velocity with 10% caustic soda acting on *B. subtilis* spores was only about one-half that of the 0·05% caustic

Table 22. *Summary of mean values of reaction velocity constants for destruction of* Bact. coli *and of* B. subtilis *spores by* NaOH

Bact. coli: Concentration of NaOH and temperature	*Bact. coli*: Mean k	*B. subtilis* spores: Concentration of NaOH and temperature	*B. subtilis* spores: Mean k
0·05% at 20° C.	0·054	5% at 30° C.	0·019
0·05% at 30° C.	0·116	10% at 30° C.	0·062
0·025% at 40° C.	0·236	2% at 50° C.	0·076
0·1% at 20° C.	0·367	1% at 70° C.	0·289
0·05% at 40° C.	0·389	5% at 50° C.	0·393
0·1% at 30° C.	0·701	2% at 70° C.	0·634

soda solution acting on *Bact. coli*. Taking n for *Bact. coli* at 2·7 it follows that at the same concentration and temperature the cells of *Bact. coli* are destroyed over three million times as fast as the spores of *B. subtilis*.

Discussion

The results we have obtained may be compared with those of recent workers. Levine, Buchanan & Lease (1926–7) and Myers (1929) studied the rate of disinfection of spores by different concentrations of caustic soda at different temperatures. Some of their figures may be compared with ours, though many are unsuitable for this purpose. Levine obtained a value of k for 2% NaOH at 50° C. of 0·069 as against our value of 0·076. Myers's value for 2% NaOH at 60° C. was 0·225. Taking $\theta^{10°\,C.}$ as 1·5, this gives a value for k at 50° C. of 0·15, which is considerably higher than that recorded by Levine or ourselves. Possibly a difference in the resistance of the spores may have been responsible for thi

discrepancy. For n Myers obtained a value of 1·67 as against our 1·75. Our results therefore are in fairly good agreement with those of the American workers.

It is not our purpose here to discuss the physical factors underlying disinfection, but our results do have some bearing on the standardization of disinfectants. A review of the literature (see Topley & Wilson, 1936) suggests that when a homogeneous suspension of spores of approximately the same age is submitted to a germicidal agency the rate of destruction appears to be similar to that in a monomolecular reaction. When vegetative organisms of varying age are used, departures from this law are frequent, [illegible] because the more recently generated [illegible]

In our experiments, [illegible] with veg[illegible] bacteria and [illegible] spores, there was an unmistakable tendency for the velocity of [illegible] reaction to be lower in the earlier than in the later [illegible] of disinfection. Observations were not made at sufficiently frequent intervals to dec[illegible] merely an initial lag phase followed by a phase of destruction at a uniform rate, or [illegible] the veloc[illegible] of the reaction increased progressively.

Accurate estimations of the reaction [illegible] in the later stages of disinfection with a powerful germicidal agent are not easy to make. [illegible] germicide in the mixture was adequ[illegible] results should [illegible] count [illegible] cep[illegible] the [illegible] of k in [illegible] stages of [illegible] would have increased progressi[illegible]. This may possibly be the [illegible] why both [illegible] *et al.* ([illegible]) [illegible] obtained a progressive increase in their reaction velocity constants throughout the period of disinfection. [illegible] values of k [illegible] beginning and at the end [illegible] than in our own [illegible] phase of [illegible]

The variation [illegible] experience [illegible] an[illegible] of the [illegible] ve[illegible] perature on the [illegible] compari[illegible] bact[illegible] of disinfe[illegible] critical. It impli[illegible] accurat[illegible] above. If a con[illegible] believe [illegible] of k taken in the middle stage of disinfection [illegible] suitable measure. So long as the initial number of organisms is the [illegible] experiment, the value of k during the middle stage of disinfect[illegible] beginning of disinfection to about the end of the middle stage, constitutes an expression of the rate of disinfection that can be measured with reasonable accuracy. A comparison of the value of k for one disinfectant over a range of destruction from 10^7 to [illegible] per ml. with another over a range from 10^7 to 10^6 organisms per ml. is not [illegible] true germicidal potency of the two reagents. Both should be compared [illegible] the same range of destruction.

[illegible] fully [illegible] this subject, since Withell (1942), in a paper wri[illegible] after our [illegible] was completed, has come to essentially the

same conclusions. His recommendation for comparing disinfectants is to choose the time necessary to destroy 50% of the organisms in the suspension. Such an end-point can be measured with considerable accuracy, and, though similar in principle, it has obvious practical advantages over the measurement of k in the middle stage of the reaction.

Finally, it is of interest to compare the germicidal activity of caustic soda with that of phenol. A comparison of our figures for caustic soda with those of Chick (1908) for phenol shows that a 0·05% solution of caustic soda at 20° C. destroyed *Bact. coli* about five times as fast as a 0·5% solution of phenol at 20° C. destroyed *Bact. paratyphosum* B, and that a 5% solution of caustic soda at 30° C. destroyed spores of *B. subtilis* nearly three times as fast as a 5% solution of phenol at 33·3° C. destroyed anthrax spores. Even allowing for the higher value of n for phenol—about 5 as opposed to 2·2 for caustic soda—the superiority of caustic soda at concentrations likely to be used in practice is very considerable.

Summary

Quantitative observations were made on the rate of disinfection of *Bacterium coli* and of the spores of *Bacillus subtilis* by caustic soda at different temperatures and different concentrations. The tests were carried out in the presence of 1/1000 milk. It was found that:

(1) With *Bact. coli* the concentration coefficient n was about 2·7, and the temperature coefficient $\theta^{10°\,C.}$ about 2.

(2) With *B. subtilis* spores the concentration coefficient n was about 1·75 and the temperature coefficient $\theta^{10°\,C.}$ about 1·5.

(3) With *Bact. coli* the values for the reaction velocity constant k tended to be irregular at 40° C. and with *B. subtilis* spores at 70° C., suggesting that at these temperatures some additional factor, presumably heat coagulation of the protein, was beginning to affect the results.

(4) With both organisms the value of the reaction velocity constant k was relatively slow at the start and tended to increase progressively during the course of disinfection. Whether it diminished again as sterility was approached could not be ascertained for technical reasons.

(5) With both organisms the presence of 1/1000 milk did not seem to affect the rate of disinfection as compared with distilled water.

(6) One experiment with *Bact. coli* suggested that the rate of disinfection was affected appreciably by the number of organisms present. Increasing the number of organisms in the suspension 100 times diminished the value of k by about one-third.

(7) The value of k was about 3,000,000 times greater with *Bact. coli* than with *B. subtilis* spores.

(8) If the mean value of n is taken as 2·2, it follows that doubling the concentration of caustic soda increases the reaction velocity by 4·6 times. If, for example, with a given concentration of caustic soda sterility was reached in 9 min., then doubling the concentration would reduce this time to 2 min., while halving the concentration would increase it to about 40 min.

(9) If the mean value of $\theta^{10°\,C.}$ is taken as 1·75, it follows that a rise of 10° C. in the temperature increases the reaction velocity 1·75 times. If, for example, with a given temperature sterility was reached in 9 min., then raising the temperature 10° C. (18° F.)

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would reduce this time to about 5 min., while lowering the temperature 10° C. would increase it to about 15 min. With temperatures, however, of over 40° C. (104° F.) the rate of destruction of vegetative organisms, and with temperatures of over 70° C. (158° F.) the rate of destruction of sporing organisms in the presence of caustic soda is probably increased by the effect of the heat itself.

(10) A comparison of our figures for caustic soda with those of Chick for phenol shows that a 0·05% solution of caustic soda at 20° C. destroyed *Bact. coli* about five times as fast as a 0·5% solution of phenol at 20° C. destroyed *Bact. paratyphosum* B, and that a 5% solution of caustic soda at 30° C. destroyed *B. subtilis* spores nearly three times as fast as a 5% solution of phenol at 33·3° C. destroyed anthrax spores. The superiority of caustic soda over phenol, particularly at concentrations likely to be used in practice, is manifest.

(11) Discussing the standardization of disinfectants, we conclude that the value of k taken in the middle stage of the reaction, or from the beginning of disinfection to about the end of the middle stage, affords the most suitable measure of comparison. This is essentially the same conclusion as that reached by Withell (1942), who uses as his index the time necessary to destroy 50% of the organisms.

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(*MS. received for publication* 28. v. 42.—Ed.)

Hooker

Industrial chemicals DIVISION

Formula NaOH Chemical Name Sodium Hydroxide

DESCRIPTION

Hooker Standard Grade Caustic Soda is available from the Industrial Chemicals Division in the East as a solution containing 50% or 73% NaOH by weight and in three flake sizes (regular, fine, crystal) or as a solid cast into drums. Liquid is produced at Niagara Falls, New York, Montague, Michigan, and Taft, Louisiana, dry forms at Niagara Falls only.

For further information on physical properties, analytical methods, handling and storage recommendations, refer to Hooker Caustic Soda Bulletin No. 115.

CHEMICAL SPECIFICATIONS

			50%	73%	Flake & Solid
Sodium Hydroxide	Equiv.	NaOH	49.0-51.0%	70.0-73.0%	97.0% min
Sodium Oxide	Equiv.	Na_2O	38.0-39.5%	54.2-56.6%	75.2% "
Sodium Carbonate		Na_2CO_3	0.20% max	0.25% max	0.70% max
Sodium Chloride		NaCl	1.10% "	1.60% "	2.15% "
Sodium Sulfate		Na_2SO_4	0.020% "	0.030% "	0.10% "
Sodium Chlorate		$NaClO_3$	0.10% "	0.12% "	None
Silicon		Si	0.010% "	0.015% "	0.02% "
Iron		Fe	0.0005% "	0.0007% "	0.0025% "
Calcium		Ca	0.0007% "	0.0010% "	0.0015% "
Magnesium		Mg	0.0010% "	0.0015% "	0.002% "
Aluminum		Al	0.0003% "	0.0005% "	0.001% "
Manganese		Mn	0.00001%"	0.00002%"	0.0005% "
Copper		Cu	0.00007%"	0.0001% "	0.0001% "
Nickel		Ni	0.00007%"	0,0001% "	0.0001% "

PHYSICAL PROPERTIES

Molecular weight	40
Melting point, 50%	55°F
Melting point, 73%	144°F
Melting point	(Anhydrous) 604°F
Boiling point	2534°F
Weight per gallon, 50%	12.8 pounds
Weight per gallon, 73%	14.4 pounds

USES

Caustic Soda is used in the manufacture of paper pulp, soap, chemical intermediates, resorcinol, indigo, sodium salts, dyes and pigments, ceramics, pharmaceuticals, cosmetics and in the perparation of boiler water softening and cleaning compounds. It is also used for regenerating water treatment units, reclaiming tin, paper and rubber, bleaching of textiles and paper, mercerizing cotton and dyeing and printing of textiles, in food processing, metal industries, petroleum refineries and many other industries.

Mlt. 11-192 1970

FOR MORE INFORMATION WRITE INDUSTRIAL CHEMICALS DIVISION, HOOKER CHEMICAL CORPORATION, NIAGARA FALLS, N. Y. 14302
SUBSIDIARY OF OCCIDENTAL PETROLEUM CORPORATION

The information presented herein, while not guaranteed, is to the best of our knowledge true and accurate. No warranty or guarantee express or implied is made regarding the performance or stability of any product, since the manner of use and conditions of storage and handling are beyond our control. No suggestion for product use, nor anything contained herein, shall be construed as a recommendation for its use in infringement of any existing patent.

Formula: KOH Molecular Weight: 56.1

Hooker Mercury Cell Caustic Potash (potassium hydroxide) is produced for special applications where a purity higher than that of standard grade is required. Mercury cell grade has a lower KCl content and is also lower in several of the trace impurities than standard grade. It is available as a solution or in dry form.

HOOKER SPECIFICATIONS

	Liquid		Dry Form
KOH*	45.0% min. - 46.0% max.	50.0% min.-51.0% max.	90.0% min.
NaOH	0.25% max.	0.25% max.	0.45% max.
K_2CO_3	0.2% max.	0.2% max.	0.8% max.
KCl	50 ppm max.	50 ppm max.	100 ppm max.
$KClO_3$	3 ppm max.	3 ppm max.	1 ppm max.
K_2SO_4	10 ppm max.	10 ppm max.	20 ppm max.
Fe	3 ppm max.	3 ppm max.	10 ppm max.
SiO_2	20 ppm max.	20 ppm max.	40 ppm max.
Ca	3 ppm max.	3 ppm max.	5 ppm max.
Mg	3 ppm max.	3 ppm max.	5 ppm max.
Hg	0.2 ppm max.	0.2 ppm max.	0.02 ppm max.

*Equivalent to total alkalinity

PHYSICAL PROPERTIES

Melting Point	45%	-22°F
	50%	48°F
	90%	427°F
	Anhydrous	716°F
Weight Per Gallon	45%	12.18pounds
	50%	12.68pounds

USES

Caustic Potash is a strong alkali which readily combines with many substances. Caustic Potash has many uses not suited to the cheaper caustic soda because of the differences in properties between potassium compounds and the corresponding sodium compounds. Some of the industries requiring caustic potash are: soap, glass, textile, pharmaceuticals, dyes, cosmetics, organic and inorganic potassium compounds, perfumes and essential oils, oil refining, electroplating, alkaline storage batteries, and other industries and processes.

PRECAUTIONARY INFORMATION

The following precautions for handling caustic potash are quoted from our drum label. This warning label has been prepared in accordance with a pattern established by the Manufacturing Chemists' Association.

The information presented herein, while not guaranteed, is to the best of our knowledge true and accurate. No warranty or guarantee express or implied is made regarding the performance or stability of any product, since the manner of use and conditions of storage and handling are beyond our control. No suggestion for product use, nor anything contained herein, shall be construed as a recommendation for its use in infringement of any existing patent.

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DANGER! CAUSTIC POTASH IS CORROSIVE
BURNS SKIN AND EYES

AVOID CONTACT WITH BODY OR CLOTHING. DO NOT TAKE INTERNALLY.

Precautions: When handling caustic potash wear goggles and face shield and avoid all contact with skin. While making solutions, add caustic potash slowly to surface of solution to avoid violent spattering.

In case of accidental contact with skin, immediately flush affected parts thoroughly with water and continue flushing with or immersion in cold water until redness fades and pain has subsided.

For eyes, flush freely with water for at least 15 minutes and get medical attention. See MCA Data Sheet SD-9.

SHIPPING INFORMATION

Classification under DOT regulations	Freight Classification	
Caustic Potash Liquid, Corrosive Liquid	Rail:	Caustic Potash
Caustic Potash, Dry Form, Non-Regulated	Truck:	Caustic Potash

Containers (Liquid)	Volume	Net Weight
Tank Cars	4,000 gal	24 ton
	6,000 gal	36 ton
	8,000 gal	48 ton
	10,000 gal	60 ton
Tank Trailer	2,500 gal*	15 ton*

*Approximate minimums

		Net Weight	Gross Weight
Drums	55 gallon non-returnable lined steel drums	660 lbs.	709 lbs.

Containers (Dry Form)

Non-returnable steel drums

	Net Weight	Gross Weight
Flake	100 lbs.	107 lbs.
	400 lbs.	421 lbs.

This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization.

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TECHNICAL REPORT SERIES
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FAO NUTRITION MEETINGS
REPORT SERIES
No. 40

SPECIFICATIONS FOR THE IDENTITY AND PURITY OF FOOD ADDITIVES AND THEIR TOXICOLOGICAL EVALUATION: SOME ANTIMICROBIALS, ANTIOXIDANTS, EMULSIFIERS, STABILIZERS, FLOUR-TREATMENT AGENTS, ACIDS, AND BASES

Ninth Report of the Joint FAO/WHO Expert Committee on Food Additives

Rome, 13 - 20 December 1965

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GENEVA
1966

peroxides are likely to be different from those from other peroxides, it was not possible to predict what biological effects they might produce. The biological evaluation of this substance was therefore postponed.

Biological information on calcium peroxides and calcium stearyl-2-lactylate was also inadequate, and further study of these additives was postponed.

Consideration was given to the use of potassum or calcium iodate as a flour-treatment agent. At the level of treatment proposed, it seemed likely that a considerable increase in the daily iodine intake would result. Although 10 ppm of iodide or iodate is the level that is commonly used for iodized table salt, there is a great difference between the daily intake of table salt and the daily intake of bread. While there may be some differences of opinion about the possible hazards of increasing the daily iodine intake to perhaps five or ten times the level usually recommended (100-200 μg/day), the Committee considered that the use as a food additive for the treatment of a staple, such as flour, of a substance having such physiological significance and potency as iodate is highly undesirable. The Committee therefore recommended that iodates should not be used for flour treatment.

Although ammonium and potassium persulfates have a long history of use, there are insufficient toxicological data for an acceptable treatment level for flour to be estimated.

Specifications, toxicological evaluation and acceptable levels of flour treatment for azodicarbonamide and stearyl tartrate can be found in the monographs.

4.4 Acids and bases

The monographs on these substances will appear in a publication entitled "Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation: Some Acids and Bases"; [1] a summary of the information is given in Annex 3.

A group of acids that are well-known substances involved in intermediary metabolism was discussed. Included were acetic, lactic and malic acids. Propionic acid (see section 4.1) might also have been conveniently added to this list. It was agreed that no limit need be prescribed for any of these acids when used as food additives, on the assumption that only reasonable quantities of them are likely to be used. The following points were, however, considered to be relevant to the food-additive use of these acids.

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[1] *FAO Nutrition Meetings Report Series*, No. 40C; WHO/Food Add./66.31 (in preparation).

As far as acetic acid is concerned, its acid taste is likely to be a safeguard against its ingestion in excessive quantities.

Fumaric acid has been shown to cause testicular damage in animals when administered in high dosage, especially if given parenterally. However, this observation was not regarded as pertinent when fumaric acid is used only in small quantities as a flavouring agent.

Both lactic and malic acids may occur in the D(−) form as well as in the L(+) form. In small infants, it has been shown that the enzyme responsible for converting the D(−) to the L(+) form is relatively deficient; consequently D(−) lactic acid may be toxic in early infancy. The Committee recommended that D(−) lactic acid should not be added to the diet of very young infants, and that, for adults, the use of the D(−) forms of lactic and malic acids should be restricted, as indicated in Annex 3 of the present report and in the monographs. In the case of malic acid, there is also the danger of contamination with maleic acid, which is a toxic substance. The Committee recommends that the maleic acid content of malic acid should not exceed 0.05%.

No details were available on the use of gluconic acid as a food additive, and it was not further considered.

Hydrochloric acid is not regarded as a toxic substance in the concentrations that are used in food technology. A specification for hydrochloric acid is included in the monograph as a safeguard against toxic impurities.

The specification, toxicological evaluation and acceptable daily intake of adipic acid are given in the monograph on this substance.

Monocalcium phosphate is used in flour. The Committee had no objection to its use, provided that this is taken into account in assessing the overall dietary load of calcium and phosphate; a limit for the latter has been recommended.

The bases used in food technology are required for pH adjustment. The amounts and concentrations used are not likely to have any toxicological significance. The Committee placed no restriction on the food-additive use of the bases listed, provided that the contribution made to the dietary load of sodium, potassium, calcium and magnesium is assessed and considered to be acceptable.

4.5 Re-evaluation

The Committee was asked to re-evaluate a number of substances. The decisions are given in Annex 1. Details on these substances will appear in a publication to be entitled "Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation: Some Antimicrobials

Annex 3

ACCEPTABLE DAILY INTAKES FOR MAN OF SOME ACIDS AND BASES

Compounds considered	Specification available	Over-all daily intake zone [a] (mg/kg body-weight)	
		Unconditional	Conditional
Acids			
Acetic acid	Yes	Not limited	
Adipic acid	Yes		0-5
Calcium phosphate, monobasic	Yes	Not limited [b]	
Fumaric acid	Yes	0-6	6-10
Hydrochloric acid	Yes	Not limited	
DL-Lactic acid	Yes		0-100 [c]
DL-Malic acid	Yes		0-100 [d]
Bases			
Carbonates of ammonium, calcium, magnesium, potassium and sodium	Yes	Not limited [e]	(p. 16)
Hydrogen carbonates of ammonium, potassium and sodium	Yes		
Hydroxides of ammonium, calcium, magnesium, potassium and sodium	Yes		
Oxides of calcium and magnesium	Yes		

[a] The first part of the acceptable daily intake zone has been termed the unconditional zone of acceptability and this represents levels of use that are effective technologically, at least for some purposes, and can be safely employed without further expert advice. The second part consists of a conditional zone of acceptability and represents levels of use that can be employed safely, but at which it is thought desirable that some degree of expert supervision and advice should be readily available.

[b] Subject to limits of phosphorus load given in the seventh report (*Wld Hlth Org. techn. Rep. Ser.*, 1964, 281, 31).

[c] Refers to content of D(−)-lactic acid.

[d] Refers to content of D(−)-malic acid. (See also p. 16.)

[e] Provided that use is in accordance with good manufacturing practice.

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LYE POISONING IN CHILDREN

G. W. KERNODLE, M.D.

GRANT TAYLOR, M.D.
AND
W. C. DAVISON, M.D.
DURHAM, N. C.

CORROSIVE burns of the esophagus, secondary to ingestion of lye, constitute one of the most difficult problems encountered in the pediatric service at Duke Hospital. The availability and the widespread use of this material account for the frequency of poisoning, especially in small children, despite the publicity regarding the dangers of its ingestion. Lye is commonly used by poor people as a cleansing agent, but caustic alkali also is present in washing powders, drainpipe cleaners and paint removers. Some persons do not realize until it is too late that

TABLE 1.—*Age of Patient at Time of Ingestion of Lye*

Age, Yr.	No. of Patients	Per Cent
0 to 1	3	2
1 to 2	68	50
2 to 5	43	32
5 to 20	13	9
Over 20	9	7
Total	136	100

a solution of lye which will "eat the stains from the boiling kettle" will have a much direr effect on the oral or the esophageal mucosa. In the great majority of cases of lye poisoning that we have seen, the blame could be placed directly on the parents who carelessly left a solution of lye in a familiar container within the reach of a thirsty child. Often the child swallows lye because he mistakes the white solution for milk.

In regard to the age of the patient at the time of ingestion of lye, table 1 reveals that the majority of lye burns occur in children. Lye poisoning is infrequent under the age of 1 year, the great majority of patients being between 1 and 5 years of age. One may note that 82 per cent of our patients were in this age group.

From the Department of Pediatrics, Duke University School of Medicine and Duke Hospital.

Read before the Section on Pediatrics at the Ninety-Sixth Annual Session of the American Medical Association, Atlantic City, N. J., June 13, 1947.

The ingestion of lye immediately causes painful and difficult swallowing. The lips, tongue and pharynx become edematous, ulcerated and covered with exudate, and, within a few hours, pharyngeal and esophageal edema makes it impossible for the child to swallow his own saliva. The immediate symptoms usually subside in five to seven days, by which time the swallowing of liquid and soft food may have greatly improved. While in the ensuing period there is little difficulty, it should be recognized that this is merely a latent interval and that in most instances, unless treatment is instituted, an esophageal stricture will develop within the following weeks or months.

Immediately after the ingestion of the alkali, an attempt should be made to neutralize the corrosive substance with a weak acid, such as diluted vinegar, lemon juice or orange juice. Removal of the caustic from the stomach by lavage is probably needless because of the neutralization of the alkali by gastric hydrochloric acid, while the resulting trauma may be harmful. Visible burns should be treated with an emollient such as olive oil, and the patient given a sedative to relieve pain. Liquids and soft foods may be given as tolerated. By the fourth day a soft rubber, eyeless catheter filled with mercury or small lead shot should be passed without force into the stomach and allowed to remain in place for two minutes. This procedure should be carried out for every patient, unless the esophagus is seen by direct illumination to be undamaged. In instances in which there is doubt, oral burns from caustic should be considered presumptive evidence of esophageal burns and sufficient indication for early esophageal dilations. The dilations are repeated daily, with a gradual increase in the size of the catheter and the period of dilation, until by the tenth day a no. 30 to 34 French catheter is kept down for five minutes. After daily dilations for two weeks, the number of them is gradually decreased to three a week for two weeks, two a week for one month, one a week for one month, two a month for three months, one a month for six months and two or three a year for several years. If increasing difficulty is encountered during the course of treatment, daily dilations are again performed. Also, fluoroscopic examination with barium sulfate or an esophagoscopic study is advisable if difficulty is encountered. Two previous articles[1] reported that 12 patients were treated by this method at Duke Hospital prior to 1944 and that in none of these did symptoms of esophageal obstruction develop. For 4 children, however, owing to the failure of their families to cooperate, the treatment was not sufficiently prolonged and strictures developed, necessitating a second admission to the hospital for gastrostomy and retrograde dilation.

1. Martin, J. M., and Arean, J. M.: Lye Poisoning and Stricture of the Esophagus, South. M. J. 32:286-290 (March) 1939. Crowe, J. T.: Poisoning Due to Lye, Am. J. Dis. Child. 68:9-12 (July) 1944.

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Since 1944 we have treated 9 patients according to the method outlined previously, and we have had 1 patient in whom, despite daily dilations, an esophageal stricture developed on the nineteenth day after the ingestion of lye. This patient was a Negro girl 14 years of age who drank a solution of lye with suicidal intent; the result was damage of moderate severity to the esophageal mucosa. In retrospect, we are of the opinion that the no. 24 French catheter used for daily dilations was not large enough to prevent formation of a stricture in this patient, but difficulty was experienced in passing a bougie of larger size. We have observed 5 patients with chemical burns of the mouth secondary to the ingestion of lye for whom no esophageal dilation was advised. The burns of these patients were limited to the lips or to the anterior portion of the oral cavity, and esophagoscopy showed no damage to the esophageal mucosa. A recent follow-up study revealed that no difficulty in swallowing had occurred in any of these patients.

TABLE 2.—*Distribution of Patients Seen*

Type of Patient	No. Seen	Per Cent
Showed definite stricture	97	71
Received early dilations	25	19
Was followed without therapy	5	3
Died during acute phase	1	1
Miscellaneous	8	6
Total	136	100

Unfortunately, at Duke Hospital the majority of the children with burns caused by the ingestion of lye were not seen until esophageal strictures developed. Table 2 reveals the distribution of patients seen. When 8 patients in the miscellaneous group whose records were incomplete or could not be found and the 5 patients who had no evidence of esophageal damage are excluded, it will be noted that only 26 of 123 patients were seen in a sufficiently early stage of esophageal damage so that prophylactic therapy could be instituted. The histories revealed that only 5 of the 97 patients who had esophageal strictures when first seen had had any form of esophageal dilation prior to admission to Duke Hospital. Several of these patients had been admitted to local hospitals for parenteral administration of fluids and sedation during the acute phase, only to be discharged without further therapy when the acute symptoms subsided and the ability to swallow liquids improved. One patient had had a gastrostomy performed for feeding purposes when the parenteral administration of fluids had become difficult. With the improvement of swallowing after the disappearance of esophageal edema, the gastrostomy opening was closed and the patient was discharged without any form of esophageal dilation. In this patient and in the

others who did not receive adequate prophylactic therapy, difficulty in swallowing recurred insidiously, increasing in severity over several weeks as the stricture developed in the burned area. At first, these patients had difficulty in swallowing solid food. Eating became slow, and food was frequently regurgitated; this state was later followed by difficulty in taking liquids. Table 3 reveals the time interval from the ingestion of the lye until the patient was seen with symptoms of obstruction. One may note that more than one half of our patients had sufficient symptoms of obstruction to apply for admission to the hospital by sixty days after the ingestion of lye.

TABLE 3.—*Time Interval from the Ingestion of Lye Until Patient Was Seen with Symptoms of Obstruction*

Time, Mo.	No. of Patients
0 to ½	7
½ to 1	37
1 to 2	20
2 to 3	7
3 to 12	21
Over 12	10
Total	102*

* This total includes 5 patients previously discussed for whom prophylactic therapy was inadequate to prevent formation of a stricture.

TABLE 4.—*Time Interval from Ingestion of Lye Until Only Liquids Could Be Swallowed (After Subsidence of Acute Symptoms)*

Time, Mo.	No. of Patients	Per Cent
0 to 1	19	60
1 to 2	7	22
2 to 3	3	9
3 to 4	1	3
4 to 7	2	6
Total	32	100

For 32 of these patients, the history is revealed that only liquids could be swallowed at the time of admission. Table 4 reveals the time interval from the ingestion of the alkali until all solid foods were regurgitated. It will be noted that without treatment the development of the stricture progresses rapidly, the majority of these patients being unable to swallow solid food thirty days after the ingestion of the alkali. Complete occlusion of the esophagus occurs rarely. Only 1 of our patients had an impermeable stricture.

Since the esophageal opening in children is small, the stricture is more frequently found in the upper third of the esophagus. Table 5 reveals the location of the strictures in our series of 102 patients. Nineteen of these patients had more than one stricture. Children whose

esophageal strictures persist for a long period have numerous systemic and other changes associated with prolonged malnutrition. Bronchitis usually is present due to the spilling of food and secretions into the trachea. It is not the purpose of this paper to discuss the treatment of esophageal stricture but instead to reemphasize the method of avoiding it and the importance of its prevention. For those in whom an esophageal stricture of moderate severity developed because of inadequate therapy, we recommend gastrostomy and retrograde bougienage as outlined by Tucker.[2] Our results with this method of treatment have been satisfactory, although dilations have had to be continued for three to five years in difficult strictures before the patients could be dismissed.

Sixteen of our 102 patients with strictures died during the course of therapy. Secondary causes of the majority of deaths were such conditions as perforation of the esophagus following direct esophagoscopic examination or attempted dilation, mediastinitis, esophagotracheal

TABLE 5.—*Strictures of the Esophagus*

Location	No. of Patients
Upper third	31
Middle third	18
Lower third	17
Upper and middle	6
Upper and lower	6
Middle and lower	7
Undetermined	14
Total	102

fistula or empyema. Our mortality has been greatly reduced since the introduction of such chemotherapeutic agents as penicillin and the sulfonamide compounds. Our only death from lye poisoning since 1943, during which time we have treated 33 patients with the effects of the poisoning, was that of a Negro child who died during the acute phase after the ingestion of a large quantity of lye.

Through the efforts of Dr. Chevalier Jackson, of Philadelphia, legislation was finally passed by Congress in 1927 requiring that caustics be plainly labeled as a poison. This measure was a great step forward in the education of the public concerning the harmful nature of this substance. Caustics must be kept away from children. Carelessness and ignorance of their dangers are too common. A survey by Taylor[3] in 1935 of the effect which legislation concerning lye has had on the incidence of esophageal strictures revealed a 50 per cent decrease in Florida,

2. Tucker, G.: Strictures of the Esophagus: Diagnosis and Treatment, Laryngoscope **41**:426-438 (June) 1931.

3. Taylor, H. M.: A Preliminary Survey on the Effect Which Lye Legislation Has Had on the Incidence of Esophageal Stricture, Ann. Oto., Rhin. & Laryng. **44**:1157-1158 (Dec.) 1935.

Pennsylvania and Arkansas during the year 1934 as compared with the incidence during the year 1926. Therefore, in conclusion, not enough has been done to reduce the incidence of esophageal stricture following the ingestion of lye. A further decrease is possible through the continued education of the public concerning the dangers of this substance and by the early institution of proper therapy. If a child swallows lye, he should be taken to his local physician immediately so that prophylactic dilations of the esophagus can be performed, unless direct vision has demonstrated beyond question that the esophageal mucosa is unharmed.

SUMMARY

One hundred and thirty-six cases of lye poisoning are reviewed. The importance of early dilation in the prevention of esophageal stricture is reemphasized.

Elon College.

ABSTRACT OF DISCUSSION

DR. JOHN AIKMAN, Rochester, N. Y.: Dr. Kernodle, Dr. Taylor and Dr. Davison have performed a great service in again calling attention to the importance of the improved methods of treatment in cases of lye poisoning to prevent stricture of the esophagus.

The use of the Salzer procedure, together with modern methods of esophagoscopy and fluoroscopy, avoids the long, painful and expensive treatment which would otherwise be necessary. Lye poisoning has occurred much less frequently since Dr. Chevalier Jackson called attention to this hazard so forcibly that laws were enacted which required proper labeling of these caustics. In fact, the number of cases seen in Durham is astonishing. It would appear to be a social problem due to poverty, ignorance and carelessness.

Dr. Davison told me that while he was in Baltimore he saw fewer than 20 cases, and I am under the impression that he said he had seen 300 or 400 cases since he had been in Durham. There was but 1 private patient in this group, whom I think he said was insolvent. It is difficult to change housekeeping habits long in existence. If families could afford to purchase commercially made soaps, these accidents with caustics would decrease. One is again impressed with the importance of preventing these and other cases of accidental poisoning in small children. Such tragedies can be reduced by education and by legislation that forces accurate labeling of drugs and poisons. Alkalis and other poisons are constantly being placed on the market for new purposes, under new names, with inadequate information on the package as to the contents and what should be done when accidental poisoning occurs. Manufacturers seem to dislike poison labels. Lists of contents often are vague and are printed in such small type that they are not easily found and read.

Study the subject of accidental poisoning as much as one wishes, and the next accident will be due to some item of which one has never heard. Children are not particular, and toilet-bowl cleaners, drainpipe cleaners, as well as dozens of other articles, cause trouble. If reports of accidental poisoning were required and if the statistics of various hospitals were collected, much useful and astonishing information would be obtained. I again plead that manufacturers be required to place readable and understandable information on the containers of poisonous materials.

I should like to ask Dr. Kernodle for his definition of lye and what alkalis he would place under that heading, and also how his treatment should be modified for accidental acid burns of the esophagus.

I have been informed that the latest figures show an increase in accidental poisoning in the United States among children, another reason that Dr. Kernodle's paper is such a valuable contribution at this time.

DR. RICHARD R. O'TOOLE, Pittsburgh: My colleagues and I do not see the great number of cases of lye poisoning that Dr. Kernodle has indicated. Unfortunately, at the Children's Hospital and at St. Francis Hospital, in Pittsburgh, in the cases we do see the symptoms have progressed to the stage at which we have to resort to gastrostomy and the retrograde form of dilation. I think that as pediatricians we have been somewhat derelict in the general management of the nutrition of these children with the effects of lye poisoning. Certainly, the story of low blood plasma protein levels and low hemoglobin levels in the malnourished child is entirely too common, and it is the same in practically every medical center from which the cases are reported.

We in Pittsburgh think we may have the solution to this problem. We have a convalescent home in Large, Pa., operated in association with the Children's Hospital. Several years ago, we had 3 children in the 2 year age group, which Dr. Kernodle has pointed out to be the most affected. In spite of what we thought of as adequate in the calculation of a diet and in nursing care, the nutrition of these children was not what it should have been. We decided to send them to the convalescent home to see what could be done. Miss Hilton, at Large, devised a unique method of managing these children. She looked over their diets, as we all looked them over, and decided that if the diets in use were inadequate she would provide better ones. She selected 6 robust convalescent 2 year old children and for two weeks observed what they ate. She carefully measured their diet, both qualitatively and quantitatively; she scraped their plates when they were finished eating and weighed and measured that. She arrived at a mean conclusion and devised a method of prorating all the foods the well children had eaten, and here, again, she was quite careful in her preparation. She divided her diet into a schedule of four feedings and fed them through the gastrostomy tube. Of course, she also supplied the necessary vitamins through the tube. The results were gratifying. Not only did these patients improve in their nutrition, but the ordinary psychiatric problems that such children usually have seemed to vanish. In addition to the necessity for the management of their nutrition, I think it should be pointed out that, too often, the mouths, jaws and teeth of these children are neglected. They are not chewing, and their jaws and teeth do not develop. Here, again, we approached this problem satisfactorily by having the children chew on an ordinary hard rubber dental ring, that is, the ring the dentist uses in his procedures. At first, it was painful, as they had not been chewing for a few weeks; but as they continued they were able to manage it well, and their mouth, jaws and teeth developed satisfactorily.

DR. G. W. KERNODLE, Durham, N. C.: In answer to Dr. Aikman's question concerning the number of patients seen, I imagine that any one associated with the pediatric ward at Duke Hospital for the past ten years would estimate that several hundred patients had been admitted during that time for treatment of the effects of lye ingestion. In reviewing the records, however, only 136 cases were found. It was noted that in the majority of these histories, there were several readmissions for various complaints or for retrograde dilations.

Since completing this paper less than two weeks ago, I have seen 3 additional patients. One of these children gave a history of the ingestion of lye one month

previously, with symptoms of obstruction developing two weeks later. The parents stated that during the two weeks preceding the admission to Duke Hospital, the patient had been seen by several physicians, each of whom stated he thought the [illegible] was secondary to tonsillitis.

I am not taking from the dictionary definition of lye, but about 100 per cent of our patients [illegible] what is known as Red Devil Lye. Various cleansing agents contain different percentages of caustic alkali.

In regard to the question concerning the effect of acid on the esophageal mucosa, in my experience it has had more effect on the pyloric region than on the esophageal mucosa. I have had 1 case of pyloric obstruction secondary to the ingestion of concentrated hydrochloric acid. I am fairly certain that a number of the department also reported a case, in which obstruction of the esophagus occurred secondary to ingestion of lactic acid U.S.P. that had been given by mistake in place of cod liver oil.

* (Alkaline steep and its effect.) Die alkalische Weiche und ihre Wirkung. (A lecture)
Kieninger, H.
Brauwelt 111 (37) 778-782 (1971) (7 ref. De)
(Inst. fur Tech. der Brauerei I, Weihenstephan, Tech. Univ., Munich, W. Germany)
Addition of 0.1% NaOH to barley steeping water improved the taste, tannin content and colur of the resultant beer. When using modern intensive steeping methods with only triple water steeping, the quality of the beer is not affected by the alkaline steep, which offers the brewer definite advantages. Data are given for results of analysis of the malt, wort and beer obtained and water consumption is also discussed. TUB-IGB

* Unable to find after several searches. Wrote Author for copy on November 12, 1973. No reply as of January 9, 1974.

[GANN, 54, 155~161; June, 1963]

UDC 616.381 003.217 006 091.83[576.312.32]. :546.4

CYTOLOGICAL EFFECT OF CHEMICALS ON TUMORS. XVI. EFFECT OF SOME INORGANIC COMPOUNDS ON THE MTK-SARCOMA III *IN VIVO*[*1]

(Plate XVIII)

Yanagi KIMURA AND Sajiro MAKINO

(Zoological Institute, Hokkaido University[*2])

Synopsis

Twenty-three kinds of inorganic compounds were examined for their inhibitory effect on the MTK-sarcoma III, a rat ascites tumor.

Damages to tumor cells by the influence of these compounds are summarized in a table. Though the degree of influence is not similar in these chemicals, $CaCl_2$, $FeCl_3$, $FeNH_4(SO_4)_2$, $ZnCl_2$ and $ZnBr_2$ were found to exert a marked damaging effect on both the cytoplasm and nuclei of the tumor cells. Most cells generally underwent metaphase block by the effect of these compounds, cells being damaged at the metaphase.

Marked decrease of dividing cells was observed in the ascites as a result of repeated application of the chemicals.

Although much has been made on experiments with inorganic compounds as carcinogens[3,13~15] or as inhibitors of tumor growth,[1,2,4~11,16,20~25,31~38,40~45,47,48] rather little has been learned on their cytological action in detail. Previous workers who dealt with the effect of certain inorganic compounds on division of *Amoeba proteus*,[46] on ascites tumor cells,[29] and on root tip meristems or petal cells of plants[12,17,18,26,27,32,50] presented evidence that these compounds have an inhibitory effect on cell division to a large or small extent. Galinsky[12] noted similar mitotic abnormalities to occur in both tumors and phosphate-treated onion roots and suggested a possible relation between them.

The present experiments were undertaken in a hope to gain some cytological information on the effect of certain inorganic compounds on tumor cells.

MATERIAL AND METHODS

Rats of both sexes for the following experiments were Wistar, Long-Evans, and Gifu-agouti strains weighing 95~130 g. The MTK-sarcoma III, an ascites tumor of

*1 Contribution No. 579 from the Zoological Institute, Faculty of Science, Hokkaido University. Supported by a grant to S. Makino from the Damon Runyon Memorial Fund for Cancer Research (DRG 563B).

*2 Kita-8 jo, Sapporo, Hokkaido (木村やなぎ, 牧野佐二郎).

s, was employed exclusively for the experiments. All the a in whom the mor was transplanted died within 9 days. The test compounds (Table I) were administered intraperitoneally to rats in each experimental group, starting on the 3rd or 4th day of tumor transfer, at dosages determined after several trials. Rats of control group received intraperitoneal injection of physiological saline solution. Cytological preparations were made according to the acetic-dahlia squash technique[28] at appropriate intervals following application of the chemicals. A part of the preparations were fixed with absolute methanol or with acetic acid-ethanol (3 : 1) and stained with May-Grünwald-Giemsa[19] or with Feulgen's reagent.[39] Autopsy was made in some cases to examine any accompanying effect of the chemicals.

RESULTS OF OBSERVATIONS

Test for a Possible Effect of pH Possible effect of pH of vehicles used upon tumor cells was examined as a preliminary test. Because of their insolubility in water,

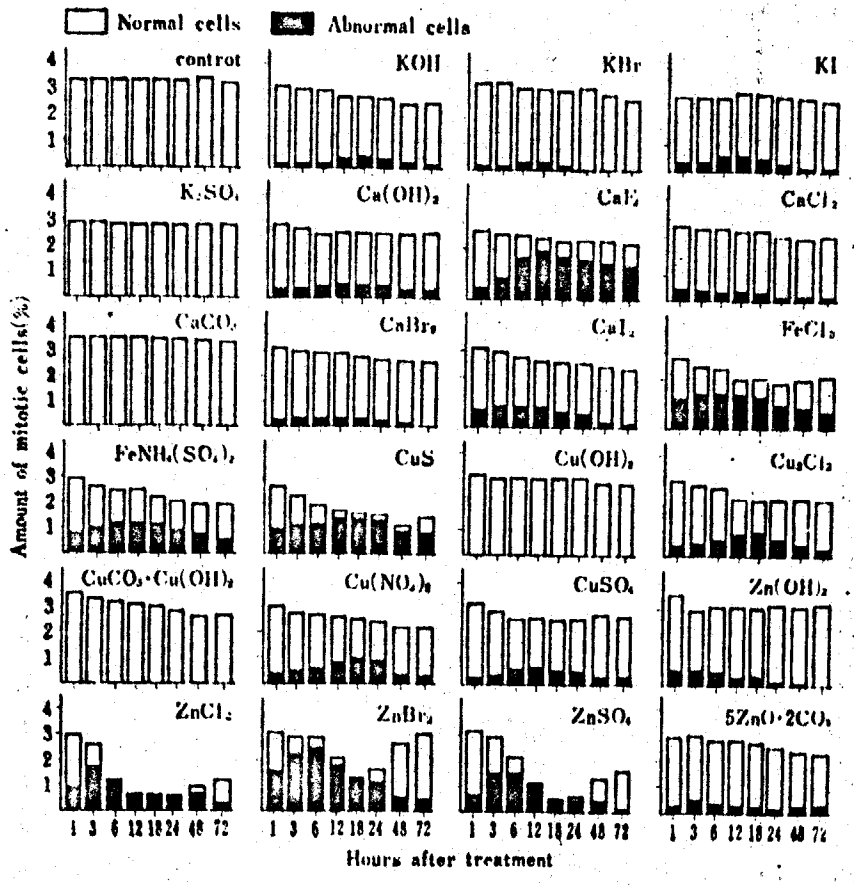

Fig. 1.

some cor[illegible]ls were dissolved by adding some alkaline or acid solution [illegible]e vehicle. I[illegible]ological saline solution was adjusted to pH 4.2, 5.2, 6.2, 7.2, a[illegible]2, by adding either *N* HCl or *N* NaOH. After adjusting the pH, the solution was injected intraperitoneally to tumor-bearing rats.

Cytoplasmic blebbing or karyorrhexis was observed rather infrequently in some tumor cells at the two extremities of pH value used. Almost all cells were viable for at least 72 hours observed after the injection of the solution.

Effect of Single Administration The reaction of tumor cells to inorganic compounds used at appropriate dose levels is presented in Table I. The reaction is represented by pycnotic aggregation of chromatin, karyorrhexis, multinucleate cells, chromosome clumping, contraction or stickiness of chromosomes, irregular chromosome distribution, lagging or bridges of chromosomes at anaphase, multipolar spindle formation, vacuolization or blebbing of cytoplasm, and disintegration of cytoplasm (Fig. 1 and Photos 1~19). Particularly striking is the contraction or stickiness of metaphase chromosomes exhibited by cells treated with KOH, KBr, KI, $Ca(OH)_2$, CaF_2, $CaCl_2$, $CaBr_2$, CaI_2, $FeCl_3$, $FeNH_4(SO_4)_2$, CuS, Cu_2Cl_2, $Cu(NO_3)_2$, $Zn(OH)_2$, $ZnCl_2$, $ZnBr_2$, $ZnSO_4$, and $5ZnO{\cdot}2CO_2$. Further, $CaCl_2$, $FeCl_3$, $FeNH_4(SO_4)_2$, $ZnCl_2$, $ZnBr_2$, and $ZnSO_4$ induced considerable cytoplasmic degeneration followed by blebbing and budding off of the cytoplasm. The above injurious effects were observed in cells tested with almost all compounds, though the degree of influence differed with different compounds. Within the scope of the present experiments, therefore, there was observed no damaging pattern specific to the agent used.

On the basis of gross cell countings, the injurious effect of compounds was evaluated from the data given in Table I as follows:

± No effect, with mitotic and cellular abnormalities only at high concentrations (lethal or sublethal) of chemicals.

\+ Slight effect, with abnormalities in less than 30% at appropriate dose levels.

++ Strong effect, with abnormalities in 30~50% at appropriate dose levels.

+++ Marked effect, with abnormalities in more than 50% at appropriate dose levels.

Effect of Repeated Administration Judging from the results of single application as described above, some compounds of calcium and zinc were selected for successive trials. They were $CaCl_2$, $CaCO_3$, CaI_2, $ZnCl_2$, $ZnBr_2$, and $ZnSO_4$. The former three seemed to be available for repeated application as they had a small injurious effect on host animals, while the latter three grossly affected the tumor cells.

Treatments were carried out daily for 7 consecutive days, starting usually on the third day after tumor transfer. Daily dosages were one-fifth to one-half of each single dosage applied in the single-administration experiments. Cell abnormalities induced here were of quite the same pattern as those resulting from the single application. Dividing cells markedly decreased in number following repeated injection

Table I. Effect and Evaluation of Chemicals in Experiment with MTK-Sarcoma III

Chemical	Dose (g/100 g)	Effect of chemicals*	Recovery of cells (hrs. after inj.)	No. of animals	Life span		Evaluation of chemicals		
					single inj.	repeated inj.	resting nucleus	chromosome	cytoplasm
KOH	0.18	4,5,6	18-24	5	8.8	—	++	+	+ ++
KBr	0.02	4,5,6,10	18-24	5	8.4	—	++	+	+ ++
KI	0.05	2,5,6	18-24	5	8.4	—	+	+	+
K_2SO_4	0.13	2	9-12	6	8	—	++	++	++
$Ca(OH)_2$	0.12	4,5	18-24	16	8	8.3	+	+	++
CaF_2	0.1	3,4,5,6,12,13	24-48	15	8.4	9.1	+	+++	+
$CaCl_2$	0.35	2,5,6,13	18-24	13	8.7	9.8	+	+	+++
$CaCO_3{\cdot}3H_2O$	0.1	3,5,9	6-12	17	8.7	9.2	++	++	+++
$CaBr_2$	0.23	3,5	18-24	13	8.7	9.8	+	+	++
$CaI_2{\cdot}6H_2O$	0.14	4,5	24-48	15	9.3	9.8	+	+	++
$FeCl_3{\cdot}6H_2O$	0.05	2,4,5,6,12,13	24-48	9	7.9	—	++	+++	++
$FeNH_4(SO_4)_2{\cdot}12H_2O$	0.06	2,5,6,10,12,13	24-44	10	8.1	—	+	+++	+
CuS	0.015	2,5,6,7,8,9	24-48	13	9.6	—	+	+++	++
$Cu(OH)_2$	0.02	2,5	6-12	5	8.8	—	++	+++	++
Cu_2Cl_2	0.012	4,5	18-24	5	8	—	++	++	++
$CuCO_3{\cdot}Cu(OH)_2{\cdot}2H_2O$	0.013	2,5	6-12	5	10.5	—	++	++	++
$Cu(NO_3)_2{\cdot}3H_2O$	0.06	2,4,5	18-24	5	8.2	—	+	+	+
$CuSO_4{\cdot}5H_2O$	0.03	1,4,11	24-48	5	8.2	—	+	+	+
$Zn(OH)_2$	0.014	2,5,9,10,13	12-24	9	7.9	8.6	++	+	++
$ZnCl_2$	0.07	2,4,5,9,10,12,13	48-72	12	9.5	10.5	+	++	+
$ZnBr_2$	0.08	2,4,5,6,9,12,13	24-48	11	8.9	10.7	++	++	+
$ZnSO_4$	0.1	2,5,6,9,10,12,13	24-48	13	9.3	10.1	+	++	+
$5ZnO{\cdot}2CO_2{\cdot}4H_2O$	0.015	2,5,13	12-18	9	9.0	9.3	++	+	+

* Cellular events corresponding to numerals given in this column are as follows:

1. lobated nuclei
2. chromatic aggregation
3. pycnosis
4. karyorrhexis
5. stickiness of chromosomes
6. contraction of chromosomes
7. scattering of chromosomes
8. lagging of chromosomes
9. clumping of chromosomes
10. chromosomic bridges at anaphase
11. multipolar spindle formation
12. blebbing of cytoplasm
13. disintegration of cytoplasm

of the chemi[illegible] while inflammatory cells showed an increase in the ascites. Pro[illegible]gation of the [illegible]pan of tumor-bearing animals, though not so marked, was observ[illegible] in all experiments.

Discussion

Makino and Tanaka,[29] in a cytological study on the injurious effect of H_2O_2, $AgCl_2$, and $CaCl_2$ on rat ascites tumor, have shown that these compounds first affected the cytoplasm, with disintegration or breaking down of tumor cells. Though the degree of influence differed with different compounds, the effect of $CaCl_2$, $FeCl_3$, $FeNH_4(SO_4)_2$, $ZnCl_2$, and $ZnBr_2$ in the present experiment appeared to follow a pattern of response similar to that described by Makino and Tanaka.[29] These compounds also attacked the cytoplasm of cells at every stage, first inducing the break down of the cell body with subsequent pycnotic disintegration of naked nuclei. Treatment with KOH, KBr, KI, $Ca(OH)_2$, CaF_2, $CaCl_2$, $CaBr_2$, CaI_2, $FeCl_3$, $FeNH_4(SO_4)_2$, CuS, Cu_2Cl_2, $Cu(NO_3)_2$, $CuSO_4$, $Zn(OH)_2$, $ZnCl_2$, $ZnBr_2$, $ZnSO_4$, and $5ZnO \cdot 2CO_2$, caused mitotic abnormalities to a large or small extent; most of the cells were arrested at metaphase showing irregular condensation and stickiness of chromosomes, and the disturbance of spindle formation. Chromosomes at anaphase showed aggregation or bridges. These results agree with those of Levan[26] who investigated the effect of some nitrates on the root tip meristems of *Allium cepa*. He observed, in several of tested compounds, the induction of pronounced stickiness and the formation of anaphase-bridge which were indistinguishable from typical colchicine-induced anomalies. He stated that, though exceptional after treatment with organic compounds, these damaging effects were rather usual following treatment with inorganic salt solutions. Yamaha[50] observed with *Allium cepa*, *Glycine soya*, *Vicia faba*, and *Vigna chinensis*, Sigenaga[32] with *Tradescantia*, Galinsky[12] with *Allium cepa*, Lorenzo-Andreu[27] with *Allium cepa*, and Hindmarsh[17,18] with *Allium cepa*, similar cell abnormalities under the influence of various inorganic salt solutions. Some of the compounds tested are chemically unrelated with each other, but they all caused a cytologically similar damaging effect. There is a possibility of a common mode of action in these chemicals, which may be related to osmosis or dehydration caused by these compounds. On the other hand, Hindmarsh[17] pointed out that stages in cell division may be linked with metabolic processes and that cytological aberrations may well result from artificial disturbance in the metabolism of the cell. Lorenzo-Andreu[27] expressed the view that these damaging effects are a function of the concentration of the agents.

The action of inorganic compounds upon cell division has hitherto been discussed from different standpoints. Hindmarsh[18] is of the opinion that the inhibition of cell division may be due to ion unbalance in the external solution, rather than to the specific effect of any inorganic ion. It is generally accepted that physiological effect

of inorganic compounds is largely due to the presence of cations [illegible]e workers have studied cation requirement in tissue culture.[31),49] Yamaha,[50] [illegible]llendorff,[30] and Lorenzo-Andreu[27] found that there was a series of actions of the corresponding cations on cell division. In the light of current knowledge, and on the basis of the results from the present study, no general statements seem to be possible on the mechanism of damaging action of inorganic compounds.

The authors are grateful to Drs. Motomichi Sasaki and Takao Matsumoto for their helpful advice and criticism.

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Explanation of Plate XVIII

Photomicrographs of MTK-sarcoma III cells.

Photos 1~3. Destructive changes of tumor cells following injection of inorganic compounds.

Photo 1. Lobated nuclei induced by KI, 12 hours after injection. ×1000.

Photos 2~4. Cells with chromatic aggregation. ×1000. 2. 3 hours after $FeCl_3$ injection. 3. 3 hours after CuS injection. 4. 6 hours after $Cu(NO_3)_2$ injection.

Photo 5. Karyorrhexis induced by $Cu(NO_3)_2$, 18 hours after injection. ×1000.

Photos 6~8. Stickiness of chromosomes. ×1000. 6. 12 hours after CaF_2 injection. 7. 6 hours after $ZnBr_2$ injection. 8. 3 hours after $ZnCl_2$ injection.

Photos 9 and 10. Contraction of chromosomes. ×100. 9. 12 hours after CuS injection. 10. 6 hours after $ZnSO_4$ injection.

Photo 11. Lagging of chromosomes induced by CuS, 18 hours after injection. ×1000.

Photo 12. Clumping of chromosomes induced by $ZnCl_2$, 3 hours after injection. ×1000.

Photos 13 and 14. Chromosome bridges at anaphase. ×1000. 13. 18 hours after $FeNH_4(SO_4)_2$ injection. 14. 3 hours after $ZnCl_2$ injection.

Photo 15. Tripolar spindle induced by $CuSO_4$, 24 hours after injection. ×1000.

Photos 16~18. Blebbing of cytoplasm. ×1000. 16. 3 hours after $ZnCl_2$ injection. 17. 1 hour after $ZnBr_2$ injection. 18. 3 hours after $ZnSO_4$ injection.

Photos 19~23. Disintegration of cytoplasm. 19. 12 hours after $FeNH_4(SO_4)_2$ injection. 20. 12 hours after $FeCl_3$ injection. ×1000. 21. 18 hours after $ZnCl_2$ injection. 22. 12 hours after repeated injections of $ZnCl_2$. 23. 12 hours after 5 repeated injections of $CaCl_2$. ×400.

Photo 24. MTK-sarcoma III cells on the 4th day after transplantation, just before injection (control). ×400.

* (Process for pasteurizing egg white.) Verfahren zum Pasteurisieren von Eiklar.
Kohl, W. F.; Sourby, J. C.; Ellinger, R. H.
(Stauffer Chemical Co.)
West German Patent Application 1 816 897 (1969)
(De)
0.2-2.0 wt.% of alkali polyphosphate which has the formula $(NaPO_3)_n$, where 3 n 40, and which may be linear or cyclic and combined with H_2O or Na_2O, are admixed with an alkaline material, e.g. NaOH or KOH, to raise the pH to 8.0-10.0. The mixture is then heated for 0.5-10.0 min to 51.5-57.0 C for pasteurization, cooled, and the pH lowered. Microorganisms, e.g. salmonellae, are destroyed without denaturing the protein. W&Co.

* Unable to find after several searches. Wrote to Patentee for copy on November 12, 1973. No reply as of January 1, 1974.

VARIABLES AFFECTING THE EFFICIENCIES OF PEELING TOMATOES WITH CAUSTIC SODA

(Order No. 70-14,067)

Loren Lemoine LUCAS, Ph.D.
The Ohio State University, 1969

Adviser: Professor Wilbur A. Gould

In 1968, tomatoes were peeled with the steam peeling method and by submergence in 16, 18 and 20 percent caustic solution at 190, 200 and 210°F. The wetting agents Tergitol and Faspeel were evaluated in conjunction with the above caustic solution temperatures and concentrations. The tomatoes processed in 1969 were peeled by steam and also by submergence in 18 and 20 percent caustic solution at 180, 190 and 200°F., utilizing a caustic applicator.

The peel loss involved with caustic peeling was increased one to two percent for a caustic solution temperature increase of ten degrees Fahrenheit. Peel loss was reduced by increasing caustic solution concentration. The use of wetting agents increased the peel loss percentage of tomatoes peeled with 16 and 18 percent caustic solutions. Loss was significantly higher for steam peeled tomatoes than those peeled with caustic solution utilizing the caustic applicator.

The caustic submergence time required to remove tomato peel was reduced with increasing caustic solution temperature and concentration.

The pH of canned tomatoes was increased as the caustic concentration of the peeling solution increased. The application of wetting agents to the caustic solution increased the pH of the tomatoes.

The total acidity of steam peeled tomatoes was higher than caustic peeled tomatoes. Increasing caustic temperature and concentration reduced the finished product acidity.

The absence of defects scores for canned tomatoes were increased as the caustic temperature was increased. Application of the wetting agent Faspeel resulted in reduced tomato absence of defect score, while the use of the wetting agent Tergitol increased the absence of defect score.

The drained weight score of canned tomatoes was increased as the caustic peeling solution concentration was increased. Tomato drained weight score was reduced as the caustic solution temperature was increased. Application of the wetting agents to the peeling solution increased the tomato drained weight score.

The wholeness score of canned tomatoes was reduced as tomato was exposed to increasing caustic solution concentration.

M $4.00; X $6.00. 116 pages.

A STUDY OF THE SYMBIOSIS BETWEEN MICROCOCCI AND LACTOBACILLI OCCURRING IN CHEDDAR CHEESE

(Order No. 70-5806)

and 6 anaerobically mannitol fermenting coagulase negative Gram-positive cocci stimulated acid production by lactobac Fourteen lactobacilli were isolated from 7 lots of cheddar cheese made in our laboratory during different seasons of t year. Thirteen of the 14 lactobacilli showed increased acid production when 5 ml 10% NFDM was supplemented with 2-: of capsular material.

A material similar to that of capsule was obtained from cheddar cheese extracts fractionated on Sephadex G-50 (fine The last peak material eluted with pyridine acetic acid buffe pH 5.1 was rich in riboflavin and olive green in color. The similarity of the two materials was established using thin la chromatography, column chromatography on Sephadex, and spectral analysis in the ultraviolet region.

The fractions got by proteolysis of casein and its fractic with the micrococcus intracellular enzymes did not stimula the lactobacilli. However, the intracellular proteinase fron the micrococci selectively attacked β-casein moiety in isoelectric and micellular casein, whereas extra-cellular proteinase degraded the α-casein preferentially. Only 3 out of micrococcus isolates produced exo-proteinase.

Cheese extract fractions of relatively high molecular we containing N-acetyl hexosamine were also found to stimulate acid production by lactobacilli. These fractions seem to ari from α-casein and contained about 25% glutamic acid of the constituent amino acids.

The stimulation of lactobacilli by micrococci capsular m terial is considered evidence of symbiosis between two orga nisms.

M $4.00; X $6.00. 96 page

Chemical Inactivation of Aflatoxins in Peanut and Cottonseed Meals[1]

G. E. MANN, L. P. CODIFER, JR., H. K. GARDNER, JR., S. P. KOLTUN and F. G. DOLLEAR,
Southern Regional Research Laboratory,[2] New Orleans, Louisiana 70119

J. Amer. Oil Chemists' Soc. 47(5): 173-76 (1970)

Abstract

Organic and inorganic reagents have been tested for destruction or inactivation of the aflatoxins present in peanut and cottonseed meals. The treatments were made in a special laboratory-scale reactor, and were evaluated by determination of the aflatoxins in the products by thin layer chromatography. In some instances, a larger pilot-plant scale reactor was used. Ammonia, methylamine, sodium hydroxide and formaldehyde reduced aflatoxin levels and appear practical for large scale treatments. Effects of various reaction parameters including time, temperature and moisture content on the efficiency of these reagents are presented.

Introduction

The best way of minimizing aflatoxin contamination in various agricultural commodities is to prevent or minimize mold infection by improvement in agricultural practices (1). This is of course impossible with large quantities of a feed or food already contaminated with aflatoxins. To "rehabilitate" this for its intended use, the aflatoxins must be eliminated or their concentration reduced to a value below objectionable levels. Furthermore, this reduction in level must be accomplished with minimum losses in total solids and in nutritive value. Also, no toxic or otherwise undesirable material should be added.

Two possible methods for reducing aflatoxin levels under these restrictions comprise solvent extraction of the commodity to selectively remove the aflatoxins, or subjecting the commodity to some chemical or biochemical treatment to inactivate the aflatoxins. Both solvent extraction and nonbiochemical treatments have been investigated at this Laboratory, and this discussion will be confined to the latter work. These treatments were designed to reduce or eliminate the aflatoxins in oilseed products, cottonseed and peanut meals in particular.

Earlier reports (2–4) indicated that aflatoxins in contaminated peanut meal were refractive to wet and dry heat, acids, alkalis, chlorine, sulfur dioxide, gamma rays and ultraviolet light. In a more recent report (5), autoclaving wet toxic peanut meal at 120 C lowered its aflatoxin content. Chlorine gas (6) and hydrogen peroxide (7) have been used to partially detoxify aflatoxin-contaminated peanut meals. Other reagents of potential value for detoxification include benzoyl peroxide, sodium hypochlorite, sodium perborate, chlorine dioxide and nitrogen dioxide (8). The use of ammonia for this purpose has been patented (9).

This Laboratory's program on inactivation of aflatoxins in contaminated oilseed meals consists of laboratory-scale trials chosen in part according to suggestions from the literature, considering the restrictions stated above. Based on these laboratory trials, selected treatments were performed on a larger scale in the pilot plant to provide sufficient material for biological evaluations. Laboratory-scale trials were generally confined to heat treatments with added moisture in the presence of reagents including acids, bases, oxidizing agents, ammonia and various amines. Other types of reagents also were tested.

Experimental Procedures

Equipment and Methods

Most of the laboratory-scale trials were performed in a bench-scale reactor designed and constructed at this Laboratory (10). This reactor is made of stainless steel, heated with steam or hot water, and equipped with an agitator system having sufficient torque to mix 700–800 g batches of meal. A reflux condenser was used to prevent excess loss of meal moisture during the treatment.

Larger amounts of meal, 15–25 lb. batches, were treated in a 10 gal Groen reaction kettle, heated with a steam jacket, and provided with Teflon-tipped agitator blades. The agitator was operated at a speed of 72 rpm during the treatments. The Groen cover was sealed for pressurized treatments, or vented to the atmosphere through a reflux condenser for treatments at atmospheric pressure.

In general, treatments were initiated by elevating the moisture content of the meal to the desired level by adding the calculated weight of water and blending in a Hobart mixer for 10–15 min. The moisture contents of the meals were determined by use of the infrared-heated Ceneo Moisture Balance. Water-soluble reagents were dissolved in the water used to hydrate the meal, and water-insoluble reagents were finely pulverized and mixed in thoroughly, or added in solvents such as acetone and ethanol. The hydrated meal-reagent mixture was transferred to the reactor and the temperature elevated to the desired value and maintained for the specified time. The gaseous reagent (anhydrous ammonia) was added to the preheated hydrated meal in the sealed reactor until the desired pressure was reached, and the temperature maintained as described above. The charge was "dumped" and spread in a thin layer to "flash off" as much moisture as possible. Small batches were air-dried under ambient conditions, and larger batches were dried in a mechanical convection oven for about 1 hr at 55 C.

The products were assayed for aflatoxins by the method of Pons, et al. (11). Briefly, the procedure involved extraction of the aflatoxins with acetone-water, (70:30 v/v), lead acetate purification, extraction into chloroform, purification of the extract on a silica gel column, thin layer chromatography on plates coated with Silica Gel GHR, and visual estimation of the fluorescence intensities of the spots under ultraviolet light. Recently, a more efficient extraction solvent [acetone-water (85:15 v/v), plus 8 ml glacial acetic acid per liter] has been used, and a Photovolt Densitometer has been employed to measure fluorescence intensities (12).

[1] Presented at the AOCS Meeting, San Francisco, April 1969.
[2] So. Utiliz. Res. Dev. Div., ARS, USDA.

TABLE I
Aflatoxin Contents of Peanut and Cottonseed Meals After Treatments With Acids and Anhydrides, Inorganic Bases and Oxidizing Agents

Reagents, per cent of meal	Meal[a]	Moisture %	Aflatoxins in product ppb
Acetic acid, 3%	PMA	30	83
Acetic anhydride, 2%	PMB	28	51
Ascorbic acid, 2%	PMA	30	57
Formic acid, 3%	PMA	30	50
Fumaric acid, 1%	PMB	28	63
Glycolic acid, 1% plus NaOH, 1%	PMA	30	91
Maleic acid, 1%	PMB	28	34
Maleic anhydride, 1%	PMB	28	33
Nitric acid, 2%	CM	22	102
Nucleic acid (yeast), 1% plus NaOH, 1%	PMA	30	66
Phosphoric acid, 3%	PMA	30	79
Succinic anhydride, 2%	PMB	28	15
Calcium hydroxide, 2%	PMB	28	11
Sodium carbonate, 2%	PMA	30	12
Sodium hydroxide, 2%	CM	22	11
Trisodium phosphate, 3%	CM	22	49
Ammonium persulfate, 2%	PMA	30	91
p-Benzoquinone, 2%	PMA	30	10
Hydrogen peroxide, 2%	PMA	30	68
Ozonized, esterified soybean oil, 2% plus NaOH, 1%	PMB	28	61
Potassium chlorate, 2%	PMA	30	91
Sodium bromite, 1% plus NaOH, 1%	PMB	28	35
Sodium hypochlorite, 1%	PMA	30	55
Sodium perborate, 2% plus NaOH, 1%	PMB	28	39

[a] Aflatoxin contents of original meals: PMA, 110 ppb; PMB, 99 ppb; CM, 214 ppb.

Materials

Most of the laboratory-scale trials were performed using a prepressed solvent extracted peanut meal specially chosen for its aflatoxin content. It was designated PMA and contained 70 ppb (μg/kg) aflatoxin B_1, 30 ppb aflatoxin B_2, and 11 ppb aflatoxin G_1. No aflatoxin G_2 could be detected. Another similar peanut meal, PMB, also was used in the trials. It contained 72 ppb aflatoxin B_1, 11 ppb aflatoxin B_2, 13 ppb aflatoxin G_1, and 3 ppb aflatoxin G_2. The cottonseed meal, CM, used for the heat effect experiments and for some of the screening tests contained 144 ppb aflatoxin B_1 and 70 ppb aflatoxin B_2. Aflatoxins G_1 and G_2 could not be detected in this cottonseed meal.

Larger scale treatments of peanut meal were performed using meal PMA described above. The cottonseed meal subjected to larger scale ammoniation and methylamine treatments was a blend of a highly-contaminated meal and an uncontaminated meal of high quality. This blend contained 304 ppb aflatoxin B_1 and 30 ppb aflatoxin B_2; no G_1 and G_2 could be detected.

The reagents used in the laboratory scale trials were from different sources and of varying purity, although efforts were made to obtain the highest quality available in each case. The anhydrous ammonia (The Matheson Company) was 99.99% pure; the methylamine (Eastman 527) was 40% in water; and the sodium hydroxide pellets (Mallinckrodt Analytical Reagent) assayed 97.0%.

Results and Discussion

Effects of Heat

Because of apparent contradictions in the literature concerning the effects of heating on the aflatoxins in oilseed meals, a study was made of the influence of moisture content, temperature and time of heating on the aflatoxin content of contaminated cottonseed and peanut meals. This has been reported in detail elsewhere (13), and can be briefly summarized here by Figure 1 which shows the effect on the aflatoxin B_1 content of time and moisture when heating at

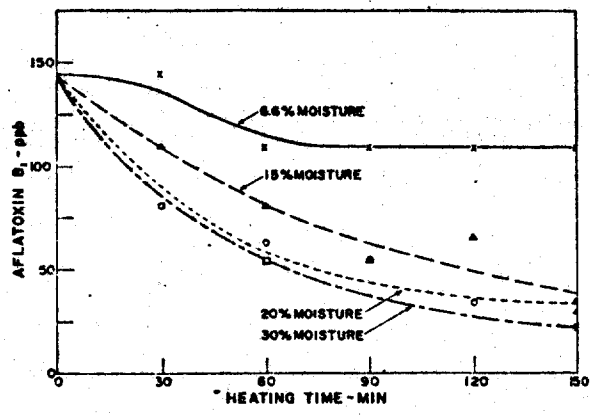

FIG. 1. Rate of aflatoxin B_1 destruction in cottonseed meal at 100 C (data from Ref. 13).

100 C, the cottonseed meal initially having 144 ppb of this toxin.

In general, more aflatoxin B_1 was eliminated by longer periods of heating for a given moisture content, and elevation of moisture also led to decrease of aflatoxin levels, particularly after longer heating periods. Although greatest reductions were obtained at 30% moisture, treatments at this or higher moisture levels were impractical because the meal tended to form a tough, plastic mass. Increasing the temperature above 100 C or extending the period of heating beyond 120 min also appeared undesirable because of excessive darkening of the meal. Hence the lowest level of aflatoxin B_1 obtained under conditions deemed practical (120 min at 100 C, 20% moisture) was 3 ppb. The meal heated under these conditions also contained 11 ppb aflatoxin B_2. Since aflatoxins G_1 were not detected in this meal, the total aflatoxin content was 44 ppb, a level higher than the maximum value of 30 ppb for peanuts and edible protein supplements established by a Protein Advisory Group sponsored by FAO/WHO/UNICEF, for those parts of the world where the need for protein is very urgent (14).

Similar experiments with peanut meal (110 ppb total aflatoxins) revealed that a moisture content of 30% could be used without serious plastic mass formation, but heating with this moisture level for 120 min at 100 C yielded a product containing 73 ppb total aflatoxins. The aflatoxins in peanut meal appear to be more refractory towards these heat treatments than the cottonseed meal aflatoxins.

Laboratory Scale Trials

Based on the moist heat treatments outlined above laboratory scale trials were performed for 120 min at 100 C. The cottonseed meal was adjusted to a moisture content of about 15% or 20%, and the peanut meal to about 30%. The reagents generally were added at the rate of 2% (or less) of the meal being treated, the addition being performed as described earlier. The results of some of the trials are presented in Tables I–III.

As shown in Table I, acids, anhydrides and oxidizing agents generally failed to yield products containing less than 30 ppb total aflatoxins, the one exception being succinic anhydride. The inorganic bases calcium hydroxide, sodium carbonate and sodium hydroxide appear quite promising, but the action of calcium hydroxide and sodium carbonate appeared erratic upon subsequent tests. Sodium hydroxide was more consistent in its ability to lower aflatoxin levels and hence was selected for further testing. In some treat-

TABLE II
Aflatoxin Contents of Peanut and Cottonseed Meals After Treatments With Nitrogen Compounds

Reagents, per cent of meal	Meal[a]	Moisture %	Aflatoxins in product ppb
tonitrile, 1% plus NaOH, 1%	PMA	30	90
iline, 1% plus NaOH, 1%	PMA	30	3
tine, 2%	PMA	30	80
line, 1%	CM	22	26
stosine, 0.5% plus NaOH, 1%	PMB	28	13
anolamine, 1%	PMA	30	33
ylenediamine, 2%	PMA	30	ND[b]
ucosamine hydrochloride, 1% plus NaOH, 1%	PMA	30	26
rine, 1% plus NaOH, 1%	CM	15	65
nine, 1% plus NaOH, 1%	PMB	28	13
droxylamine, 2%	CM	22	23
ine hydrochloride, 1% plus NaOH, 1%	PMA	30	29
thylamine, 1.25%	PMA	30	ND
rrole, 2%	PMA	30	85
ourea, 2%	PMA	30	32
methylamine, 1.25%	CM	15	29
cil, 1% plus NaOH, 1%	PMB	28	13
ea, 2%	PMA	30	19
monium carbamate, 2% plus NaOH, 1%	PMA	30	10
monium carbonate, 1% plus NaOH, 1%	PMA	30	33
(ammonium phosphate), 2%	PMA	30	29
ssium cyanate, 2% plus NaOH, 1%	PMA	30	56

[a] Aflatoxin contents of original meals: PMA, 110 ppb; PMB, 99 ppb; CM, 214 ppb.
[b] ND, None detected.

ents, this base was added together with other agents in effort to enhance the effectiveness of the atter.

Table II indicates that, in general, lower aflatoxin vels were obtained using nitrogen compounds as mpared with the reagents described in Table I. he outstanding reagents appear to be ethylenedimine and methylamine yielding products in which aflatoxins could be detected. Considerations based n cost and probable toxicity led to the choice of ethylamine for further examination. Similar conderations led to the rejection of other treatments niline plus sodium hydroxide, cystosine plus sodium ydroxide, etc.) which appeared to lower the aflatoxin vel markedly. Erratic results were obtained when rea was used in other treatments.

Most of the aldehydes examined are listed in able III.

Formaldehyde was selected for further testing since appeared to eliminate aflatoxins completely. The her treatments which seemed effective (crotonaldeide plus sodium hydroxide, 2-furaldehyde plus dium hydroxide, etc.) were deemed impractical for mmercial purposes because of cost.

It has been observed in this Laboratory that mmonia acts most effectively when applied to atoxin-contaminated meals under pressure at vated temperatures. The data in Table IV were tained from a series of larger scale ammoniation periments using contaminated peanut and cottond meals. These data show the effects of moisture, mmonia pressure and temperature on the lowering aflatoxin content by this treatment. Compared th peanut meal, it appears that more drastic amoniation conditions were required to inactivate the latoxins in cottonseed meal.

Data on methylamine and sodium hydroxide treatents of contaminated peanut and cottonseed meals given in Table V. In laboratory scale treatments peanut meal with sodium hydroxide alone, 2.0% this reagent and 30% moisture were required to arkedly reduce the aflatoxin content. Similar reults were obtained when contaminated cottonseed al was treated with sodium hydroxide. Treatment

TABLE III
Aflatoxin Contents of Peanut Meals After Treatments With Aldehydes

Reagents, per cent of meal	Meal[a]	Moisture %	Aflatoxins in product ppb
Acetaldehyde, 2%	PMA	30	102
Acrolein, 1%	PMA	30	91
Aldol, 1%	PMA	30	57
Benzaldehyde, 1%	PMA	30	90
Butyraldehyde, 1% plus NaOH, 1%	PMA	30	93
Crotonaldehyde, 1% plus NaOH, 1%	PMA	30	11
Dialdehyde starch, 1% plus NaOH, 1%	PMA	30	102
Formaldehyde, 2%	PMA	30	ND[b]
2-Furaldehyde, 1% plus NaOH, 1%	PMA	30	5
Glutaraldehyde, 1%	PMA	30	91
DL-Glyceraldehyde, 2% plus NaOH, 1%	PMA	30	90
Glyoxal, 0.5% plus NaOH, 1%	PMA	30	76
Heptaldehyde, 1% plus NaOH, 1%	PMA	30	73
Isobutyraldehyde, 2%	PMB	28	49
Paraldehyde, 1% plus NaOH, 1%	PMA	30	20
Phenylacetaldehyde, 1% plus NaOH, 1%	PMA	30	24
Propionaldehyde, 1% plus NaOH, 1%	PMA	30	81

[a] Aflatoxin contents of original meals: PMA, 110 ppb; PMB, 99 ppb.
[b] ND, None detected.

of the peanut meal with 0.60% methylamine plus 1.0% sodium hydroxide seemed more effective than either reagent acting alone. In larger scale treatments of cottonseed meal with methylamine and sodium hydroxide, practical considerations led to lowering the moisture contents to 15% or less, and shortening the reaction periods to 15 or 30 min. With this meal under these conditions, a higher concentration of methylamine, 2.0%, was required for drastic lowering of aflatoxins, and addition of sodium hydroxide seemed necessary to reach nondetectable levels.

Data on the laboratory scale treatments of contaminated peanut meal with formaldehyde and formaldehyde donors are presented in Table VI. The effectiveness of formaldehyde appeared enhanced by increasing moisture content and reaction times, and by the addition of 1.0% sodium carbonate. Similar tendencies have been noted in some of the treatments previously described. Formaldehyde treatments also lowered aflatoxin levels in contaminated cottonseed meal.

All treatments darkened the meals to some extent, the effect being greater with cottonseed meal. Ammonia and methylamine yielded darker products from cottonseed meal than did sodium hydroxide and formaldehyde, but the discoloration did not seem excessive. Meal texture was seemingly unaltered by the treatments. Of the four reagents, only methylamine yielded products with off odors, a slight "fishy" aroma noticed when higher concentrations of the reagent were used.

Contaminated peanut meal has been treated with ammonia, methylamine and sodium hydroxide, and the products subjected to preliminary biological eval-

TABLE IV
Effect of Ammoniation on Aflatoxins in Peanut and Cottonseed Meals

Moist %	Time min	Aflatoxins, ppb				
		66 C		93 C		
		15 psig	30 psig	15 psig	30 psig	45 psig
Peanut meal (110 ppb)						
8.6	15	36	30	41	5	
	30	44	18	24	ND	
15.0	15	41	ND[a]	5	ND	
	30	26	10	5	ND	
Cottonseed meal (334 ppb)						
10	15			....	....	12
15				81	69	6
10	30			....	....	5
15				....	....	4

[a] ND, None detected.

TABLE V

Effect of Methylamine and Sodium Hydroxide Treatments on Aflatoxins in Peanut and Cottonseed Meals (Treatment temperature 100 C)

Methylamine %	NaOH %	Moisture %	Time min.	Aflatoxins ppb
Peanut meal (110 ppb)				
0.0	0.0	22	120	75
0.0	0.0	30	120	73
0.0	1.0	22	120	34
0.0	1.0	30	120	22
0.0	2.0	22	120	18
0.0	2.0	30	120	4
0.0	3.0	22	120	4
0.0	3.0	30	120	7
0.70	0.0	30	120	32
1.25	0.0	30	120	ND[a]
0.60	1.0	30	120	10
Cottonseed meal (334 ppb)				
1.0	1.0	15	30	55
1.5	0.0	15	15	53
1.5	0.0	15	30	53
1.5	1.0	15	15	29
1.5	1.0	15	30	27
2.0	0.0	15	15	16
2.0	0.0	15	30	17
2.0	1.0	15	60	ND
2.0	1.0	15	30	ND
2.0	1.0	15	15	16
2.0	1.0	15	15	38[b]
2.0	1.0	10	30	32

[a] ND. None detected.
[b] Treatment temperature 85 C instead of 100 C.

uations. Details of the treatments, the composition of the products, and the biological evaluations have already been published (15), and the evaluations may be summarized as follows: (a) the treated meals did not produce symptoms of aflatoxicosis in ducklings; (b) the treatments did not seem to introduce any markedly toxic agents; and (c) some reductions in protein efficiency ratios (rat) resulted from the treatments, ranging from 18.1% for ammoniation to 28.0% for methylamine treatment. The formaldehyde-treated product has not been subjected to preliminary biological evaluation.

Ammoniation yielded a product of very low aflatoxin content in the shortest period of treatment at the lowest temperature, with only moderate alterations in meal properties. Ammoniation, however, requires a pressurized reactor capable of handling solids, equipment not generally found in oilseed mills. The methylamine and sodium hydroxide treatments do not require pressurized equipment, and hence might be performed in readily available apparatus such as the "five high" cooker. There is considerable reason to believe that these detoxification treatments might be performed on a commercial scale.

TABLE VI

Effect of Formaldehyde and Formaldehyde Donor Treatments at 100 C on Aflatoxins (110 ppb) in Peanut Meal

Reagents, per cent of meal	Moisture %	Time min	Aflatoxins ppb
Formaldehyde, 0.5%	30	60	18
Formaldehyde, 0.5% plus	15	30	34
	15	60	37
	15	120	37
Sodium carbonate, 1.0%	30	30	26
	30	60	8
	30	120	Trace
Paraformaldehyde, 0.5% plus	15	120	25
Sodium carbonate, 1.0%	30	30	16
	30	60	13
	30	120	11
Dimethylol urea, 0.5% plus	30	60	21
Sodium carbonate, 1.0%	30	120	19

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SPERMATOCYTE CHROMOSOME ABERRATIONS IN TWO SPECIES OF GRASSHOPPERS AT TWO DIFFERENT IONIC ACTIVITIES

By

G. K. MANNA AND PRANAB K. MUKHERJEE*
Department of Zoology, University of Kalyani, Kalyani, Nadia, West Bengal, India

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Introduction

In an early preliminary note Manna and Ray (1964) reported that changes in the spermatocyte chromosomes of the grasshopper, *Spathosternum prasiniferum*, could be introduced by injecting acidic and alkaline solutions. Since the discovery of mutagenic action of nitrogen mustard in animals by Auerbach and Robson (1947) and urethane in plants by Oehlkers (1943), a large number of chemicals has been tested for inducing cellular damages. The effects of various chemicals used by different workers in different plant and animal materials have been ably reviewed by many workers (Auerbach, 1951; Biesele, 1958; D'Amato, 1950; Grundmann, 1964; Ligsti and Dustin, 1957; Kihlman, 1961; Sharma and Sharma, 1960; Wilson, 1960). Further, Kihlman (1956) showed that at least the effect of some of the chemical mutagens as for example, maleic hydrazide, may indirectly be connected with the hydrogen ion concentration of the treated cell. In order to understand many biological problems centering round the evolutionary conception, the study of structural changes of chromosomes is of fundamental importance. Such structural changes often have to be produced by artificial means since spontaneous ones occur very rarely. Of the two important means of inducing chromosomal changes, the ionizing radiations (see Hollaender, 1954; Bacq and Alexander, 1955) and the radiomimetic chemicals, the latter originally was thought to be of greater utility particularly for localized breaks. However, this expectation has not been realized in all cases from the factual data, although the other implications of such studies are still important. The pH dependence effect in the chromosomes opens up an interesting field of investigation. The effect of chemicals in the animal chromosomes has not been tested as widely as that of the plant materials, and in recent years a new problem has been presented by virus-associated mammalian chromosome breakage (see A[illegible], 1965).

Further, the response of animal chromosomes in relation to breakage or other changes through different chemical treatments appears to be relatively poor in comparison to that of plant materials. Thus Raychaudhuri (1961) suggested that this difference in response might be attributable to the difference in the metabolism of the two classes of materials. However, with the accumulation

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of more data on animal materials, the response between plants and animals may not appear to be so wide. In recent years more records of the chemical effect in animal materials similar to that of plants are being published (Hsu and Somers, 1961; Manna and Mazumder, 1964; Manna and Parida, 1965a, b, 1966 and unpublished; Sarkar, 1956).

The reports on the spermatocyte chromosome changes of grasshopper induced by various chemical agents are very limited. Yosida (1950), Manna and Parida (1965a) studied the effect of colchicine on the male meiotic cells treated in various ways and fixed at different hours of post-treatment period. Sarkar (1956) studied the effect of nitrogen mustard in the spermatocyte cells of the grasshopper, *Gesonula punctifrons*. Recently Manna and Mazumder (1964) have reported the specific type of break in the X-chromosome of the first meiotic prophase cells in one of the two species of grasshoppers treated with ethyl alchohol. Manna and Parida (1965b, 1966 and unpublished) are making a series of investigations on the effect of radiomimetic chemicals and mitotic poisons in the spermatocyte cells of different species of grasshoppers.

The present investigation was undertaken with a view to find out the effect of the acidic and alkaline solutions of some known pH upon the spermatocyte cells of two species of grasshoppers.

Material and Method

Two species of adult male grasshoppers, *Spathosternum prasiniferum* and *Oxya velox*, constituted materials for the present study. Specimens were injected with water, 0·67% normal saline and various kinds of solutions having the pH adjusted at some particular level as detailed below (table 1). The pH level of the solutions was tested prior to the injection. Each experiment was repeated twice or more but in the table they have been added together. The effects produced by solutions of different pH were repeatable.

The alkaline solution of pH 9 was prepared by adding sodium hydroxide to 0·67% saline solution. Each specimen of *Spathosternum prasiniferum* was injected abdominally with 0·02 ml. of the solution. In one series, acidic pH solutions of 3 and 4 were prepared by mixing hydrochloric acid with 0·67% normal saline solution. Approximately 0·02 ml. of the solution was injected into the abdomen of *S. prasiniferum*. In another series individuals of *Oxya velox* were injected with an acidic solution of pH 4 prepared by diluting acetic acid in distilled water (0·04 ml acetic acid solution gives a pH of about 4).

The data (table 1) show that the mortality was higher among specimens treated with HCl than those with acetic acid solution. However, the size of *Spathosternum prasiniferum* was relatively smaller than *Oxya velox*. At present the mortality rate cannot be definitely correlated with size of the species or with the use of strong or weak acid. In the HCl-treated series no individual could survive beyond 4 hours at pH 3 and 18 hours at pH 4 after treatment while in the acetic acid series, some of them could survive upto 72 hours or more.

Table 1

Species used	Series	Solution injected	Total sp.	No. of expt.	No. of sp. surv.	Hour of fixation	Mortality %
	Control	0·67% saline	15	3	15	4	Nil
	pH 9	NaOH	30	3	30	4	Nil
	Control	0·67% saline	25	3	25	24	Nil
	pH 9	NaOH	27	3	22	24	19%
Spathosternum prasiniferum	Control	0·67% saline	20	2	20	4	Nil
	pH 3	HCl	20	2	7	4	65%
	Control saline	0·67%	10	2	10	4	Nil
	pH 4	HCl	20	2	8	4	60%
	Control	0·67% saline	20	2	18	18	10%
	pH 4	HCl	20	2	7	18	65%
	Control	Dist. water	5	2	5	24	Nil
	pH 4	Acetic acid	10	2	8	24	20%
Oxya velox	Control	Dist. water	5	2	8	48	Nil
	pH 4	Acetic acid	10	2	5	48	50%
	Control	Dist. water	5	2	5	72	Nil
	pH 4	Acetic acid	10	2	4	72	60%

Further, the higher mortality of grasshoppers due to the injection of the solutions of different pH would be very much apparent when the mortality data is compared with that of control specimens (table 1). Almost cent percent survival of the control specimens indicates that the death of the treated specimens was neither due to the shock of the injection nor due to the fluid used as diluting agents, e.g., 0·67% saline or distilled water.

Testes of each specimen were dissected out and were immediately fixed in

acetic-alchohol mixture. They were squashed and stained either in iron-alum haematoxylin or in Feulgen stain.

Observations

Control Series:

The behaviour of the meiotic chromosomes of the normal individuals of the two grasshoppers has been described by Ray Chaudhuri and Dutt (1947). The cytological behaviour of the different sets of materials of the control series resembled those of the normal individuals and showed no abnormality in the chromosomes. In both, the spermatogonial chromosome number has been found to be 23 and at meiotic prophase there are 12 elements, 11 of them autosomal bivalents and the remaining one the unpaired sex chromosome. At metaphase I the bivalents undergo much condensation and the univalent sex chromosome lies at any place near the equatorial region. First division anaphase is reductional.

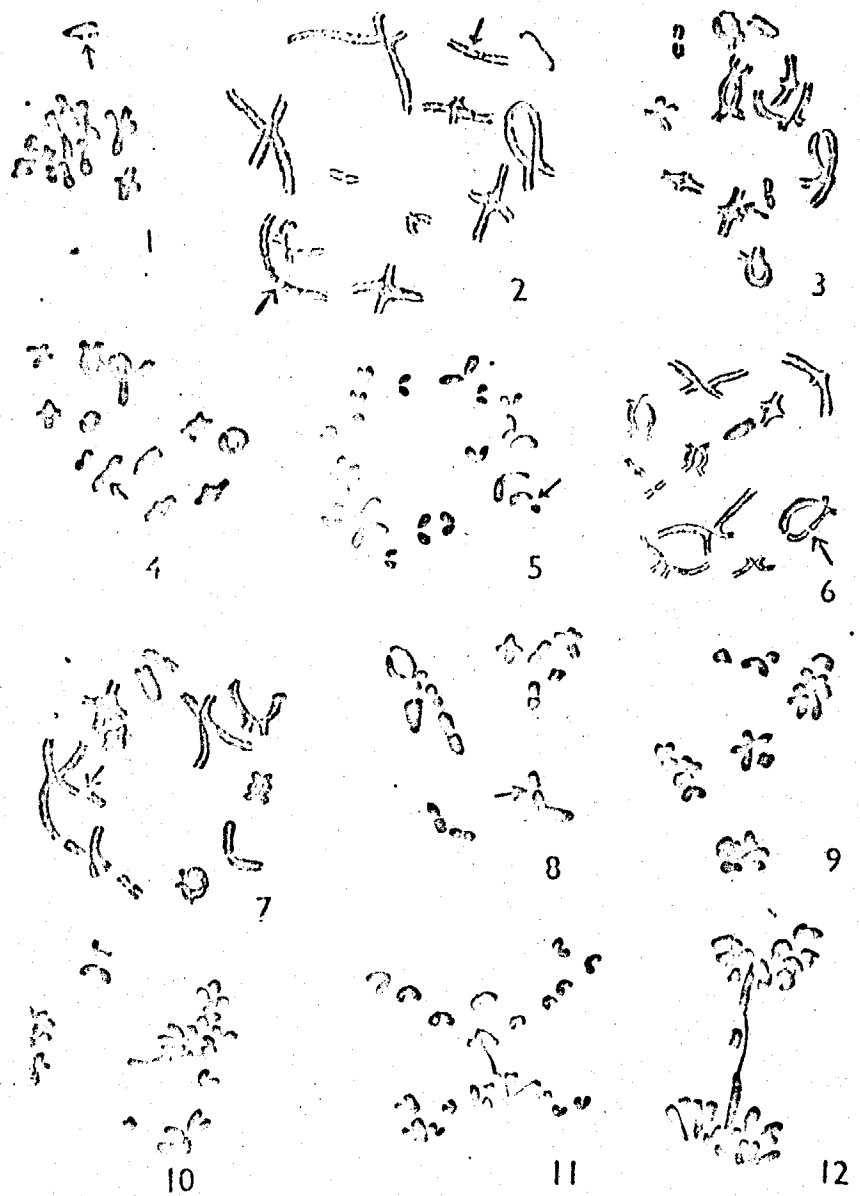
For quantitative study, about one hundred or more meiotic cells of each of dividing stage were examined but no cell with chromosomal aberrations like those of treated ones (*vide infra*) was encountered. However, some abnormalities like clumping of chromosomes, sticky anaphase bridge or polyploid cells were encountered in very rare instances.

Treated Series:

Sodium hydroxide solution of pH 9 in Spathosternum prasiniferum: Specimens injected with this solution and testes fixed at 4 hours after injection had very negligible number of cells with chromosome abnormalities. In 100 first spermatocyte late prophase cells not a single cell was found to contain chromosomal aberration of any kind. In 145 metaphase I, only one cell contained a chromatid type break in the X chromosome (fig. 1). Among the limited number of cells available for study, second division metaphase chromosomes were not affected at this hour of fixation. The tissues fixed after an interval of 24 hours of the injection showed marked changes in the spermatocyte chromosomes. Chromatid type breaks were encountered at the different stages of division (figs. 2-5; table 2) while the chromosome type breaks (figs. 6-8) appeared to be more limited in occurrence. First spermatocyte prometaphase stage was found commonly with damages in the spindle structure due to which at metaphase I the bivalents were lying in groups. Since the present study was made from squash preparation,

(Figs. 1-12 are of *S. prasiniferum* treated with sodium hydroxide solution of pH 9. ca. 1,600).

Fig. 1. Metaphase I with chromatid type break in the X-chromosome. Fig. 2. Diplotene with 2 bivalents showing chromatid and chromosome type break. Fig. 3. Diakinesis with a chromatid type break in a bivalent. Fig. 4. Metaphase I with a chromatid type break in a bivalent. Fig. 5. Polyploid metaphase II with disorganised spindle and a break in a chromosome. Fig. 6. Diplotene with a chromosome type break in a bivalent. Fig. 7. Diakinesis with a chromosome type break in a bivalent. Fig. 8. Metaphase I with disorganised spindle, bivalents arranged in group, and one of them with a chromosome type break. Fig. 9. Anaphase I with asynchronous division of chromosomes. Fig. 10. Anaphase I with unequal distribution of chromosomes. Fig. 11. Anaphase I with a sticky bridge. Fig. 12. Anaphase I with a bridge and a fragment.



no quantitative data of this type of abnormality was taken as such an abnormality might have also occurred due to the mechanical pressure. However, with the same technique in the control specimens the occurrence was extremely rare.

In the treated specimens the spindle was seen to be affected both at metaphase I and at later stages of division. Various types of abnormalities such as the asynchronous separation of the chromosomes (figs. 9-10), distribution of chromosomes in small groups (fig. 8), extreme stickiness or clumped condition, and sticky bridges (fig. 11) were observed. Some second division metaphase cells were found to be of polyploid constitution which could have originated due to the destruction of the first anaphase spindle (fig. 5). In some cases the chromosome bridge at first division anaphase was formed by structural rearrangement (fig. 12). Second division metaphase and anaphase chromosomes as well as the spindle were also affected with the alterations in pH, resulting in breakage, maldistribution, stickiness and clumping of chromosomes.

Table 2

Frequency of distribution of different types of abnormalities at different stages of meiosis in Spathosternum prasiniferum *injected with NaOH solution of pH 9 and fixed 24 hours after treatment*

	Stage of division and number of cells examined						
	Diplo. (68)	Diak. (74)	Meta. I (246)	Ana. I (182)	Meta. II (176)	Ana. II (70)	Total (816)
Chromatid break	2	2	4	—	4	—	12
Chromosome break	4	1	1	—	—	—	6
Multipolar spindle	—	—	4	6	6	—	16
Polyploid cell	—	1	4	—	2	—	7
Chromosome bridge	—	—	—	4	—	—	4
Lagging and maldistrib.	—	—	—	6	—	4	10
Total	6	4	13	16	12	4	55
Percentage	8·8%	5.4%	5·5%	8·8%	6·8%	5·7%	6·7%

An analysis of the data (table 2) indicates that the percentage of different types of abnormal cells was quite appreciable and all of them contributed to the chromosomal anomalies. The frequency of chromatid and chromosome type breaks was 18 out of 564 cells examined (3·2%).

Hydrochloric acid solution of pH 3-4 in S. prasiniferum: No individual could survive beyond 4 hours after the injection of the solution of pH 3. The effects of

pH 3 and 4 were not qualitatively different. The effect of the acidic pH solutions on the chromosomes as well as on the spindle was similar to those produced by alkaline pH solution. The changes involved were chromosome type and chromatid type breaks, maldistribution of anaphase chromosomes, general stickiness and clumping of the chromosomes, production of polyploid cells, disorganization of the spindle, etc. It appeared that polyploid cells due to fusion of cell boundaries were more prevalent than in other series.

No quantitative data has been recorded from the tissues treated with solution of pH 3 since the specimens could not survive for longer period after treatment. Frequency of distribution of abnormalities in the specimens treated with pH 4 has been recorded in table 3.

Table 3

Frequency of distribution of different types of abnormalities at different stages of meiosis in S. prasiniferum *injected with HCl of pH 4 and fixed at 18 hours after treatment*

	Stages of division and the number of cells examined						
	Diplo. (40)	Diak. (83)	Meta. I (210)	Ana. I (222)	Meta. II (146)	Ana. II (17)	Total (718)
Chromatid break	1	2	1	—	1	—	5
Chromosome break	—	3	—	—	—	—	3
Multipolar spindle	—	—	—	15	—	—	15
Polyploid cell	—	—	5	5	1	—	11
Chromosome bridges			—	4	—	—	4
Lagging and maldistrib.	—		—	2	—	—	2
Total	1	5	6	26	2	—	40
Percentage	2·5%	6%	2·8%	11·8%	1·4%	—	5·5%

A comparison of the percentage values of the different types of abnormalities (tables 2 and 3) showed that the effect of the solution of pH 9 was relatively more than that of pH 4. However, when the percentages of total abnormal cells were compared the difference between the two was negligible.

Acetic acid solution—pH 4 in O. velox: The types of abnormalities observed qualitatively here were more or less like those of *S. prasiniferum* and they did not vary much in the materials fixed at different intervals. The most frequent type of abnormalities was break or gap (figs. 13-15) encountered generally at the late prophase stage of first spermatocyte division. Sometimes instead of having a clear break in a chromosome a constriction was found at particular regions

(fig. 16). Further evidence of breakage was seen from the occurrence of fragmented element in some nuclei (figs. 14, 17). However, the presence of the extra element was not only due to the fragments but also due to the failure of bivalent formation by some chromosomes (fig. 17). A differential despiralization effect in some chromosomes was also encountered (fig. 18). In this effect, sometimes the end of the chromosome appeared to be stretched out and relatively understained. The most common effect observed at the late prophase or metaphase I was the stickiness of the chromosomes. In a number of cases interbivalent connection in the form of thin chromatin threads between the ends of bivalents was observed (fig. 19). The bivalents also were clumped in groups in some cases (fig. 20). Other types of abnormalities were polyploid cells, anaphase bridges, etc. (figs. 21-22).

A quantitative study of the frequency of some types of abnormalities in the materials fixed at 24, 48 and 72 hours has been made in table 4.

Table 4

Frequency of distribution of some types of chromosome aberrations in the first meiotic division of Oxya velox *treated with acetic acid solution*

Stage	Hour of fix.	No. of cells	Types of abnormalities					
			Chroma. break	Chr. break	Constric.	Fragment	Total	Percentage
Diplotene	24	193	2	16	—	3	21	10·8%
	48	30	4	1	1	—	6	20·0%
	72	44	—	3	—	—	3	6·8%
Diakinesis	24	414	7	8	2	1	18	4·3%
	48	173	1	1	—	—	2	1·1%
	72	240	3	4	—	1	8	3·3%
Metaphase	24	170	2	3	1	—	6	3·5%
	48	54	—	—	—	—	0	—
	72	107	1	1	—	1	3	2·7%
Total		1425	20	37	4	6	67	

An analysis of the data roughly indicates that at the diplotene stage the frequency drops down with the enhancement of the fixation period after reaching a peak period at 48 hours. However, this was not evident at diakinesis and metaphase I. The aberration frequency was higher at the diplotene stage. The different types of abnormalities observed show that a good number of cells suffered chromosomal damages as a result of acetic acid solution treatment. Four cells at

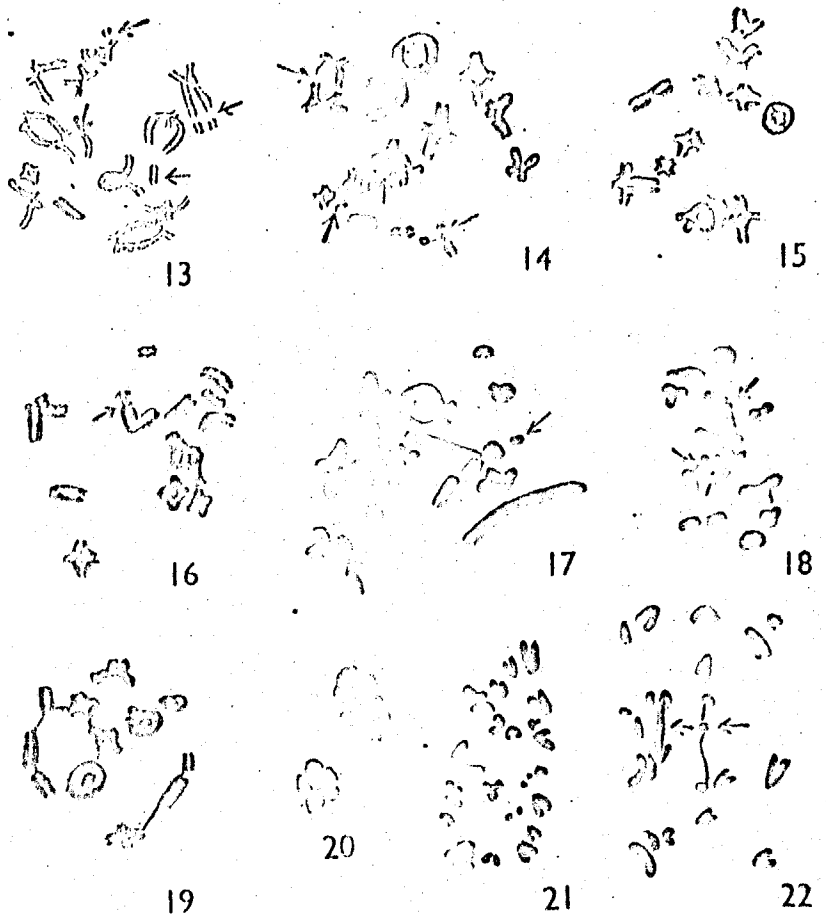


Fig. 13-22 are of *Oxya velox* treated with acetic acid solution of pH 4. × ca. 1,600).

Fig. 13. Diplotene with chromosome type breaks and fragments. Fig. 14. Diakinesis with chromosome type breaks, fragments and satellite like structure. Fig. 15. Diakinesis with a chromatid type break. Fig. 16. Diakinesis bivalent with a constriction. Fig. 17. Metaphase I bivalent with chromatid type break and some univalents and fragments. Fig. 18. Metaphase I bivalent with a chromosome type break, another bivalent with despiralised condition and some extra fragments. Fig. 19. Diakinesis with chain of inter-bivalent connections. Fig. 20. Metaphase I with extreme clumping effect and bivalents are clumped in groups. Fig. 21. A polyploid metaphase II. Fig. 22. Anaphase I with bridge and fragments.

diakinesis stage and 1 cell of metaphase II (out of 140 cells) were polyploid, 3 cells at anaphase I (out of 144) and 2 cells at anaphase II (out of 144) had chromosome bridges. Therefore, out of a total 1,853 cells 77 or 4·1% cells were with some sort of chromosome aberrations, and thus the total effect (4·1%) was little less than that of HCl treatment (5.5%).

This was perhaps due to the fact that the acetic acid treatment did not

produce a significant effect on the spindle structure, and hence cells with multipolar spindle and polyploid cells were rare or absent in comparison with HCl-treatment effects.

Discussion

In the present study although the adult specimens were injected with known strengths of acidic and alkaline pH solutions yet the induced change in the pH of the spermatocyte cells or at the testes itself has not been determined. It is, therefore, difficult to state the extent to which the cytological aberrations are due to the change in pH of the cell. Thus, for the sake of accuracy the changes brought about by the pH of the solution should for the present be taken as the changes due to pH shocks until it is established that the injected solutions actually changed the pH of the cells to the level of the solution injected.

Since the control specimens injected with only 0·67% saline solution or distilled water and fixed at different intervals of time did not show any cellular changes comparable to those of pH treated ones, possibly the changes observed in the treated series are due to the pH shocks for mixing the chemicals like sodium hydroxide, hydrochloric acid and acetic acid for pH adjustments and not for the injection of the saline solution or the distilled water. The effect of pH shocks upon the spermatocyte cells appears to be quicker since specimens fixed 4 hours after treatment showed qualitatively more or less the same type of chromosomal and spindle abnormalities as those after 18 or 24 hours. However, the frequency of these abnormal types was very low in the materials fixed at 4 hours.

The different kinds of abnormalities observed at different pH may broadly be grouped into two categories namely, (*i*) effects on the spindle and (*ii*) effects on the chromosomes. Qualitatively, the types of effects observed in testes cells due to the pH alterations are similar to those produced by ionizing radiations or radiomimetic chemicals.

If the effect on the spermatocyte cells produced by hydrochloric acid solution with pH adjusted between 3 and 4 (Manna and Ray, 1964) is compared with that of 0·04 M acetic acid solution having the same pH of 3-4, there appears to be some difference with regard to the spindle abnormalities. Hydrochloric acid solution produced relatively more spindle abnormalities while the spindle abnormalities in acetic acid treated cells were negligible. In the former, 19 out of 222 first division anaphase cells were with multipolar spindle, lagging chromosomes, etc. while in the latter such abnormalities were not observed among 144 anaphase cells. Data for some other type of spindle abnormality such as spindle disorganization has not been scored for reasons stated before. But the nature of effects on the chromosome is more or less same between the two types of treatments.

Apparently, different chemical solutions of pH between 3-4 and 9 affect the chromosome of grasshoppers in producing similar kinds of effect. Also the response is same even in species belonging to different genera as *Spathosternum prasiniferum* and *Oxya velox* in the present study.

In the quantitative data, the chromosome aberrations could be observed more in the diplotene stage than in any other stage of spermatocyte division. This may either be due to the fact that this stage is most vulnerable or else breaks are produced in equal quantity even at later stages though not detectable due to technical disadvantages. For example, at diakinesis or metaphase the spiralized and over-condensed state of chromosomes makes the detection difficult in many cases. Another reason for obtaining the low frequency could be that the affected cells were fixed before reaching diakinesis or metaphase stage if there was no inhibitory effect. The frequency of abnormal cells at stages later than diplotene was not higher when the fixation hour was extended to 72 hours. Thus it seems more likely that the diplotene stage is relatively vulnerable and perhaps the effect on the chromosome was produced at still earlier stage.

The sodium hydroxide, hydrochloric acid and acetic acid solutions produced chromosome as well as chromatid type breaks. The frequency was found to be higher in most of the cases at the diplotene stage. In the series with acetic acid treatment, the minimum hour of fixation in the present study was 24 hours while in the other two chemicals it was 4 hours after treatment. This indicates that the cells with chromosome type or chromatid type break are likely to have been affected by the chemical at some earlier stage of division. In that case, the occurrence of chromosome and chromatid type breaks may be taken to imply that breaks were produced before and after the chromosome reproduction. Since the chromosome type breaks induced by chemical agents are not likely of the 'isochromatid' type that are produced by ionizing radiations, it is presumed that the bivalent showing the chromosome type break originated before the chromosome reproduction while the chromatid type break in the chromosome was after its reproduction.

The different types of aberration produced by the treatment with acetic acid solution are somewhat comparable to the effect produced by the ionizing radiations or radiomimetic chemicals. However, the effect seems to be more intense in case of ionizing radiations or of some radiomimetic chemicals. With radiations, in most cases the frequency of aberrations is proportional to the dosage used. However, such results could not be verified because of some obvious limitations. The basic difference between the chromosome aberrations induced by the ionizing radiations and those produced by the chemical agents is that though in both the cases chemically active radicals are produced which indirectly bring about chromosome breakages yet the energy behind the active radicals produced by the ionizing radiations is far greater than those produced by the ionizations of radiomimetic chemicals. Further, on the basis of the dissociation constants of hydrochloric acid and acetic acid the latter is no doubt the weaker acid. As such it was desired to note whether the latter chemical is weaker in action as compared to HCl in inducing chromosome aberrations. In the grand total of HCl treated materials, 5·5%, and in the acetic acid treated material 4·1% cells were abnormal. This comparison should be regarded as a gross one due to various reasons. Apparently there

is no difference in the quality and quantity of the effect between the two kinds of treatments. This is not contrary to expectation, since the two acid solutions are made upto the same pH. The 0·04 M acetic acid solution would dissociate incompletely to give pH 3-4 which was the same for the HCl solution. Although total hydrogen ions are higher in acetic acid, yet the number of active hydrogen ions in both the solutions having the same pH, would be the same. Thus the effect is also expected to be the same. This is in agreement with the present result. Since higher concentration, of either acetic acid or HCl, produce detrimental effect in the tissues, the effect of higher concentrations could not be studied.

Chromosome breaks through chemicals are possibly effected by breakage of hydrogen bond of DNA during the chromosome reduplication. But this assumption is irreconcilable with the fact that chromatid breaks originate after chromosome reduplication. It may be suggested that the change in the ionic activity around the nucleus would lead to the exchange of ions between the nucleus and its surrounding which may result in various chromosomal and spindle abnormalities as manifested in these experiments. It implies that the action of acetic acid is different from the effect of HCl solution. Though both may produce similar type of chromosome aberrations, the former is less effective in causing damage to the spindle structure. The spindle structure is known to be mainly formed of fibrous protein. At present it is not known if the fibrous protein of the spindle is comparable to that present in chromosome structure.

Summary

Adult male grasshoppers, *Spathosternum prasiniferum*, were injected abdominally with 0·02 ml. NaOH solution of pH 9 and HCl solutions of pH 3 and 4 and fixed at intervals of 4, 18, 24 hours. Specimens injected with 0·67% saline solution served as the control. Similarly specimens of another species, *Oxya velox*, were injected with 0·04 M acetic acid solution of pH 3-4 and fixed at 24, 48, 72 hours. Specimens injected with distilled water served as the control.

Survival percentage was low in the specimens treated with solutions of different pH and it was still lower in hydrochloric acid solutions. Specimens treated with acetic acid solution could survive longer than those treated with sodium hydroxide or hydrochloric acid solution.

The nature of damages caused in the spermatocyte cells due to the treatment with solutions of different pH have been studied both qualitatively and quantitatively. All types of treatments produced chromatid and chromosome type breaks, fragments, constrictions, chromosome bridges at anaphase, interbivalent connections, polyploid cells, multipolar spindle, etc. The frequency data for some of the abnormalities have been scored and no appreciable difference in the frequency was observed between the different types of treatment. The frequency of abnormalities associated with spindle structure was found to be less in acetic acid-treated materials. Diplotene stage was found to be more susceptible to damages in the chromosomes.

The probable mechanism in the production of the various kinds of abnormalities by the solutions of different pH has been discussed. Since in the control specimens meiotic abnormalities similar to those in treated ones were absent, it has been assumed that the changes were caused due to the quantitative alterations of the active ions.

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ACIDIFICATION OF LYE PEELED TOMATOES AND SODIUM LEVEL OF THE CANNED TOMATOES

RICHARD F. MATTHEWS

Food Science Department, IFAS
University of Florida
Gainesville

ABSTRACT

Studies on the peeling of tomatoes (cultivar Homestead) were conducted to determine the effects of acid treatment following lye peeling on the quality of canned tomatoes and the effect of lye peeling on the sodium content of canned tomatoes. The treatments were: (1) lye peel, (2) lye peel-acid dip, (3) lye peel-acid added, (4) lye peel-acid tablet and (5) steam peeled.

Acid addition did not affect total solids or color. Sodium content and yield of the canned tomatoes were higher for all lye peel treatments than for the steam peeled tomatoes. Mean percent titratable acidity values for the five treatments were (1) 0.39, (2) 0.39, (3) 0.50, (4) 0.51 and (5) 0.42. pH values for treatments were (1) 4.41, (2) 4.41, (3) 4.24, (4) 4.14 and (5) 4.35. The pH of a #303 x 406 can of tomatoes was reduced 0.17 units by the addition of 0.46 g citric acid and 0.27 units by the addition of a citric acid-calcium sulfate-sodium chloride tablets containing 0.52 g effective citric acid.

INTRODUCTION

Lye peeling of tomatoes is used in many areas of the United States in the processing of tomatoes for juice, puree and whole-pack tomatoes. Considerable labor savings have been estimated for lye peeling over hand peeling (3). Lye peeling of Florida tomato cultivars has been evaluated for its effect on percent titratable acidity, pH, color and total solids of canned whole tomatoes (5). There were no negative effects of lye peeling on these factors, however, there was an indication of slightly higher pH values for the lye peeled tomatoes. As pH increases, microbiological spoilage problems increase and above pH 4.5 the safety of foods processed at 212°F may be questionable. Acidification of lye peeled tomatoes before heat processing was suggested as a safety measure in processing. This work investigated the acid dipping of tomatoes after lye peeling and the addition of acid to the peeled tomatoes in the can.

EXPERIMENTAL METHODS

Tomatoes, cultivar Homestead, were obtained from commercial growers. All tomatoes were hand harvested when mature-ripe and were processed at Gainesville. Two separate lots of tomatoes were obtained. Lot 1 was approaching an over-ripe stage and was considerably riper than Lot 2.

The tomatoes were dipped for 20 seconds into a 21 percent lye solution maintained at 195°F. The tomatoes (20-lb lots) were lightly agitated during the immersion period; they were removed and allowed to drain for 45 seconds and were then immersed in a rinse water bath for 30 seconds. All lye peeled tomatoes received the same treatment to this point.

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Treatments—

1. Lye Peel—The lye peeled, rinsed tomatoes were put through a peeling reel to remove loose skins. After being cored and having the remaining peel removed, the tomatoes were packed into #303 cans.

2. Acid Dip—The lye peeled, rinsed tomatoes were dipped 30 seconds in a two percent citric acid solution and allowed to drain. They were then put through a peeling reel to remove loose skins. The tomatoes were cored, remaining peel was removed, and the fruit was packed into #303 cans.

3. Lye Peel-Acid Added—The lye peeled, rinsed tomatoes were put through a peeling reel to remove loose skins. The tomatoes were cored, remaining peel was removed and the fruit was packed into #303 cans. Citric acid ($0.5gC_6H_8O_7 \cdot H_2O$) was added to the tomatoes in each can.

4. Lye Peel-Acid Tablet—These were treated in the same manner as the Acid Added treatment except a 50 grain tablet (Calcium-Acidulant Type A-Morton Salt Co.) was added to each #303 x 406 can. Tablet composition was:

Sodium Chloride (Culinox 999 salt)	60.9%
Anhydrous Citric Acid	19.5%
Calcium Sulfate ($CaSO_4 \cdot 2H_2O$)	15.0%
Sodium Bicarbonate	3.5%
Polyethylene Glycol	1.0%
Magnesium Stearate	0.1%

Part of the citric acid is neutralized by the sodium bicarbonate, leaving an effective citric acid content of 0.52 g.

5. Steam Peel—The fresh tomatoes were put through a tunnel which was open to the atmosphere and into which steam heated to 25 psi was released. Temperature measured in the tunnel was 190°F. The tomatoes were in the steam portion of the tunnel for 90 seconds and were then sprayed for 45 seconds with cold water. The tomatoes were cored, the remaining peel removed and the tomatoes packed into #303 cans.

All cans of tomatoes were finish filled with juice prepared from tomatoes of the respective treatment and exhausted in a water bath to 100°F. They were then sealed and processed in a 212°F still cook for 40 minutes and water cooled immediately to approximately 100°F.

All evaluations, except for percent yield, were made on the canned products within 30 to 60 days after canning. Determinations were made by blending the entire contents of a can of tomatoes and examining aliquots of the blend for pH, total titratable acidity, total solids and color. Three cans from each replicate treatment were analyzed.

Analytical Procedures—Procedures used for the determination of pH, titratable acidity, total solids, color and percent yield were the same as previously described (5). Ascorbic acid was analyzed as outlined in Section 20-8 of the Laboratory Manual for Food Canners and Processors (6).

Sodium determinations were made on an ammonium oxalate extract of blended tomatoes. Ten g of sample was transferred to a 100 ml volumetric flask and the flask brought to volume with 0.24 N ammonium oxalate solution. The flask was shaken at five min intervals for 30 min. The solution was filtered until 35-40 ml of filtrate was collected. The filtrate was analyzed for sodium by flame spectrophotometry.

The experiment was replicated three times, data was subjected to analysis of variance and significance was determined at the 0.05 level.

Results and Discussion

pH—The steam peeled tomatoes had a mean pH of 4.35, which was significantly lower than the pH of the lye peeled tomatoes and of the lye peeled-acid dipped tomatoes (Table 1). Acid dipping following lye peeling was no more effective in reducing the pH of the tomatoes than water rinses and sprays. The lye peeled-acid added tomatoes had a pH of 4.24. This was a reduction of 0.17 units per 0.46 g added citric acid.

Table 1. pH OF LYE AND STEAM PEELED TOMATOES.

TREATMENT	LOT I	LOT II	MEAN
1. Lye Peel	4.47	4.34	4.41 a
2. Lye Peel +Acid Dip	4.45	4.37	4.41 a
3. Lye Peel +Acid Added	4.29	4.18	4.24 c
4. Lye Peel +Acid Tablet	4.20	4.07	4.14 d
5. Steam Peel	4.41	4.29	4.35 b

Means followed by the same letter are not different at the 0.05 level of significance.

The citric acid-calcium sulfate-sodium chloride tablets added to the tomatoes reduced the pH to 4.14, a reduction of 0.27 pH units for 0.52 g of added effective citric acid (Fig. 1). This added pH reduction by calcium-citric acid tablets over citric acid has been attributed by one worker (4) to the common ion effect of the added calcium. The addition of salt has also been demonstrated to reduce the pH of acidified tomatoes (8).

Titratable Acidity—There was no difference between the titratable acidities of the steam peeled tomatoes (0.42 percent), the lye peeled tomatoes (0.39 percent), and the lye peeled-acid dip tomatoes (0.39 percent) (Table 2). The lye peeled-acid added tomatoes and the lye peeled-acid tablet added tomatoes were significantly higher in titratable acidity than the other treatments. Their mean values were 0.50 and 0.51 percent titratable acidity; this is an increase over the titratable acidity of the steam peeled tomatoes of 0.08 percent for the lye peel-acid added tomatoes and of 0.09 percent for the lye peel-acid tablet (Fig. 2).

Yield—The yield of lye peeled tomatoes was 76.3 percent and the yield for the steam peeled tomatoes was 67.9 percent. In previous work steam peeling produced the higher yields (5). The improved yields from lye peeling are no doubt attributable to the shorter lye peeling immersion time (20 seconds vs. 30 seconds for previous work) used in this experiment.

Table 2. PERCENT TITRATABLE ACIDITY OF LYE AND STEAM PEELED TOMATOES.

TREATMENT	LOT I	LOT II	MEAN
1. Lye Peel	.32	.45	.39 b
2. Lye Peel +Acid Dip	.33	.44	.39 b
3. Lye Peel +Acid Added	.43	.56	.50 a
4. Lye Peel +Acid Tablet	.42	.59	.51 a
5. Steam Peel	.34	.49	.42 b

Means followed by the same letter are not different at the 0.05 level of significance.

Peel Removal—Two procedures were used in determining the degree of peel removal from tomatoes. With Lot 1 the peel remaining on the tomatos after lye or steam peeling was removed by hand and weighed. The amount of peel remaining on lye peeled tomatoes was 0.28 lbs per 20 lbs of tomatoes treated. After steam peeling the peel remaining was 1.23 lbs per 20 lbs of tomatoes treated.

With Lot 2 each tomato was rated visually as to whether or not 95 percent of the peel had been removed. It was found that 81.3 percent of the lye peeled tomatoes had 95 percent peel removed, but only 8.7 percent of the steam peeled tomatoes had a 95 percent peel removed. Lye-

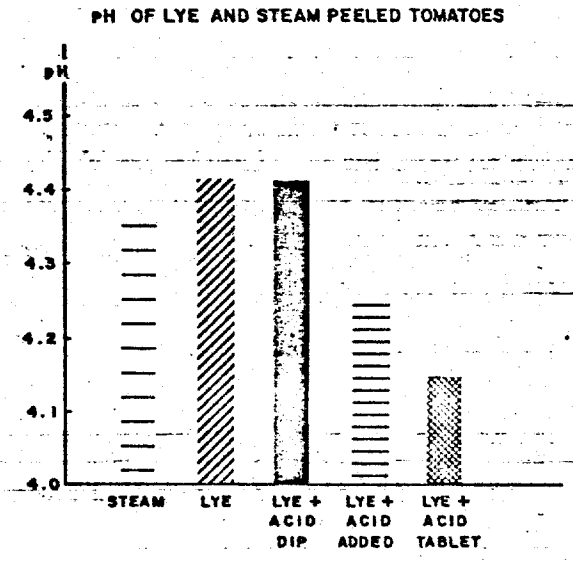

Figure. 1.—Effect of acidification on the pH of lye peeled tomatoes.

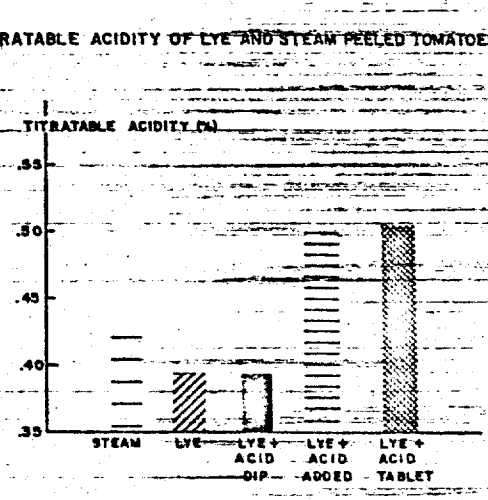

Figure 2.—Effects of acidification on percent titratable acidity of lye peeled tomatoes.

Table 3. SODIUM CONTENT OF PEELED TOMATOES (ppm).

TREATMENT	LOT I	LOT II	MEAN
1. Lye Peel	112	90	101 ab
2. Lye Peel +Acid Dip	103	77	90 b
3. Lye Peel +Acid Added	123	107	115 a
4. Steam Peel	60	38	49 c

Means followed by the same letter are not different at the 0.05 level of significance.

peeling is more efficient than steam peeling as measured by peel removal.

Sodium Content—The steam peeled tomatoes had significantly lower sodium content (49 ppm) than any lye peeled treatment (Table 3). The lye peeled tomatoes and the lye peeled tomatoes with acid added were highest in sodium content. The acid dipped tomatoes had 90 ppm sodium and the acid-dipping appeared to aid in the removal of sodium from the tomatoes. The increased sodium due to lye peeling would restrict the use of lye peeled tomatoes in low sodium diets.

Ascorbic Acid—Mean ascorbic acid content ranged from a high of 14.3 mg/100 g for steam peeled tomatoes to a low of 11.7 mg/100 g for lye peel with acid dip (Table 4). There was no difference in ascorbic acid content between the lye peeled and the steam peeled tomatoes. All treatments following lye peeling resulted in lower ascorbic acid levels.

Total Solids—Mean total solids for all treatments ranged from 5.8 to 6.0 percent and there was no effect of treatment on the total solids (Table 5).

Table 4. ASCORBIC ACID CONTENT OF LYE AND STEAM PEELED TOMATOES (mg/100 g).

TREATMENT	LOT I	LOT II	MEAN
1. Lye Peel	15.5	11.8	13.7 ab
2. Lye Peel +Acid Dip	12.4	11.0	11.7 c
3. Lye Peel +Acid Added	13.3	12.0	12.7 bc
4. Lye Peel +Acid Tablet	13.9	11.1	12.5 bc
5. Steam Peel	16.1	12.5	14.3 a

Means followed by the same letter are not different at the 0.05 level of significance.

increase of 0.06 units from lye peeling. The addition of tablets containing citric acid-calcium sulfate-sodium chloride reduced the pH more than citric acid alone.

The yield of tomatoes obtained by lye peeling was higher and the peel removal from tomatoes was more complete than that obtained by steam peeling. There was no significant treatment effect on the color or the total solids of the canned tomatoes.

ACKNOWLEDGEMENT

Appreciation is expressed to Dr. H. L. Breland for the sodium analyses and to Mr. Richard Johnson for product analyses.

Table 5. TOTAL SOLIDS OF LYE AND STEAM PEELED TOMATOES (percent).

TREATMENT	LOT I	LOT II	MEAN
1. Lye Peel	5.8	5.7	5.8 a
2. Lye Peel +Acid Dip	5.8	5.8	5.8 a
3. Lye Peel +Acid Added	5.9	5.9	5.9 a
4. Lye Peel +Acid Tablet	5.9	6.0	6.0 a
5. Steam Peel	5.9	5.8	5.9 a

Means followed by the same letter are not different at the 0.05 level of significance.

Color—Mean color, as measured by the Gardner a/b ratio, ranged from 1.24 to 1.30 and there was no treatment effect on the color of the canned tomatoes (Table 6).

CONCLUSION

Lye peeling increased the pH and the sodium content of canned tomatoes to levels higher than obtained by steam peeling. Water rinses and sprays were as effective in reducing the pH of lye peeled tomatoes as dipping in a two percent citric solution followed by water rinsing.

The addition of 0.46 g of citric acid to a #303 x 406 can of lye peeled tomatoes reduced the pH 0.17 units and more than offset the pH

Table 6. COLOR OF LYE AND STEAM PEELED TOMATOES (Gardner - a/b ratio).

TREATMENT	LOT I	LOT II	MEAN
1. Lye Peel	1.27	1.20	1.24 a
2. Lye Peel +Acid Dip	1.42	1.12	1.27 a
3. Lye Peel +Acid Added	1.41	1.12	1.27 a
4. Lye Peel +Acid Tablet	1.35	1.15	1.25 a
5. Steam Peel	1.44	1.16	1.30 a

Means followed by the same letter are not different at the 0.05 level of significance.

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LYE PEELING OF FLORIDA TOMATOES — EFFECTS OF TIME, TEMPERATURE AND CONCENTRATION

RICHARD F. MATTHEWS

Food Science Department, IFAS
University of Florida
Gainesville

HERBERT H. BRYAN

Sub-Tropical Experiment Station
Homestead

ABSTRACT

Lye peeling studies on the tomato cultivar Homestead 24 and breeding line 2086 were conducted to determine the effects of time, temperature, and lye concentration on canned tomato yield and quality. Two periods of time (30 and 45 seconds), two temperatures (195 and 210 F), and two lye concentrations (17 and 21 percent) were evaluated for each variety. An additional time period (60 seconds) was evaluated with Homestead 24. Effects on product yield, total solids, pH, total acid and color were measured. With both varieties yield decreased with increasing time or with increasing temperature. The major portion of the peel was removed by all treatments. Time, temperature, or lye concentration had no effect on total solids, pH, total acidity or color.

INTRODUCTION

The peeling and coring of tomatoes for processing is a labor-intensive operation in the canning of Florida tomatoes. In some tomato processing areas of the country lye peeling has replaced steam peeling and considerable labor savings have been estimated (5).

Tomatoes have been lye peeled either before or after coring and the use of wetting agents has increased the peeling efficiency of lye solutions (7).

This study was undertaken to determine the effect of major peeling variables on canned tomato yield and quality and to determine the adaptibility of some Florida tomato varieties to lye peeling. The Homestead variety was selected since it represents the main tomato variety now available to Florida canners. Breeding line 2086 was evaluated as it is one of the new lines for mechanical harvest.

EXPERIMENTAL METHODS

Tomatoes, cultivar Homestead 24, were obtained from the Gulf Coast Experiment Station, Bradenton and experimental line 2086-S1-D1-BGBK-CAVStw from the South Florida Field Laboratory, Immokalee. All tomatoes were mature-ripe, hand harvested and processed at Gainesville within 3 days of harvest.

A 3 x 2 x 2 factorial experiment with two replications was conducted with cultivar Homestead 24. Three lye immersion time periods (30, 45 and 60 seconds), two lye concentrations (17 and 21 percent), and two temperatures (195 and 210 F) were investigated. A 2 x 2 x 2 factorial experiment with two replications was conducted with breeding line 2086. Two time periods (30 and 45 seconds), two lye concentrations (17 and 21 percent), and two temperatures (195 and 210 F) were investigated.

The experiments were analyzed separately by analysis of variance. Varieties were compared for identical conditions by t-test procedure. Significance was determined at the 0.05 level.

The lye solution was maintained in an 80-gallon steam-jacketed kettle and a wetting agent (0.3 percent Faspell, Wyandotte Corp.) was used for all treatments. Tomatoes (7-lb. lots) were placed in a wire basket and immersed in the lye solution. Light agitation was maintained during the immersion period. The tomatoes were removed and allowed to drain for exactly 45 seconds, immersed in rinse water for 30 seconds, and then conveyed through water sprays (top and bottom) for 45 seconds.

The lye peeled tomatoes were cored, and the remaining peel and defects were removed. Percent yield was calculated on this material.

For comparison with lye peeled tomatoes, lots of tomatoes were steamed for 60 seconds in a steam-saturated conveyor tunnel and then sprayed with cold water for 30 seconds.

The tomatoes were hand packed into 303 cans (10.5-11.0 oz. per can), filled with juice prepared from tomatoes of the same treatment, and exhausted in a water bath to 100°F. They were then sealed and processed in a 212°F still cook for 30 minutes and water cooled immediately to approximately 100°F.

All evaluations, except for percent yield, were made on the canned products within 60 to 90 days after canning. Determinations were made by blending the entire contents of a can of tomatoes and examining aliquots of the blend for pH, total titratable acidity, total solids and color. Two cans from each replicate treatment were analyzed.

1. pH—measured on an aliquot of the undiluted blended tomato.
2. Titratable acidity—based on a weighed aliquot and expressed as percent (w/w) calculated as citric acid (4).
3. Total solids—based on a refractive index determination as outlined in Tomato Products section of reference 8.
4. Color—Determined by Hunter Color and Color Difference Meter on blended tomato sample which had been deaerated under vacuum. Standard values were Rd=7.0, a=33.9 and b=16.2. Color was reported as the ratio of a/b values, and the higher the ratio the redder the tomato.
5. Percent yield—weight of peeled and cored tomatoes divided by the weight of original tomato sample.

Results

Peel Removal—All lye treatments resulted in removal of the major portion of the peel. The peel had a tendency to stick around the stem end and, to a much lesser degree, at the blossom end. This was more pronounced with yellow shoulder tomatoes. The treatments of 60 seconds and 210 degrees with Homestead 24 were much too harsh and in some instances penetrated the locular cavity. The mildest treatment (30 seconds, 195 F, 17 percent lye) satisfactorily removed the peel from the main body of the tomato.

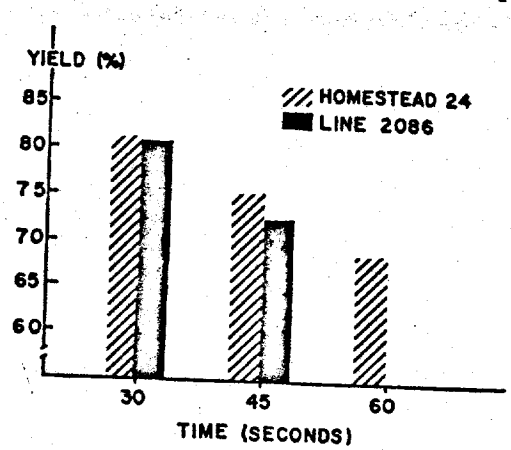

Fig. 1.—The effect of lye immersion peeling time on the yield of tomatoes for canning.

Yield—The effect of time on yield (Table 1) was significant for both Homestead 24 and line 2086. Yield decreased as the period of time increased (Figure 1) and the decrease was linear for Homestead 24. Highest average yield for both varieties was at 30 seconds (81.0—Homestead 24 and 80.6—2086).

Temperature effect was significant for both varieties, with a decrease in yield with increased temperature. Yields at the two lye concentrations were not different. Variety response for yield was compared at the 30 and 45 second time levels and was not different. Yield of steam peeled tomatoes (83.9 percent—Homestead and 84.6—2086) was higher than the yield for lye peeled tomatoes.

Percent Titratable Acidity—The average percent acid was 0.31 for both Homestead 24 and

Table 1. Percent yield of lye peeled and steam peeled tomatoes.[1]

Variety	Lye peeled: Time (seconds) 30	45	60	Lye percent 17	21	Temperature (°F) 195	210	Steam Peeled
Homestead 24	81.0a	75.1b	68.6c	74.8a	75.1a	77.0a	72.8b	83.9
2086	80.6a	72.5b		76.5a	76.6a	78.8a	74.4b	84.6

[1] Means, within horizontal groups, followed by the same letter are not different at the 0.05 level of significance.

Table 2. Percent titratable acidity of lye peeled and steam peeled tomatoes.[1]

Variety	Lye peeled							Steam Peeled
	Time (seconds)			Lye percent		Temperature (°F)		
	30	45	60	17	21	195	210	
Homestead 24	.31a	.31a	.32a	.31a	.32a	.31a	.32a	.33
2086	.32a	.31a		.32a	.31a	.31a	.32a	.34

[1] Means, within horizontal groups, followed by the same letter are not different at the 0.05 level of significance.

line 2086 (Table 2). There was no significant main effect or interaction for time, temperature, or lye concentration for Homestead 24 or 2086. The average percent acid for steam peeled Homestead tomatoes was 0.33 and for 2086 was 0.34; these values were not different from the lye peeled tomatoes.

pH—Mean treatment values for pH ranged from 4.52 to 4.59 (Table 3). There was no main effect on pH with either Homestead 24 or line 2086. The average pH values of steam peeled (4.52) and lye peeled (4.58) Homestead tomatoes were not different. However, there was a difference (10 percent level) between the average pH value of steam peeled (4.44) and lye peeled (4.55) line 2086 tomatoes.

There was a Time x Temperature interaction for pH with Homestead tomatoes.

At the low (195°) temperature, the average pH increased as the period of time increased. At the high (210°) temperature, the average pH decreased as the time increased.

Color—The range of means for color ratio was 1.64 to 1.81 (Table 4). There were no main effects on color for either Homestead or variety 2086, nor was there any difference between the average color of steam peeled tomatoes (1.73 for Homestead, 1.73 for 2086) and lye peeled tomatoes (1.74 for Homestead, 1.68 for 2086).

There was a Time x Lye interaction for color in variety 2086. At the low (17 percent) lye concentration, the average color increased as the period of time increased. At the high (21 percent) lye concentration, the average color decreased as the period time increased.

Total Solids—Mean total solids for the variety Homestead 24 was 5.72 percent and for line 2086 was 5.62 percent (Table 5). There were no main effects of time, temperature, or lye concentration on total solids, nor was there any difference between the average total solids of steam peeled tomatoes (5.7) and total solids of lye peeled tomatoes.

There was a Time x Lye interaction for total solids with variety 2086. At the low (17 percent) lye concentration, the average solids increased as the period of time increased. At the high (21

Table 3. pH of lye peeled and steam peeled tomatoes.[1]

Variety	Lye peeled							Steam Peeled
	Time (seconds)			Lye percent		Temperature (°F)		
	30	45	60	17	21	195	210	
Homestead 24	4.58a	4.58a	4.57a	4.58a	4.58a	4.59a	4.56a	4.52
2086	4.52a	4.59a		4.54a	4.56a	4.54a	4.57a	4.44

[1] Means, within horizontal groups, followed by the same letter are not different at the 0.05 level of significance.

Table 4. Color (a/b ratio) of lye peeled and steam peeled tomatoes.[1]

Variety	Lye peeled: Time (seconds) 30	45	60	Lye percent 17	21	Temperature (°F) 195	210	Steam Peeled
Homestead 24	1.72a	1.81a	1.67a	1.80a	1.68a	1.69a	1.78a	1.73
2086	1.69a	1.66a		1.68a	1.67a	1.71a	1.64a	1.73

[1] Means, within horizontal groups, followed by the same letter are not different at the 0.05 level of significance.

percent) lye concentration the solids decreased as time increased.

Discussion

The large effects of lye immersion time and temperature on yield, point out areas requiring close processing control. Since the major portion of peel was removed by all treatments, it is probable that treatment times shorter than 30 seconds would give satisfactory peeling and increased yields. Highest yields were obtained at 30 second immersion times and 195 F.

With the precautions that were taken to wash the lye solution from the peeled tomatoes there was no difference in pH or total acidity between steam peeled and lye peeled tomatoes. However, since the pH of the canned product (mean value 4.58) was so high acidification of lye peeled tomatoes should be evaluated.

Product quality of lye peeled tomatoes as measured by pH, total acidity, solids and color did not differ from the steam peeled tomatoes.

Acknowledgments

Appreciation is expressed to Dr. John A. Cornell, Statistics Department, IFAS for the analysis of data and Dr. J. W. Strobel, Gulf Coast Experiment Station for supplying tomatoes.

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Table 5. Percent total solids of lye peeled and steam peeled tomatoes.[1]

Variety	Lye peeled: Time (seconds) 30	45	60	Lye percent 17	21	Temperature (°F) 195	210	Steam Peeled
Homestead 24	5.71a	5.60a	5.77a	5.72a	5.72a	5.73a	5.71a	5.65
2086	5.65a	5.59a		5.65a	5.59a	5.59a	5.65a	5.70

[1] Means, within horizontal groups, followed by the same letter are not different at the 0.05 level of significance.

UNITED STATES PATENT OFFICE

2,529,405

STABILIZED COMPOSITION

Brack B. McHan, Quincy, Ill., assignor to Calcium Carbonate Company, Chicago, Ill., a corporation of Illinois

Application June 1, 1949, Serial No. 96,487

3 Claims. (Cl. 99—2)

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This invention relates to improvements in the stabilization of chemical compounds and is a continuation-in-part of my allowed co-pending application Ser. No. 661,247, filed April 11, 1946 (issued as Patent No. 2,479,583 on August 23, 1949), which in turn is a continuation-in-part of my previous application Ser. No. 478,938, filed March 12, 1943, which issued on July 2, 1946 as Patent No. 2,403,010. More particularly, it pertains, for example, to the stabilization of calcium compounds, such as limestone, to be used as an ingredient in food products, or the like, without fear that it will materially destroy the vitamin content or develop rancidity in the remaining ingredients, and this is a principal object of the invention.

Calcium carbonate, in the form of ground limestone, is a commonly used ingredient in feed mixtures. It is used in percentages varying from 1 to 5 per cent. Calcium carbonate is a necessary component of the diet. The addition of calcium carbonate to food is deemed necessary in order to maintain the correct balance or ratio of calcium and phosphorus and to insure the normal development of the skeletal structure as well as to maintain the necessary balance of calcium in the blood stream and the other organic portions of the normal animal structure.

To supply the necessary calcium requirement it is common practice of feeders of all classes of livestock to introduce into the mixed feed a necessary quantity of calcium. This is usually introduced in the form of pulverized limestone. It is also conventional practice to fortify feed products with various vitamin concentrates in order to insure a necessary amount of these very important ingredients being present at all times. Vitamin A may be added in the form of carotene, various fish oils, or alfalfa meal. Vitamin D is usually added through the medium of one of the fish liver oils, or by means of any other suitable vehicle. Most of the common forms of feed stuff, such as alfalfa hay or meal, corn meal (especially yellow corn meal), grains and grain products, carry varying percentages of various vitamins and oils. Vitamins are very readily destroyed by processes of oxidation. As an example, the vitamin A content of alfalfa meal will be reduced as much as 50 per cent in less than two months time due to oxidation of the vitamin A molecule.

The oils present in the natural feed stuffs also undergo processes of oxidation resulting in the development of varying degrees of rancidity. The rancid odor developed through oxidation of the

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oils impairs the attractiveness and palatability of the food, and greatly reduces its biological activity by the accompanying destruction of vitamins. The development of rancidity is accompanied by the development of substances known as peroxides; these peroxides in turn further oxidize the vitamins, oils, and natural enzymes of feed materials.

Generally, it is an object of the invention to overcome the foregoing difficulties and disadvantages by effectively lowering the oxidation-reduction potential of useful compounds, both inorganic as well as organic, the latter comprising, in particular, animal and vegetable products.

More specifically, it is an object of the invention to stabilize calcium carbonate by combining sodium hydroxide therewith over its reactive area and reacting the sodium hydroxide with the calcium carbonate to provide a composition having as its essential property an adsorption isotherm of such characteristic that oxygen adsorbed by the calcium carbonate is replaced and further adsorption of oxygen by the carbonate is prevented, to the end that the composition, when used, for example, as an ingredient of food, will not destroy vitamins nor initiate rancidity development.

Other objects of the invention will in part be obvious and will in part appear hereinafter.

The invention accordingly comprises the several steps and the relation of one or more of such steps with respect to each of the others, and the product possessing the features, properties, and the relation of constituents, which are exemplified in the following detailed disclosure, and the scope of the application of which will be indicated in the claims.

The sole figure is a graphic illustration of the effect of the various reagents herein, singly and in combination, on the peroxide value showing the peroxide development plotted against time in days and depicts the high oxidizing capacity of untreated calcium carbonate and the reducing effect of each of the reagents when used singly, and still further the great reduction in oxidizing value when used in combination.

The problem of rancidity development and destruction of vitamins in food products has long confronted the industry and solutions thereto have constantly been sought. No one familiar with the chemistry of calcium carbonate would attribute thereto a high oxidizing capacity. Indeed, a contrary view has been inevitably obtained. The molecule of calcium carbonate is an extremely stable one with the oxygen molecules

so tightly bound that they would not be transferred in any ordinary processes of oxidation. This statement is susceptible to both chemical and thermodynamic proof. Hence, this material has heretofore been eliminated as being among one of the chief causes that give rise to the problem.

The present invention, however, is based on my discovery that calcium carbonate is the primary cause of vitamin destruction and rancidity development in this connection. I have found that during the processes of grinding and attrition of the calcium carbonate, the surfaces of the particles presented unsaturated chemical bonds which picked up, at activated points, oxygen atoms from the air. Also, that the oxidizing action of the adsorbed oxygen on the surface of the carbonate particles could completely be nullified or replaced by some substance preferentially adsorbed over the oxygen, and that the chemical nature of this substance must have an adsorption isotherm that would effectively prevent the carbonate from adsorbing further oxygen on storage.

It was further observed that on many of the surfaces of the calcium carbonate particles the oxygen atom may be closely compacted and in a highly reactive state. When a particle of calcium carbonate, carrying its charge of adsorbed oxygen atoms, comes into contact with an oxidizable substance, these oxygen atoms are transferred and the substance oxidized. It has also been noted that very quickly after mixing an oxidizable substance with calcium carbonate, such, for example, as vitamin A, fish oils, or carotene, that 90 per cent plus will be oxidized. This, however, does not neutralize the oxidizing capacity of the calcium carbonate as these activated points on the crystal simply pick up more oxygen atoms from the air and again transfer them to oxidizable substances. This process goes on until the oxidizable substance has been completely saturated with oxygen.

As a result of these combined discoveries and observations the problem at hand has been solved by a process of subjecting the calcium carbonate to the action of a suitable stabilizing agent which, by adsorption, effectively will reduce the oxidation-reduction potential, either before, during or after grinding of the calcium carbonate. An actual process of treatment will now be described as set forth in the following examples:

Example 1

Four pounds (i. e. 0.2 per cent) of NaOH were intimately mixed with one ton of calcium carbonate during the process of grinding. This caused a drop in the oxidation-reduction potential of the calcium carbonate sufficiently low so as effectively to inhibit its normal oxidizing reaction on such substances as vitamins and oils for periods as long as nine months. The following table compares the development of rancidity in 2,000 A, 400 D fish oil by untreated limestone and limestone treated in accordance with this example. In this table, the redox potential is designated as E_H. It is significant to note the development of high peroxide values with untreated limestone as compared with the relatively low peroxide values developed with the limestone treated with 0.2 per cent NaOH.

TABLE 1

Time	Qcy No. 4 Reg. Control E_H=0.3033	Qcy No. 4 Reg. 0.2% NaOH E_H=0.3272
Day of Prep.		
1 day	2.18	0.0
2 days	14.9	0.80
3 days	17.1	1.30
4 days	16.8	1.50
5 days	12.2	2.17
6 days	12.6	2.80
7 days		2.20
8 days	10.85	2.46
10 days		
13 days	9.11	2.66
14 days		
16 days		2.60
17 days		
20 days	4.47	
21 days		
23 days		3.17
24 days		
27 days	4.11	
28 days		
31 days		2.02
34 days		
35 days		
36 days		2.70
37 days		
40 days		
46 days	1.75	
50 days		
54 days		
56 days		2.82
58 days		
61 days		
66 days	1.48	
73 days		
91 days		
92 days		
93 days		
95 days		
4 months	1.69	3.99
6 months	1.56	2.27
9 months	1.39	1.93
	1.53	1.76

It has been found that the NaOH is effective in treating limestone in accordance with this invention in percentages ranging from 0.1 to 0.5 per cent based on the weight of the limestone. The preferred range of concentration is from 0.1 to 0.3 per cent by weight of the limestone.

Example 2

Sodium thiosulfate, 0.5 per cent, was mixed with one ton of calcium carbonate during the process of grinding. This caused a drop in the oxidation-reduction potential of the calcium carbonate sufficiently low effectively to inhibit its oxidizing reaction on such substances as vitamins and oils. Vitamins and vitamin carrying products when mixed with calcium carbonates thus treated remained 70 per cent to 80 per cent stabile up to six months time, whereas in untreated calcium carbonate they would be completely destroyed in less than a week. Oils and food products containing oils or fats when mixed with the treated calcium carbonate retained their original fresh odor without any development of rancidity for over a year.

With respect to the relative weights of materials used, it has been found that sodium thiosulfate is desirably effective in percentages ranging from 0.1 per cent to 0.5 per cent, which is equivalent to two pounds and ten pounds of sodium thiosulfate per ton of limestone respectively. While this is a preferred range, desirable results have been secured from percentages varying from 0.01 per cent up to and in excess of 1.0 per cent.

The data presented in the two following tables will serve further to show the advantages to be obtained in treating limestone in accordance with my invention.

TABLE 2

Rancidity development in 1% A—D oil mixtures effect of sulfur, sodium thiosulfate and sodium hydroxide on peroxide values

Reagent	Peroxide Value After Number of Days Shown										
	1	2	3	5	10	15	20	30	40	50	60
Control	14.9	17.1	16.8	12.5	8.4	5.6	4.5	3.4	1.7	1.8	1.6
0.2% Sodium Thiosulfate	0.4	0.2	0.2	5.0	6.8	2.7	2.6	2.0	1.6	1.9	1.4
0.2% Sodium Hydroxide	0.8	1.3	1.8	2.3	2.7	2.6	3.1	2.2	2.8	2.8	2.8
0.2% Sodium Thiosulfate +0.2% Sodium Hydroxide	0.4	0.1	0.0	0.0	0.2	1.2	2.4	2.7	1.1	0.9	1.0
0.1% Sodium Thiosulfate+0.2% Sulfur+0.2% Sodium Hydroxide	0.0	0.0	0.0	0.0	0.2	0.6	0.2	----	----	----	----

TABLE 3

Oxidation—reduction values for various reagents

E_H = Oxidation Reduction Potential (Redox)

Reagents	E_H
Control	0.3933
0.2% Sodium Thiosulfate	0.3113
0.2% Sodium Hydroxide	0.3972
0.2% Sodium Thiosulfate+0.2% Sodium Hydroxide	0.2888
0.1% Sodium Thiosulfate+0.2% Sulfur+0.2% Sodium Hydroxide	0.3108

From all of the foregoing uses, and others, it appears that any chemical compound or reagent, used in accordance with the teachings of the present invention, and which possesses the property of a high degree of adsorption on limestone particles, that is, will have an adsorption isotherm of such characteristic that it would cause the replacement of adsorbed oxygen, and would effectively prevent any further adsorption of oxygen by the calcium carbonate particles, should act as an effective stabilizer.

The oxidation-reduction potential, or redox potential, in all cases was measured by means of a potentiometer and it should be noted that in order to secure the best results, a redox potential value of preferably around +0.35 should not be exceeded.

It should further be noted that the present invention contemplates adaption to other fields of industrial usage. The property of undergoing spontaneous oxidation when exposed to the atmosphere is by no means peculiar to edible fats, but is exhibited also by many other substances of biological and industrial importance. For example, cases outside of the field of the preservation of food are the aging or perishing of rubber, the formation of gum in gasoline, production of sludge in mineral, lubricating and transformer oils, and the oxidation and resinification of essential oils.

In addition to the foregoing, prevention of the development of rancidity and vitamin loss and vitamin-carrying substances are important in the pharmaceutical trade as, for instance, the inclusion of vitamins in tablets or capsules where various substances are used as carriers.

The invention can also be adapted in the field of paint and paint oils where many calcium carbonates when mixed with certain types of oils accelerate the oxidation thereof. In addition to calcium carbonate, other finely ground powders, such as Lithopone, White Lead, Titanox, China Clay, and Barytes, can thus be treated.

Since changes when carrying out the above process, and certain modifications in the composition which embody the invention may be made without departing from its scope, it is intended that all matter contained in the above description shall be interpreted as illustrative and not in a limiting sense.

It is also to be understood that the following claims are intended to cover all of the generic and specific features of the invention herein described, and all statements of the scope of the invention which, as a matter of language, might be said to fall therebetween.

Particularly, it is to be understood that in said claims, ingredients or compounds recited in the singular are intended to include compatible mixtures of such ingredients wherever the sense permits.

Having described my invention, what I claim as new and desire to secure by Letters Patent is:

1. A stabilized composition of matter comprising discrete particles of calcium carbonate, and sodium hydroxide as an oxidation-reduction potential reducing substance, said substance preferentially being adsorbed over oxygen reaction areas of said calcium carbonate.

2. A stabilized composition of matter comprising discrete particles of calcium carbonate and from 0.1 to 0.5 per cent of sodium hydroxide as an oxidation-reduction potential reducing substance, said substance being preferentially adsorbed over oxygen reaction areas of said calcium carbonate.

3. A stabilized composition of matter comprising discrete particles of calcium carbonate and from 0.1 to 0.3 per cent of sodium hydroxide as an oxidation-reduction potential reducing substance, said substance being preferentially adsorbed over oxygen reaction areas of said calcium carbonate.

BRACK B. McHAN.

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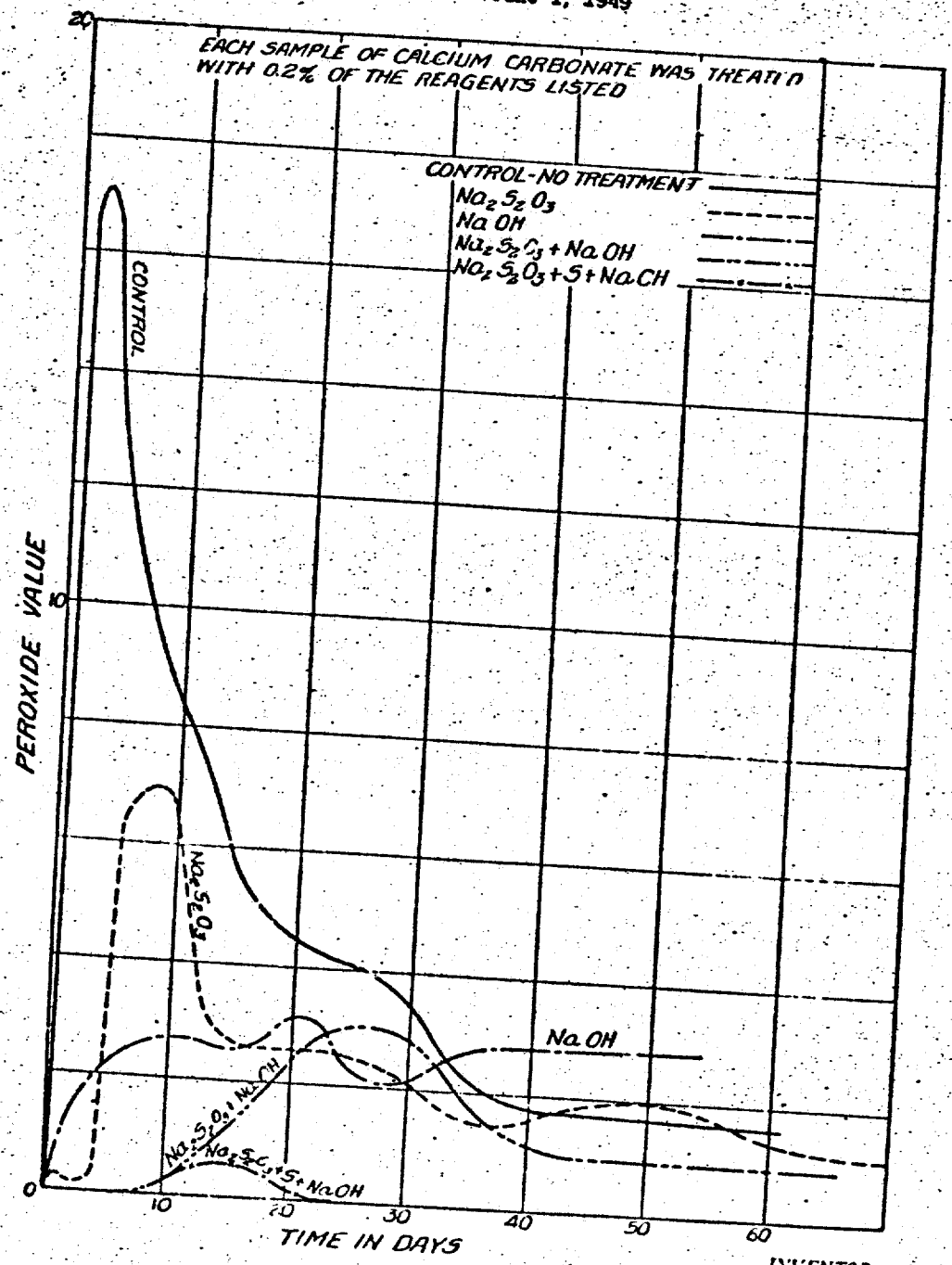
Nov. 7, 1950

B. B. McHAN

STABILIZED COMPOSITION

2,529,405

Filed June 1, 1949



INVENTOR.
Brack B McHan.

BY
Cromwell, Greist & Warden
Attys

EXPERIMENTAL ATTEMPTS TO MODIFY THE SEX RATIO

in Rabbits and Pigs

HUGH C. MCPHEE AND ORSON N. EATON
Bureau of Animal Industry, U. S. Department of Agriculture

THE control of the sex determination mechanism is of such universal interest that reports of success are almost certain to be received with uncritical enthusiasm by a large audience. The basic reason for this in the human family is mainly one of sentiment which expresses itself in the desire for a daughter or a son at a particular time. Although the livestock breeder is motivated less by sentiment and more by financial gain, it is probable that his eagerness for a successful method of controlling sex is even greater. Thus a large section of the population is always eager to grasp any new sex control theory. It is unfortunate that of many hundreds of these theories propounded to date, hardly any have any merit whatsoever and none yet has passed the crucial test of success in practice, in spite of lurid claims. Such procedures have only served to befuddle the public mind and to make it suspicious. In this way probably more harm than good in advancing the cause of science has been done by enthusiastic sex controllers.

Previous Reports

Publications on the subject appearing prior to 1933 were reviewed by Cole and Johanson[1] who concluded that all claims that the sex ratio in mammals could be controlled were premature.

Schröder[2] proposed a theory based on the idea that x and y bearing chromosomes would be attracted to opposite poles when an electric current passes through the solution containing the spermatozoa. When rabbit semen that collected at the cock was used, approximately 80 per cent females were obtained; and when semen from the cathode was used 70 per cent males were obtained. About 3,000 rabbits were obtained in this work over a period of six years. Schröder also studied the movements of semen under the microscope. She observed that some rabbit sperm moved toward the anode and some toward the cathode when current was passed through the solution. In semen of the cock, where all spermatozoa carry the *x* (or *z*) chromosome, she observed that all spermatozoa moved toward the anode. Schröder's results appear to be associated with the acid-alkaline balance, since the pH of extracts (physiologic solutions) from the anode varied from 6 to 7.5, while those from the cathode varied from 4 to 4.5. These are interesting findings and should be checked by other laboratories.

Warren[7] summarized the results of attempts made by the Applied Research Laboratories, Dayton, New Jersey, and which had been sponsored and previously reported, as results became available, in the New York *Daily News*. This work was based on the theory that the acid-alkaline balance of the vagina would have an effect on the spermatozoa, an acid condition resulting in a higher proportion of female offspring and an alkaline condition giving a preponderance of males. Rats were used and positive results were reported. These results were reviewed by Cole[3] and he concluded that the experiments should be repeated in one or more independent laboratories before they could be accepted as conclusive. Results have now been reported from three institutions and the results are conflicting. Roberts[5] used essentially the same technique as that described by Warren[7] and his results indicated that douching female rats with lactic acid was effective in increasing the proportion of males. Cole *et al*[1] conducted similar experiments with large numbers of

rats and with a limited number of rabbits and the results were entirely negative. These workers also inseminated a number of rabbits artificially, using semen that had the pH altered by the addition of lactic acid, KH_2PO_4 and $NaHCO_3$. This was done since it was felt that the douching procedure might not be affecting the pH of the semen. The resulting sex ratios in the three groups did not differ significantly from each other, or from an expected 50:50 ratio. Quisenberry and Chandiramani[4] also report a series of experiments similar to those described above in which both rats and rabbits were used. In their work with rats no significant departure from the control sex ratios was obtained. With rabbits a significant alteration of the sex ratio was obtained but the authors point out that the number of offspring was small and that the work was being continued.

Following the publication of these conflicting results the Bureau of Animal Industry of the U. S. Department of Agriculture was requested to undertake some investigations to determine whether the sex ratio can be altered significantly either by douching the female with acid and alkaline solutions or by changing the pH of semen used for artificial insemination.

Material and Methods

In order to test further the possibility of controlling sex by controlling the pH with such materials as lactic acid and sodium bicarbonate, two series of experiments were undertaken with rabbits and swine. The procedures in the two experiments are outlined below.

Rabbits were divided into several groups. Ten males were selected as sires. Each male was to be bred to nine females. These females were divided into three groups, one to be bred normally, one in which the females were to be acid-douched immediately before breeding, and one to be alkali-douched in like manner. The same females were always to be mated with the same male and were to receive one each of the three treatments for successive matings. The young were removed from the doe within a day or two after birth and killed by anesthesia. They were slit down the abdomen, the abdominal viscera pushed aside, and examination made of the gonads to determine sex. Testicles and ovaries are both in the abdominal cavity at this age, but can be easily distinguished by position, form and place and character of their attachment to the body wall. The doe was rebred under the next treatment from one day to a week following parturition. If she failed to produce a litter the same treatment was repeated until a litter was produced or until it was determined that the doe was not going to breed further. This made it necessary to replace some of the does after but one litter had been born. Some does died after producing one or two litters and were replaced by others. Several does produced from four to six litters, thus repeating one or more of the treatments. Three of the original males also had to be replaced because of failure to produce litters.

The acid solution used was three per cent lactic acid. Because of its irritating effect on the vagina, and because the females thus treated failed to conceive in the same proportion as the normally bred and the alkali-douched females, the acid solution was changed to a two per cent solution. For the alkali douch a five per cent sodium bicarbonate solution was used throughout the experiment.

The females were douched by placing them on their backs on a V-shaped animal board; a small catheter attached to a syringe containing five cc. of the solution was introduced into the vagina. After allowing the animal to lie quietly on her back for about one minute, she was taken to the male's cage for breeding. None or very little of the solution was observed to be lost in the handling of the doe.

In the swine experiment sows were all bred by artificial insemination in order that studies might be made on the pH of the semen. This value was determined for the natural semen as it came from the boar, for the semen after mixing with the acid or alkaline solution

be used as the means of shifting the sex ratio, again for the solution voided from the vagina after insemination. The Beckman pH meter was used in making the determinations. Observations were made on the motility of the spermatozoa in the solution returned from the vagina. Amounts of three per cent lactic acid solution used with the semen varied from 0.1 to 0.7 ml. per 10 ml. semen. The alkali solution used was N/10 NaOH, additions varying in amount from 0 to 0.24 ml. per 10 ml. semen. Of 41 [illegible] in this experiment, 11 in the acid-treated group and seven in the alkali-treated groups failed to conceive [illegible] two or three trials.

Results

Table I shows the result of the three treatments with rabbits. Approximately the same number of litters was born under each treatment. The number of litters and of young born in the acid-treated group was somewhat smaller than in the other groups, making the average litter size also smaller. This may have been due in part to a greater strength of the acid solution used during the [illegible] of the experiment. At first a [illegible] lactic acid solution was used, but after the irritating effect on the [illegible] was observed, and it was noted that [illegible] inseminations resulted, the acid solution was changed to two per cent. Although this change resulted in a higher percentage of conceptions, the lower litter size persisted. The percentage of males produced in the untreated group was higher than in the acid- and alkali-treated groups, though not significantly [illegible] percentage of males and females in the two latter groups was practically equal.

Table II shows the result of the experiment when tabulated according to sire. Ten sires are listed in the table. Actually in the experiment it was observed that three of the males were not [illegible] good breeders, showing a [illegible] in the females and failing to produce litters. These were replaced by males 9, 13 and 17. Only one of the three discarded males (No. 8) sired a litter. This was in the alkali-treated group and consisted of four young, three females and one dead, in such a condition that the sex could not be determined. This litter was not included in Table II.

Each male sired approximately the same number of litters and these were almost equally divided between the three treatments. The average number of young per litter for each sire varied from 6.5 to 8.4. The differences, however, were not significant. Of the ten males listed, seven sired an excess of males; the other three an excess of females.

Statistical Analysis

The data were treated statistically to determine effect of sires, dams and treatments on the percentage of males, on litter size and on percentage of young born dead. In the analysis of the relation between treatments and males and females, only those females have been used which produced litters under each treatment. This naturally eliminates part of the data, since some females died before producing litters under all three treatments, or failed to produce after giving birth to one or two litters. This loss of data was not great, however, except for the females mated to male No. 3, where more than half the females failed to produce three litters.

The females in each sire group were first analyzed. For nine of the sire groups the variance in sex ratio (percentage of males) between treatments and between females was no greater than would be expected by chance. In the group of females mated to male No. 15, the uniformity of sex ratio between treatments was much greater than would be expected by chance. This is one of the groups in which an excess of females was born. The variance in sex ratio due to treatments compared with all females combined showed no significant differences. Likewise the variance in sex ratio between sires and treatments showed no significant differences.

While litter size varied considerably, the variances due to treatment and to sires did not exceed the variance due to chance.

It was noted in an earlier paragraph that the percentage of young born dead was much lower in the acid treated group than in the untreated and alkali-treated groups. The variance in percentage born dead between males was not significant. The variance between treatments was significant for the five per cent level and closely approached significance for the one per cent level. Just why the acid treatment should reduce the number born dead is not clear.

The sex ratio during each five-day period showed a nearly parallel fluctuation between groups from a high to a low percentage of males. Thus, one period with a high percentage of males was followed by a high percentage of females. The fluctuation has the appearance of occurring periodically at about 20- to 25-day intervals. The cumulative sex ratio for these five-day periods showed that while the numbers of observations were small, the sex ratio was more aberrant, but as the numbers increased a fairly constant sex ratio was reached and maintained throughout the remainder of the experiment. At no time during the experiment was there a definite trend toward an excess of one sex or the other in the two treated groups.

Only a few litters of swine were produced in this experiment. The results are given in Table III. Since almost all the litters were produced by different sires and dams, analysis could be made only between the treatments. The differences proved not to be significant. The number of each sex required to give the normal sex ratio for swine of 52 per cent males is given in the table for comparison.

The pH of the semen as it came from the boar averaged 7.25. Treatment with acid lowered the pH to an average of 5.79, but the fluids of the vagina raised it to a pH of 6.06 as determined by the solution voided from the vagina after treatment. Alkali treatment raised the pH to 8.65 and after return from the vagina the pH of the solution was 8.59. The range of pH of the semen for individual males ranged from 6.90 to 7.48.

Summary and Conclusions

Experiments on the value of acid and alkaline douche treatments in altering

TABLE I. [illegible] of litters, of young, and sex-distribution of [illegible] treated rabbits.

Group	Number of litters	Number of young	Average litter size	Number of males	Number of females	Sex undetermined	Percentage of males
Untreated	104	818	7.9	427	372	19	53.5
Acid	102	714	7.0	362	351	1	50.1
Alkali	115	815	7.4	417	396	36	50.2
Total	321	2347	7.4	1206	1119	41	51.8

*Sex was undetermined because in some cases young had been partially eaten by the doe and in other cases the young were partially decomposed.

Table II. [illegible] of [illegible] treated rabbits of [illegible]

Sire number	Number of litters	Number of young	Average litter size	Number of males	Number of females	Sex undetermined	Percentage of males
3	21	167	7.1	46	70	2	39.7
6	24	178	7.4	110	63	5	63.7
11	30	207	6.9	104	99	4	51.7
12	30	220	7.3	121	96	3	[illegible]
14	33	220	6.7	111	100	9	[illegible]
15	34	271	8.4	172	142	17	60.7
16	27	216	8.0	106	104	1	51.0
17	30	197	7.0	96	96	5	50.0
18	32	207	6.5	[illegible]	110	7	50.3
9, 13	32	228	7.3	130	110	-	53.2
Total	321	2378	7.4	1206	1179	53	50.6

*One litter of four in the alkali-treated group was stillborn and [illegible]
**Sire 8 was removed from the experiment and replaced by male No. 9.
The litter sired by No. 8 is not included in this table.

TABLE III. Results of sex-control experiments with swine

Season	Number litters	Initial pH of semen	pH before insertion	pH of returned material	Volume returned (ml)	Gestation period (days)	Number females	Number males	Total	Percent males	Expected* females	Expected* males
Acid Treated												
Fall 1941	6	7.14	8.59	7.35	74	113	26	17	43	39.5	21	22
Spring 1942	7	7.26	8.71	8.62	51	114	37	41	78	52.6	37	41
Total	13	7.15	8.65	8.59	43	114	63	58	121	47.9	58	63
Acid Treated												
Fall 1941	5	7.19	5.71	5.11	48	115	11	11	4	47.5	19	21
Spring 1942	6	7.21	5.84	6.05	54	115	41	46	55	44.8	28	30
Total	11	7.21	5.79	6.06	51	114	52	45	98	45.9	47	51

*Number expected for normal sex ratio of 52 per cent males.

the sex ratio have been conducted with rabbits and swine. In all, 2,383 rabbits and 219 swine were born.

The results fail to show any significant modification of the normal sex ratio for the species as a result of the acid and alkali treatments.

At no time during the course of the experiments was there any indication of a trend such as was present throughout the Dayton laboratory experiments.

It is concluded that acid and alkaline douche treatments of the female and alteration of the pH of the semen are without effect on the sex ratio.

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Consistency of Tomato Products

II. Effect of pH During Extraction on Tomato Juice Consistency

Food Technol. 21(6), 923-6(1967) (Eng)

J. C. Miers, J. R. Wagner, and D. W. Sanshuck
Western Regional Research Laboratory, Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Albany, California 94710

SUMMARY

The effects upon tomato juice consistency of modifying the pH of tomato tissue during maceration and heating have been investigated. Tomatoes were extracted at pH levels ranging from 1 to 9 and then heated to 95°C. Extraction at low pH levels and then heating, yields juices having much higher consistencies than juices prepared at the natural pH. Their consistencies change little when the pH is restored to normal by adding NaOH.

Extraction at higher pH levels, and then heating, also yields juices having higher consistencies. But in this case tomato gels are formed when pH is decreased by adding HCl. To achieve the above effects, immediate contact between the tomato tissue as it is macerated and the acid or base is essential to assure pH control during breaking and heating. The effects obtained at low pH levels appear to be related to rapid inhibition of both pectinesterase (PE) and polygalacturonase (PG). The effects at high pH levels appear to be related to PG inhibition and increased PE activity.

INTRODUCTION

Consistency is one of the main criteria in the marketing of strained tomato products. Thorough discussions of research previously undertaken to increase consistency of tomato products have been published by Kertesz and Loconti (1944) and Cooler (1962). It is now generally recognized that increasing the retention of undegraded pectin in tomato products increases the consistency. But when tomatoes are macerated at ambient temperatures, pectic enzymes, pectinesterase (PE) and polygalacturonase (PG), are released from the tomato cells and quickly degrade the pectin which results in a large loss in consistency in

the final product.

To prevent enzymatic action on the tomato pectin and the consequent loss in consistency, the tomatoes are heated as quickly as possible to 190°F or higher before, during, or immediately after chopping. The heating inactivates the pectic enzymes and the resulting tomato products contain more undegraded pectin and have higher consistencies.

However, instantaneous heating of whole or chopped tomatoes to the required temperature is not feasible by available methods. Therefore, enzymic degradation of pectins and loss of consistency cannot be completely prevented by heat alone.

Investigation by Wagner and Miers (1966) of the use of chemicals to inhibit enzymatic action in tomato macerate showed that acidification below pH 1.50 was an effective method for pectic enzyme inhibition. When acidification was combined with heat, they obtained juices of much higher consistencies than were obtained by heating alone.

Since the acid treatment plus heat produced a remarkable increase in consistency an investigation of the effect of pH control over the range from 1 to 9 was made. The experiments reported here indicate that a faster and better way of controlling pectic enzymes appears to be technically feasible and not only permits the development of much higher consistency in strained tomato products, but also may offer other processing advantages. The experiments to be discussed involve the control of pH by appropriate additions of concentrated hydrochloric acid or sodium hydroxide.

EXPERIMENTAL METHODS

The tomatoes used in the laboratory and pilot plant experiments were obtained from local canning companies in season or from a produce wholesaler.

Sample preparation. For any series of comparisons on a laboratory scale, 20 or more clean whole tomatoes were halved or quartered and the portions distributed to separate sub-samples weighing one kilogram. Each subsample, control or treated, was blended in a gallon-size laboratory blender. This blender bowl was fitted with a removable steam heating coil and a flat close fitting lid. The subsample was dropped into the bowl [illegible] required [illegible] volts) which chopped the tomato pieces into a moderately fine macerate.

The blender was stopped and the steam coil was put into the bowl, the lid put on, and a thermometer put through the lid into the macerate. At 1 min from the start, the steam was turned on and the macerate mixed at reduced speed (70 volts) while being heated. When the temperature reached 200°F (100 ± 5 sec) the steam was turned off but the mixing continued until 3½ min had elapsed. The hot macerate was then put through a laboratory size pulper using an 0.033-in. screen. The hot juice was cooled to 25°C (77°F) and the consistency obtained with an efflux pipet, described by Wagner and Miers (1966), which had a discharge time of 8.1 sec for 96 ml of water at 25°C.

Modifications of the standard treatment. The standardized procedure outlined above for the blending and mixing of the tomato subsamples was modified as necessary. For instance, the effect of delay in adding the HCl was studied by withholding the acid for time intervals up to 30 sec from the time the blending was initially started. In such cases, the motor speed was reduced after 10 sec of blending at 10,500 rpm. Mixing was always continued 5 sec beyond even the longest delay period. The hot juice from the pulper was cooled to 25°C and the consistency measured with a GOSUC efflux pipet.

The GOSUC efflux pipet was developed by Gould *et al.* (1964) at Ohio State Agricultural Experiment Station where it was used for evaluation of tomato juice made by conventional procedures. For such products they used a small stainless steel orifice 1 in. long, having a 5/64 in. opening.

It was found that an 0.125 in. orifice opening was better suited to this study since it permitted the direct comparison of a wider range of product consistencies. When using the latter orifice the pipet was adjusted so that discharge time for 200 ml of water at 25°C was 15 sec. For measurement of tomato juice consistency, the sample was sucked up to the top of the reservoir and the time determined for 100 ml of juice to flow from the pipet. Three measurements were made and timed with a stopwatch.

Tomato gels. Tomato juices, which were made at higher than normal pH, formed gels when restored to the normal pH of the tomato. Such gels were made by adding concentrated HCl to 200 g of [illegible] pH extract which [illegible] added before extraction. Although the extract was hot when the HCl was added, some small and large lumps of gel formed. The larger lumps were mashed and aliquots of the hot mixture were poured into each of two 50 ml beakers which were covered and refrigerated overnight. Next morning the beakers were removed from the refrigerator and the gels allowed to reach room temperature. The gel strength of the upper surface was determined with a Bloom gelometer using the 1 [illegible] plunger (Fellers and Griffiths, 192[illegible]).

Determining the effects of acidification. In one small pilot plant experiment, 200 lb of Ace tomatoes were pulped through an 0.033-in. screen. The juice was pumped into a heat exchanger at 210°F, then cooled quickly to 85°F in two heat exchangers. Another 200 lb lot was blended in 6.6 lb portions with 54 ml HCl in a large laboratory blender for 30–40 sec. All the acidified blended portions were accumulated in a large tub. After blending, the acidified macerate was pulped and the juice heated and cooled in the same processing line. After cooling to 85°F, the pH of the acid juice, 1.03, was increased to 4.42 by stirring in 12N NaOH.

Both juices were concentrated under 2-in. Hg pressure to pastes in a small evaporator. The consistencies of these pastes and several dilutions made from them were measured in a Stormer viscosimeter at 25°C by determining the weight in grams necessary to turn a special helical spindle 100 revolutions in 30 sec.

Determining the effect of pH upon yields. In another pilot plant experiment, triplicate 150-lb batches of tomatoes were processed at the natural pH, at pH 1.50, and at pH 6.14. These tomatoes were put through a Fitzpatrick Comminutor at low speed using a 1-in. screen. The moderately coarse macerate fell into a tub to which no chemical was added for the first batch, concentrated HCl was added in portions at a rate of 5.5 ml per pound for batch 2, and 3N NaOH was added in portions at a rate of 6.5 ml per pound to batch 3.

After comminuting and mixing, each batch was heated to 200°F in 4½ min and then put through the pilot plant pulper using an 0.033-in. screen. Weights of the recovered juices and pomaces were determined so that yield estimates could be calculated. Moisture contents in the pomaces were determined by drying duplicate samples [illegible] at 100°C [illegible] hours.

pH was determined [illegible] with a glass electrode pH meter. °Brix, determined at 25°C with a refractometer equipped with a °Brix scale, was corrected for any added acid

or base. Tomato solids were obtained from the National Canners Association °Brix-solids tables (1966).

RESULTS AND DISCUSSION

Consistency variance and extraction pH. Typical changes in consistency with extraction pH for two lots of tomatoes are shown in Fig. 1. Consistency at the normal pH of 4.40 was relatively low, approximately 25 sec. As the extraction pH was lowered or increased from 4.40, the consistency was remarkably increased—increases of 2, 3, and even 4-fold in efflux time were observed at pH 1.

Effects of acidification. When concentrated HCl (12 ml per subsample) was withheld for varying times from 0 to 30 sec after macerating the subsamples of two different lots of tomatoes, a significant loss in juice consistency resulted from even a 5-sec delay in one set of subsamples (Fig. 2).

With increasing delays the consistencies of the acidified juice samples displayed a downward trend approaching the comparatively low consistency of the control samples prepared without added acid. These data show that the acid should be mixed as quickly as possible with the tomato macerate in order to obtain products having the highest consistency. Presumably, any delay in contact between macerated tissue and alkali in the preparation of high pH extractions, would also result in juices of less than maximum consistencies.

Since the degree of acidity affects flavor, juice prepared at either low or high pH levels would need to be restored to the natural pH range of tomatoes for most uses. The net effect of using HCl or NaOH to neutralize the previously added acid or alkali is equivalent to the addition of NaCl which is a common ingredient in tomato products.

Table 1. Consistency and gel strengths.

Variety	Extraction pH	Adjusted pH	Consistency at extraction pH (sec)	Av gel strength at adjusted pH (g)
VF-36	5.90	4.64	88	257
VF-36	7.02	4.50	74	133
	8.93	4.35	95	103
Green wraps (ripened)	5.45	4.10	113	58
	5.99	4.23	54	155
	6.87	4.15	59	194
	7.15	4.29	64	290
	8.13	4.10	58	251

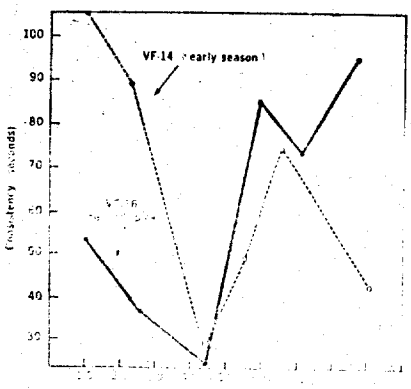
Extract gels. As previously reported (Wagner and Miers, 1966), acid extracted juices remain in the high consistency range after restoration to the natural pH. When concentrated HCl was added to those extracts prepared at the higher pH levels, another phenomenon occurred. In nearly all cases, the extracts then formed gels, many of which were exceptionally strong and easily self-supporting.

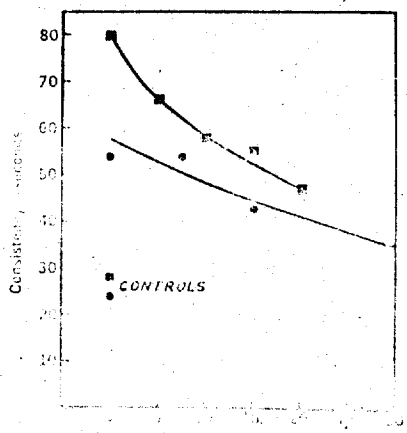
As can be seen in Table 1, the gel strength at the normal pH cannot be predicted from the consistency of the extract at the extraction pH even for subsamples from the same lot of tomatoes. In another variation, part of a lot of tomatoes can be extracted at low pH of 1 to 1.5 and the other part at a higher pH of 6 to 7. Then the juices can be mixed to obtain a gelled tomato juice in the normal pH range. Gels obtained in this manner easily slid out of a 250-ml beaker and remained self-supporting.

Normal and acid extracted pastes. In the pilot plant, the two pastes made from Ace tomatoes were remarkably different consistency-wise. The paste from the juice extracted at normal pH had 27.5% tomato solids and the paste from the acid extracted juice contained 18.3% tomato solids. The consistencies of these two pastes were approximately the same even though the tomato solids differed by a factor of 1.5.

When these pastes were diluted to several lesser concentrations and the consistencies of the dilutions plotted against their tomato solids contents, the curves shown in Fig. 3 were obtained. These curves show that at equal tomato solids contents above 7.5%, the puree or the paste from the acid extracted juice was 2 to almost 3 times thicker than the product made at the normal pH. These curves indicate a distinct benefit, consistency-wise, when tomato concentrates are prepared from tomatoes processed with dilute HCl.

Evidence of another effect is shown in Table 2, which gives the average data on the extractions of tomatoes at the 3 pH's listed. The extract yields (corrected for addition of acid or base) at the high and low pH's were larger and the pomaces were less than





the consistency of two different tomato samples. No acid was used in the control subsamples. GOSUC pipet used.

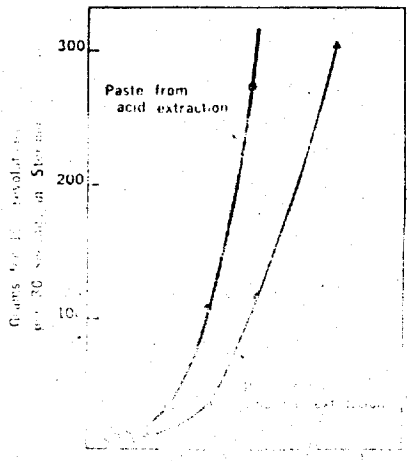


Fig. 3. Consistency at several dilutions of pastes prepared from acid extraction juice and from control juice.

Table 2. Pilot plant yields for tomatoes extracted at three pH levels.

Extraction pH	Juice recovered (%)	Pomace (%)	Moisture in pomace (%)
4.24	92.5	3.4	79
1.50	94.5	1.3	69
6.14	93.5	1.5	69

that at the normal pH. The pomaces from the high and low pH runs appeared much drier and less sticky than the pomace from the run at pH 4.24. Analysis showed the moisture content to be 10% less in the pomaces from the high and low pH runs. These data indicate a potential benefit yield-wise when tomatoes are extracted at pH lower or higher than normal.

pH and pectic enzyme activity. The pH-dependent effects noted in the above experiments appear to involve changes in the activity of the pectic enzymes. Patel and Phaff (1960) reported that a purified tomato polygalacturonase (PG), the enzyme that depolymerizes pectin, had two peaks of activity; one at a pH of 2.5 and the other at 4.5. Their PG preparation was completely inactive below a pH of 1.5 and above a pH of 5.5.

The other well-known tomato pectic enzyme, pectinesterase (PE), which removes the methyl groups from the carboxyl groups, was reported by McColloch and Kertesz (1947) [they used the synonymous term pectinmethylesterase (PM)] to have only slight activity below 4.5 and increasing activity through pH 8. Earlier work by Speiser *et al.* (1945) indicates that PE may still be quite active at pH 9.5. The pH versus activity curves for PG and PE are combined in Fig. 4.

Below pH 1.5 there is very little if any enzyme action by either PG or PE. Therefore at such pH's, more native pectin should be retained than at the normal pH in the presence of active PG and PE. Now if the pH were increased from normal to 5.5 or above, PG would not be active, but PE activity would increase as the pH increased from 5.5 to 8.0. This would increase demethylation and minimize depolymerization.

Consistencies at various pH extraction levels. The high consistencies of juices extracted at the lower pH levels could be due to extraction of more and/or larger molecular weight high methoxyl pectin. High consistencies at the upper pH levels are probably due to the extraction and formation of more and/or larger molecular weight low-methoxyl pectin. This low-methoxyl pectin may react with the natural calcium content to form the gels obtained when the pH was restored to the normal.

It is also possible that increased extraction of other large molecular weight materials under conditions of high and low pH are contributing to the high consistency values observed. Williams and Bevenue (1954) found that 19% of the tomato solids were insoluble in 80% alcohol. Of this AIS, only 22% was pectic substances and 61% was other polysaccharides, much of which was strongly lyophilic. The remaining 17% of the AIS was protein.

Possible increases in extraction of such components at high and low pH levels could account for the increases in consistency. Luh *et al.* (1954) state that non-pectic AIS also makes important contributions to the consistency. Research now in progress in our laboratory which will define more completely the substances causing the increase in consistency and the formation of gels will be reported in another paper.

These observations suggest a new opportunity for control of consistency through the production of high consistency juice which could be used as such, or blended with other juices to obtain intermediate levels of consistency. pH control seems to offer an enlarged opportunity for new products from tomatoes—especially those juices produced in the pH range of 5.5 to 9.0.

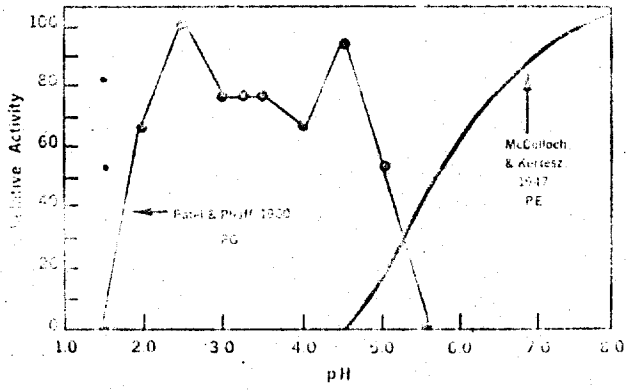


Fig. 4. Relative activity of tomato enzymes at different pH's.

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Ms. [illegible]

Presented by J. C. Miers at the Northern California Section, Institute of Food Technologists, Alhambra Motor Inn, Stockton, California, December 2, 1966.

Although hydrochloric acid and sodium hydroxide have been classified GRAS (Federal Register 1/31/61) their use in this study does not imply approval for use in foods [illegible]

[illegible]

Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Department of Agriculture to the exclusion of others that may be suitable.

Engineering

Peeling Grain

A. I. Morgan, Jr., E. J. Barta, and P. W. Kilpatrick

■ A NEW WHITE, unmilled cereal can be produced from wheat and several other grains by chemical peeling. The product retains all the endosperm, including the nutritious aleurone layer, but loses most or all of the colored bran layers.

The bran is loosened by strong warm lye, then removed by vigorous water washing. Warm dilute acid restores the surface whiteness. The grain is dried in warm air.

Peeled Wheat

One purpose of peeling wheat is to provide a nutritious white grain retaining its normal shape, for use in the developing countries of the world. Peeled wheat retains the high protein value of wheat. It is rather shiny, with a thin brown stripe along one side where some of the bran remains in the crease of the grain. This product can be conveniently steamed in about 25 min to a firm, white, free-flowing edible grain, free of surface stickiness. Peeled wheat has a pleasing texture when chewed. Because only 15% of the bran remains there is no sensation of toughness. Fig. 1 shows raw peeled wheat on the left; Fig. 2 shows cooked wheat.

Peeled Bulgur

To provide an attractive new wheat food primarily for sale in America and Europe, the wheat is parboiled by a mild version of the bulgur process before peeling. This product, peeled bulgur, shown raw on the right in Fig. 1, is completely free of crease bran. It has a slightly yellowish cast, compared with the creamy-white color of raw peeled wheat. The peeled bulgur can be steamed in only 15 min to a white, firm food. Many of the kernels open out somewhat during cooking.

Table 1 compares the characteristics of peeled wheat and bulgur at 14% moisture. The whitest peeled grains come, naturally, from white common or club wheats. Because of its abundance, however, hard red winter wheat has been emphasized in this work. When peeled, red wheat appears slightly colored in comparison with peeled white common or club wheats.

Table 1. Product characteristics.

Property	Original wheat	Peeled wheat	Peeled bulgur
Color	Brown	White	Yellow-white
Colored bran content	9%	1%	0%
Cooking time	40 min	25 min	15 min
Yield	100%	91%	89%
Density	64 lb/bushel	61 lb/bushel	40 lb/bushel
Protein[a]	12%	11%	11%
Fat[a]	1.4%	1.3%	1.0%
Thiamine[a]	0.39 mg/100 g	0.23 mg/100 g	0.12 mg/100 g
Riboflavin[a]	0.15 mg/100 g	0.09 mg/100 g	0.10 mg/100 g
Germ	Present	Variable	Absent

[a] Analyses made by N. J. Tennis, of this laboratory.

Storage stability tests are still under way at the time of this writing. We foresee no particular problems.

Other Cereals

Some other grains which have been successfully peeled are barley and oats. Neither of these, however, provides quite as white a product as does wheat. Oats and barley yield a very bulky, fibrous bran in addition to the peeled cereal. Both oats and barley, when peeled, may be advantageously milled to flour, especially for breakfast cereals.

Background of Process

A number of attempts have been made to peel wheat. The dry or mechanical methods have been judged hopeless for various reasons, particularly because of losses of endosperm (Grosh, 1960). The authors of this article took part with T. Wasserman at this laboratory in tests with an Engleberg mill on wheat at various moistures, with and without abrasives. Not only is endosperm lost in this kind of dry peeling, but the kernel becomes nearly round.

Pomeranz (1961) reviewed wet peeling methods, using water and steam. Most of these methods were proposed as preliminaries to milling the wheat into flour. Their objective was to increase flour yields and to better retain nutrients located near the outside of the endosperm. Millers have rejected them for various reasons.

Peeling wheat by chemicals has been proposed occasionally. Gums, surface active agents, fat solvents, sulfate ion and strong acid are among the chemicals mentioned. Rao *et al.* (1953) proposed 3*N* hydrochloric acid for peeling wheat to produce a light-colored

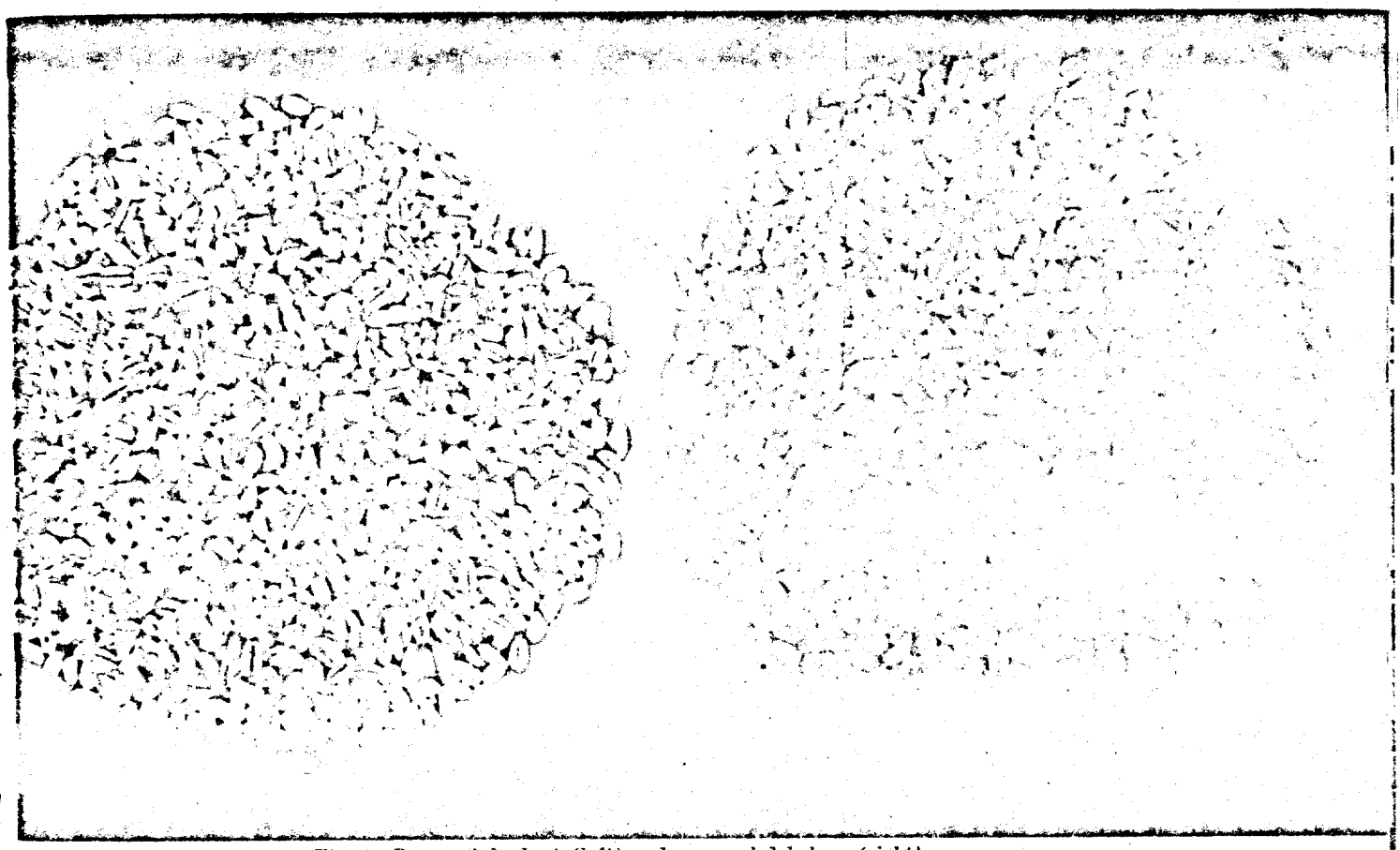

Fig. 1. Raw peeled wheat (left) and raw peeled bulgur (right).

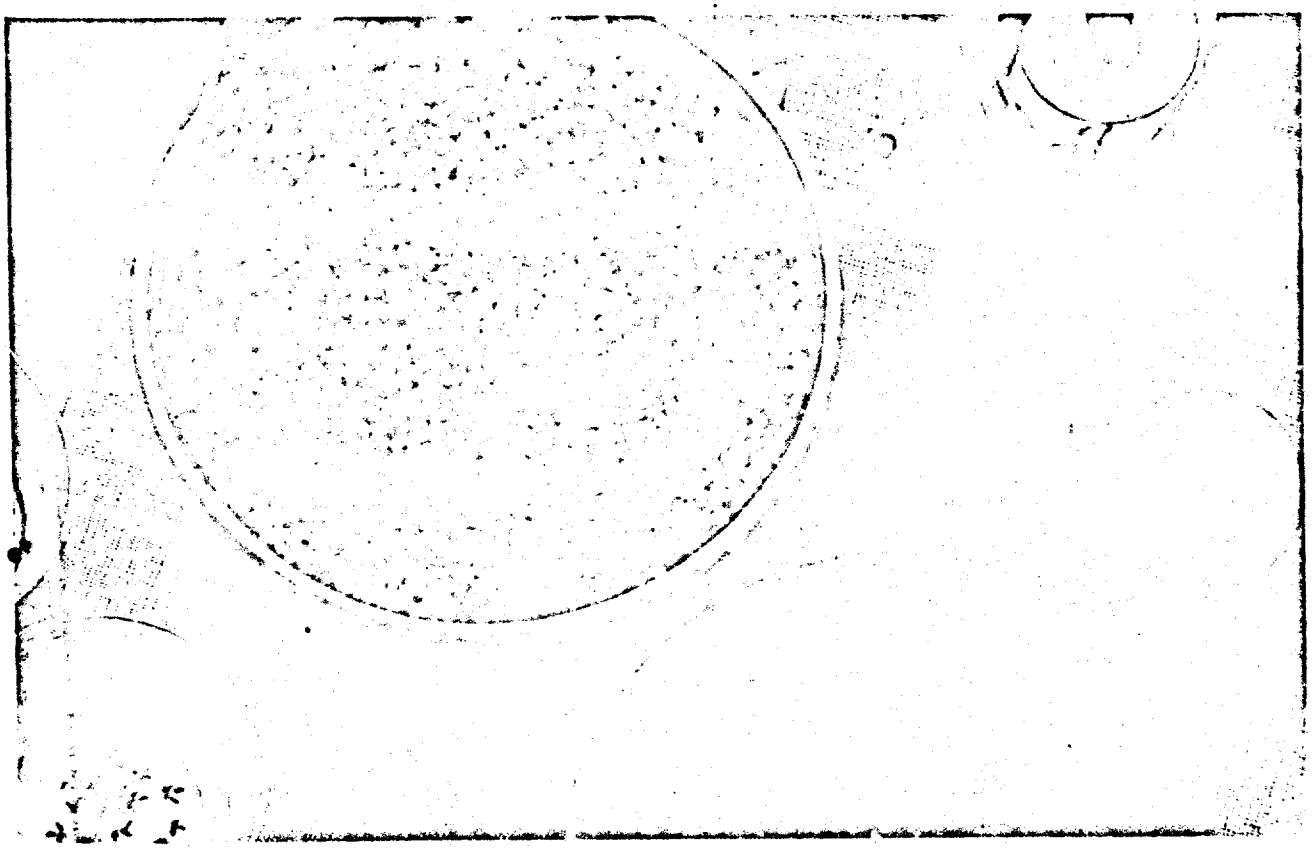

Fig. 2. Cooked peeled wheat.

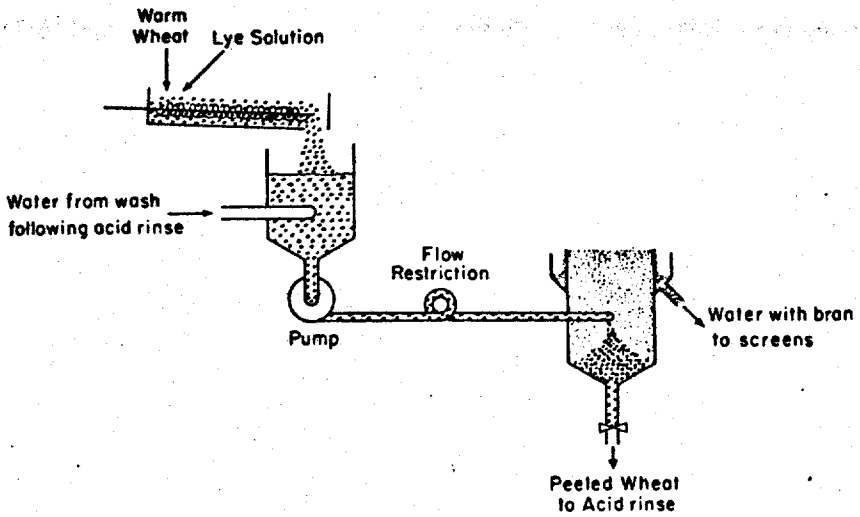

Fig. 3. Process schematic diagram.

product. His method was not effective, at least on American hard red winter wheat.

In proposing a combination of lye and steam peeling for potatoes, Adams *et al.* (1960) suggested that the method would work with grain.

Pretreatments

Some water-soluble nutrients (thiamine, nicotinic acid, pyridoxin) lie in or near the aleurone layer in wheat. Rohrlich *et al.* (1961) recommended that, before milling, wheat be soaked in water for several hours at 140°F to allow these nutrients to diffuse farther into the kernel. Soaking is even more appropriate as pretreatment for wheat peeling, since the wheat is to be wet anyway. Such soaking, however, does not contribute to the peeling process itself. It does seem to reduce losses of germ during peeling.

For peeled bulgur the wheat should be soaked in water for 1 hr at temperatures rising from 135 to 180°F, held 30 min at 180°F, and then steamed at 212°F for 15 min (Smith, 1964). The hot wet bulgur is then peeled.

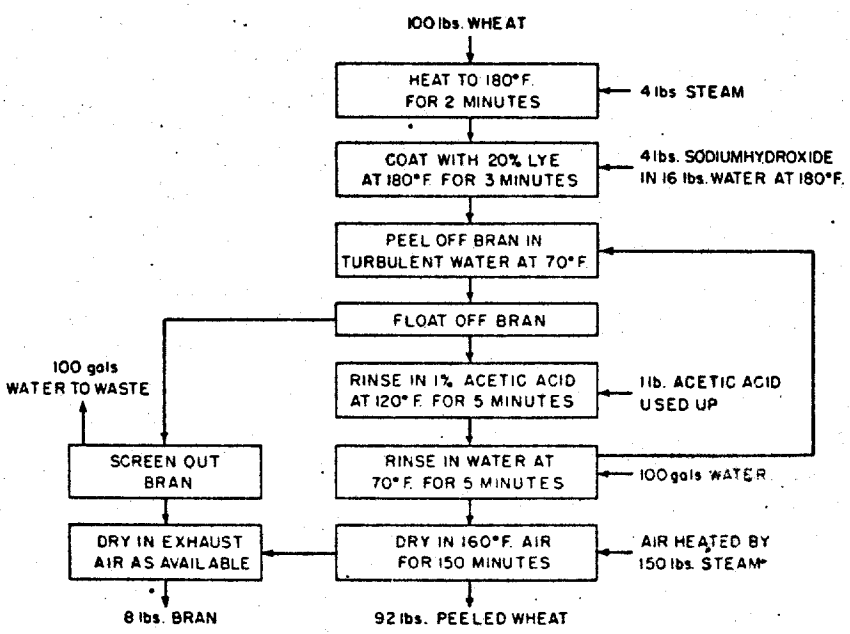

Fig. 4. Process block diagram.

Heating

The grain must be at 180°F warmer to react with the lye. Sin only a small amount of lye is us the grain should be warm as well the lye. One simple heating method to pass steam through a layer of gra for several minutes. Grain temper ture above 180°F does not injure t peeling process. The heating has additional advantages of inactivati enzymes and killing microorganisms.

Lye Coating

Treatment with 20% sodium h droxide at 180°F for 3 min loose the pericarp from the seed. The l reacts with components of the bra making the used lye dark and visco It is therefore economical to wet t grain with just enough lye for t purpose rather than to submerge t grain in excess lye. To do this, v put the warm grain and 20% of i weight of hot 20%-solids lye solutic (5*N* NaOH) into a ribbon mixer. T mixer must wet each kernel quick and uniformly with lye and keep that way for 3 min. Mixer desig should ensure positive control of trea ment time by moving the grain un formly through the mixer.

Peeling

The grain drops into excess roon temperature water after lye coatin The bath removes excess lye and limi lye penetration. This same water ha been used in a later step to wash off th excess acid. The slight acidity of th water helps to neutralize the lye an to reduce water consumption. Th slurry of water and wheat should the be pumped through a turbulent r striction to peel off the loose bra This restriction can be a loop o tubing, as shown in Fig. 3, whic augments the turbulence produced b the pump.

Flotation

The turbulent stream of water an wheat enters a larger vessel, wher the peeled grain sinks and the bra floats up with the overflowing wate For easier disposal, the bran can b screened out of the water stream Separation of bran from grain is com plete for wheat and barley. However much bran sinks with oats and mus be removed after drying. Fig. 4 show the peeling process.

Acid Rinse

At this stage, the peeled grain i often discolored by the action of th lye on the aleurone layer. Rinsing th

grain 5 min in 1% acetic acid solution at 120°F removes the color. The acid solution does not become foul or dirty. The same acid is used continuously, therefore, by adding about 1 lb of acid per 100 lb of grain treated to make up for the acid neutralized by alkali in the grain. Rough handling of the grain is likely to rub off the germ at this stage. A screw trough conveyor transports it gently. If the germ is to be removed to increase storage stability, more violent mixing may be used here. Sulfurous acid will whiten grain more than acetic. The SO_2 will destroy the thiamine completely, however, and there may be some difficulty in eliminating the SO_2 taste from the peeled grain.

Final Rinse

The grain is lifted out of the acid rinse and washed with room-temperature water. This may be done countercurrently in another trough with a continuous screw, or on a mesh belt under a water spray. The use of acetic acid does not demand a thorough final rinse, because remaining traces of the acid can be evaporated during drying.

Drying

When the grain is separated from the final rinse water, the kernels contain 35–45% moisture. To eliminate spoilage, drying back to 10–12% moisture should begin immediately. An effective method is warm air flowing through a mildly mixing bed of grain. Many types of grain dryers are satisfactory. The bran collected at the flotation step can also be dried in air exhausting from the grain dryer.

Costs

Lye and acetic acid are among the cheapest chemicals. Water, power, steam, and waste disposal requirements are modest, and depend on the equipment chosen. Equipment needed is simple and conventional, being confined to pumps, tanks, troughs, and dryers. Our estimates, in advance of continuous pilot-plant construction, indicate a cost of about one cent per pound of wheat peeled.

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THE AUTHORS are with Western Regional Research Laboratory, Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Albany, California.

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EXPERIMENTAL PRODUCTION OF MALIGNANT GROWTHS BY SIMPLE CHEMICALS

JOSEPH K. NARAT

Associate in Surgery, Loyola University, Chicago, Ill.

Among the various problems of cancer research the question of the experimental production of cancer has been brought into the foreground in the last decade. The pioneer work in this field has been done by Fibiger,[1-3] who in 1914 published reports of his experiments with rats, in whose stomach he succeeded in producing cancer by feeding the animals with cockroaches infected with a roundworm *Spiroptera neoplastica* (*Gongylonema neoplasticum*).

A great interest in the subject of experimental cancer production was elicited by Yamagiwa and Ichikawa,[4-9] who in 1915 reported successful results of production of follicuło-epithelioma in rabbits by prolonged application of coal tar to rabbits' ears, after futile attempts by many investigators, Cazin,[10] Hanau,[11] Jores,[12] Brosch,[13] and Bayon,[14] to employ coal tar for production of cancer.

The experiments of Yamagiwa and Ichikawa were repeated on mice in 1918,[15] by Tsutsui, and more recently by Fibiger and Bang,[16-19] Roussy,[20] Deelman,[21] Bierich,[22-23] Russell,[24] Murray and Woglom,[25] Bloch and Dreifuss,[26-27] Jordan,[28] Roffo,[29] Burckhardt and Müller,[30] and many others. The possibility of voluntary production of blastomata was thus established.

Some investigators, as Bloch, Jordan, etc., started experimenting with different fractions of distillation of coal tar in order to find in the complicated mixture represented by coal tar, a chemically well defined substance which possesses the ability to produce cancer. Leitch[31] used crude shale oil in producing tumor formations. Applications of potassium arsenite by Leitch and Henneway[32] led to formation of a growing tumor in one mouse. Cancer was produced by Passey[33] by means of

soot mixed with quicklime. Stabr[34] succeeded in producing epithelioma on the tongue of rats by feeding them with oats. Bullock and Curtis[35] induced formation of sarcoma in the liver of rats through mediation of *Cysticercus fasciolaris*, the larval stage of *Taenia crassicollis* of the cat. Infestation of the rat by the larvae follows the ingestion by the rat of the food contaminated with cat feces containing the eggs of this tapeworm.

In the fall of 1922 we started a series of experiments based upon the assumption that any chemical substance able to cause irritation of the skin can lead to the formation of a cancer after prolonged and repeated applications. It was decided to investigate whether substances of chemically simple structures might not act as tumor producing or carcinogenic agents just as well as coal tar.

Caustic potash, caustic soda, hydrochloric acid, sulphuric acid, benzol, xylol, turpentine, and several other substances were tried. These preliminary experiments showed that it was difficult to find a suitable concentration of the liquids, as too weak solutions did not irritate the skin sufficiently, and too strong ones caused such serious lesions that it was impossible to repeat the applications frequently enough without producing deep ulcers and losing the animals. The determination of the necessary concentration of the irritant caused a great waste of time and animals. Therefore, it was decided to limit the experiments to the use of caustic potash and hydrochloric acid.

There was a considerable difference in the sensitiveness to the irritants in various strains of mice, and even in animals belonging to the same strain. Therefore, it was necessary to find a suitable concentration of the irritant for each animal. The average concentration was 3 to 6 per cent NaOH and 3 to 5 per cent HCl. The applications were repeated at intervals of 1 to 2 days till the first appearance of macroscopic lesions, and renewed on the average twice weekly later on. The frequency of the applications was regulated according to the condition of the produced lesion: no time was given to the organism of the mouse for reparation hyperplasia of epithelium and other healing processes; on the other hand, the production of deep lesions was

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FIG. 1. PACHYDERMIA. Ten weeks after beginning of applications of KOH
FIG. 2. PAPILLOMA. Fourteen weeks after beginning of applications of KOH

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FIG. 3. PAPILLOMATOUS GROWTH. Sixteen weeks after beginning of applications of HCl
FIG. 4. TUMOR. Five months after beginning and three weeks after cessation of painting with HCl. Microscopical examination revealed a cancer

ance; these elevated nodules were noticeable in 4 to 5 months. Pericentric formation of these new growths had been observed in nearly every case. They soon were covered with thick horny masses, thus resembling the cutaneous horns encountered in human subjects.

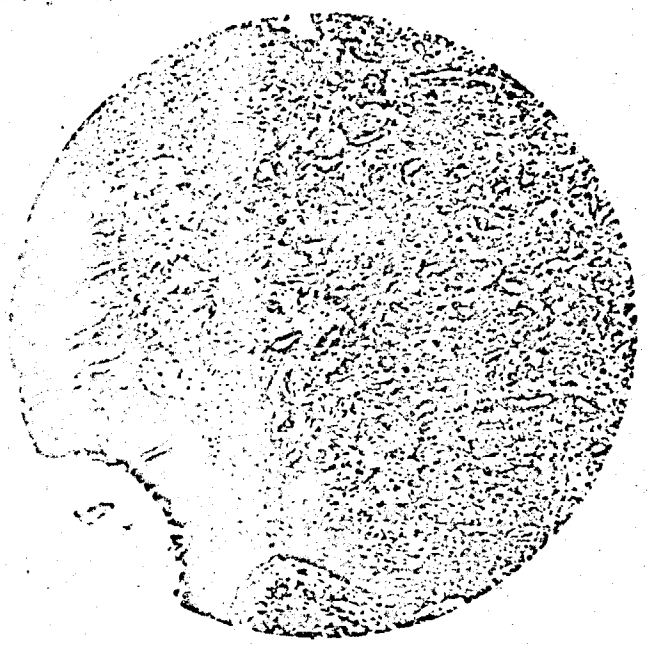

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FIG. 7. [illegible] TISSUE. Ten weeks after the beginning of applications of KOH. [illegible] masses removed; the edge of the ulcer visible on the left side

After injection of the chemicals had been discontinued, three different changes were observed.

1. In about 56 per cent of the mice which survived the repeated irritation with KOH or HCl spontaneous retrogressive changes took place. In 2 to 3 weeks the painted areas were transformed into scars; in many cases the skin regained nearly normal appearance and seemed to have recuperated completely; a permanent alopecia was the only remaining visible result of the experiment.

2. In about 29 per cent of the cases the treated areas assumed the appearance of chronic eczema; superficial ulcerations,

excoriations and formation of scabs and crusts were observed. These skin affections remained stationary; they did not yield to the treatment with salves used in therapeusis of eczema in human beings.

3. In about 15 per cent of the cases, the painted areas continued their evolution after cessation of the irritation. They gradually increased in dimension for a certain time and invaded

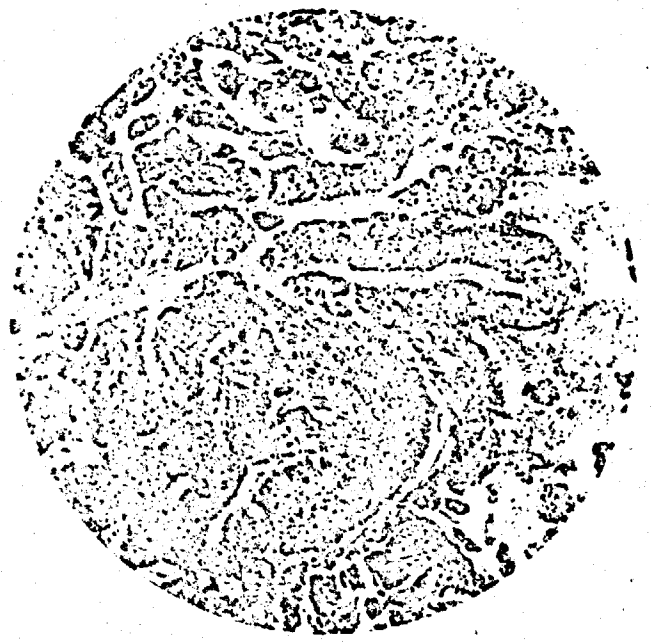

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FIG. 8. TUMOR. Forty-two weeks after beginning of applications of HCl. Atypical proliferation of epithelium

contiguous tissues. They penetrated subcutaneous fat, muscles, and finally broke into the peritoneal cavity; metastases in the lymph-glands or other organs were never found. The tumors caused a marked emaciation and cachexia of the animals. All attempts of auto- and homo-transplantation of the tumors failed. Recurrences were observed after a wide excision of the tumors in six animals, out of nine in which this experiment was made. In order to follow up the development of histo-

ained unsuccessful. 359 mice were used for experiments; 62 lived longer than 6 months from the time the application of chemicals was started. 118 of them were males; 144 females; the age varied from 2 to 7 months at the beginning of injections. out of these 262 mice 61 belonged to two strains in which neither

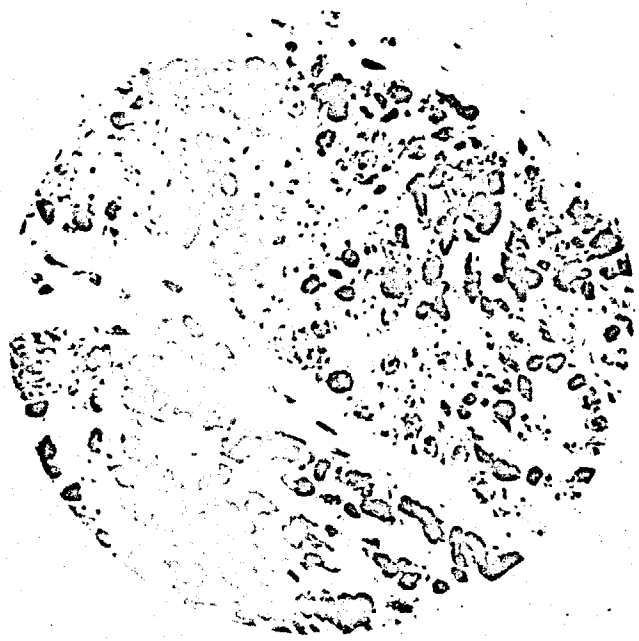

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FIG. 12. [illegible] Same as Fig. 11. Magnification 325 diameters. Note the enormous number of mitoses

KOH nor HCl nor tar was able to produce a malignant growth. The statistical data concerning the remaining 201 mice are shown in the following table:

[illegible]	[illegible]	[illegible]	Occurrence of Cancer	
[illegible]	[illegible]	[illegible]	15 = 15.11%	7 males = 13.46% 8 females = 17.02%
KOH	[illegible]	[illegible]	12 = 11.81%	4 males = 13.79% 8 females = 15.38%
[illegible]	[illegible]	[illegible]	8 = 38.09%	5 males = 36.36% 4 females = 40.00%

COMMENT AND CONCLUSIONS

In opposition to statements made by several authors (Lipschütz[36–39]) we found that not only the individual animal's susceptibility but also the question of strain plays an important rôle in experimental production of cancer.

In two strains of mice the applications of HCl, KOH, or tar produced in a number of cases typical malignant growths. In two other strains there was nothing in the microscopic appearance or clinical character of the lesions to indicate that the tumor was malignant. Buschke and Langer[19] also reported unsuccessful results of experimental production of tar cancer in mice. This observation proves that an experimentally produced cancer is not a localized lesion but that more complicated processes are involved in the formation of a malignant growth.

The systemic nature of cancer has to receive serious consideration. Possibly metabolic, endocrine or vegetative nervous disturbances play an important rôle in the origin of a cancer, be the irritant of a chemical, physical, or a parasitic nature. It is a remarkable fact that the age of the animal is a negligible factor in the incidence of experimental lesions, contrary to the occurrence of cancer in human beings. The sex does not appear to exercise much influence upon the incidence of experimental tumors.

Considerable difficulty was encountered in forming a judgment as to the benignity or malignancy of some of the produced lesions. Extreme caution should be observed before reports of the production of cancer by artificial means are accepted. The exact nature of some of the reported tumors in regard to malignancy remains obscure in our opinion. However, in a number of mice in our experiments the tumors showed morphological evidence of malignancy, such as anaplasia, atypical proliferation of epithelium, invasive growth, as well as biological and clinical evidence: cachexia, emaciation of the animals, leading to their death; progression of growth after the irritant had been suspended. At the last international conference for the experimental study of cancer at Amsterdam in 1923, the Danes expressed the opinion that every papilloma in mice is

malignant.[31] Absence of lymphatic metastases is a striking feature of tumors in mice when compared with corresponding growths in man, which is true in all forms of cancer in mice, according to Maud Slye.[32] Through courtesy of Dr. Jaffe we had the opportunity of examining the original slides of tar cancer produced by Yamagiwa and Ichikawa. These slides presented exactly the same histological picture as the tumors produced by the application of KOH or HCl. Therefore we feel entitled to regard the tumors produced in our experiments as cancer. Thus chemically simple substances can be used in experimental cancer research instead of chemical compounds of not yet isolated substances. The soundness of Virchow's irritation hypothesis has been confirmed by the results of our experiments. The greatest importance of experimental production of tumors lies in the possibility of observation step by step of all stages of tumor development.

SUMMARY

1. Repeated applications of hydrochloric acid or caustic potash to the skin of mice over prolonged periods resulted in production of papillomatous and ulcerative lesions. The tumors seemed to be of inflammatory character and possibly presented precancerous alterations in epithelium.

2. In some strains applications of KOH or HCl elicited formation of tumors which presented morphologically as well as biologically the characteristics of a cancer.

3. Some strains of mice were refractory to experimental production of cancer, disregarding whether tar, KOH, or HCl was used.

In conclusion we desire to acknowledge our indebtedness to Dr. William F. Petersen of University of Illinois and to Dr. Lloyd Arnold of Loyola University for giving us the conveniences to conduct these experiments.

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* Method for removing the peel from fruit and vegetables.
Plotkin, M.
Israeli Patent 30 124 (1971) (En)
Pears, peaches, apples, tomatoes etc. are immersed for a short period, preferably 5-9 sec, in an aqueous solution of $CaCl_2$, NaOH or glycerine, while the solution is at its bp of 120 C. W&Co

* Unable to find after several searches. Wrote U.S. Patent Office for a copy, if abailable, December 12, 1973.

ance; these elevated nodules were noticeable in 4 to 5 months. Pluricentric formation of these new growths had been observed in nearly every case. They soon were covered with thick horn masses, thus resembling the cutaneous horns encountered in human subjects.

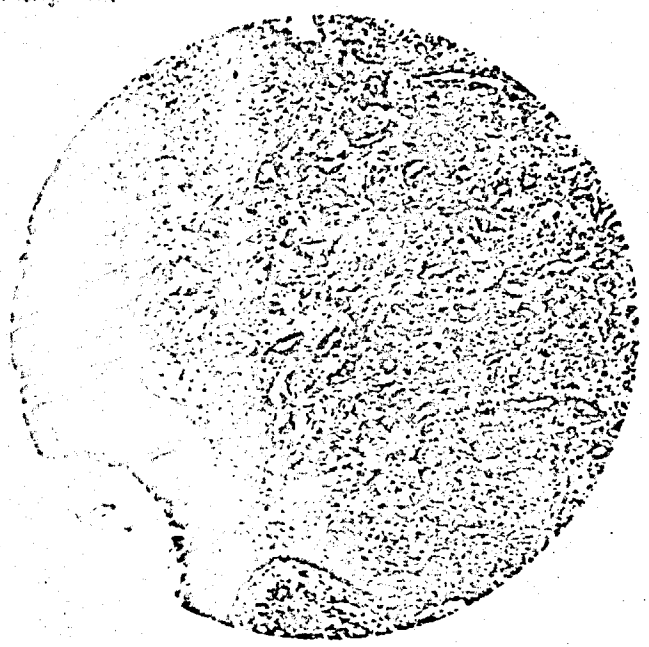

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FIG. 7. [illegible] TISSUE. Ten weeks after the beginning of applications of KOH; horn masses removed; the edge of the ulcer visible on the left side.

After interruption of the chemicals had been discontinued, three different changes were observed.

1. In about 50 per cent of the mice which survived the repeated irritation with KOH or HCl spontaneous retrogressive changes took place. In 2 to 3 weeks the painted areas were transformed into scars; in many cases the skin regained nearly normal appearance and seemed to have recuperated completely; a permanent alopecia was the only remaining visible result of the experiment.

2. In about 29 per cent of the cases the treated areas assumed the appearance of chronic eczema; superficial ulcerations,

excoriations and formation of scabs and crusts were observed. These skin affections remained stationary; they did not yield to the treatment with salves used in therapeusis of eczema in human beings.

3. In about 15 per cent of the cases, the painted areas continued their evolution after cessation of the irritation. They gradually increased in dimension for a certain time and invaded

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FIG. 8. TUMOR. Forty-two weeks after beginning of applications of HCl. Atypical proliferation of epithelium

contiguous tissues. They penetrated subcutaneous fat, muscles, and finally broke into the peritoneal cavity; metastases in the lymph-glands or other organs were never found. The tumors caused a marked emaciation and cachexia of the animals. All attempts of auto- and homo-transplantation of the tumors failed. Recurrences were observed after a wide excision of the tumors in six animals, out of nine in which this experiment was made. In order to follow up the development of histo-

avoided. The applications were repeated for about 4 to 6 weeks from the time of the first appearance of papillary growths and then discontinued. The total period of applications ranged from 25 to 46 weeks. The span of life of mice under laboratory conditions is frequently much shorter than the time required

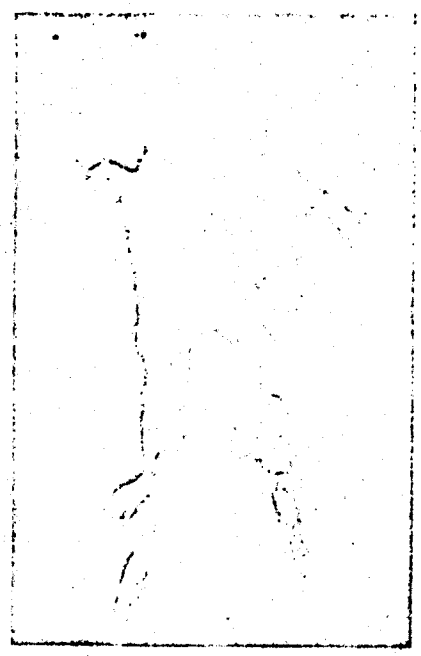

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Fig. 5. Tumor. Seven months after beginning and four weeks after cessation of painting with KOH. Histological diagnosis: cancer

for production of tumor. The longevity of rats induced us to use them in addition to mice for our experiments but neither cancer nor sarcoma developed in any rat. Enormous hyperkeratosis was the only result of the treatment.

Crude coal tar (*Ol. Lithanthracis*) was used as a control in order to determine the susceptibility of the animals of the same strain to the tar cancer.

The skin on the dorsum in the sacral region was painted without any previous epilation. Every mouse was kept in a

separate cage. White and gray mice, both sexes of four different strains were used; their ages varied at the beginning of the experiment from 1 to 6 months.

The macroscopic as well as microscopic effects of hydrochloric acid, caustic potash, and crude coal tar were nearly the same, so that they can be described together. The only noticeable difference was in the amount of horn substance which was much greater when hydrochloric acid was used as compared with effects of caustic potash and even tar.

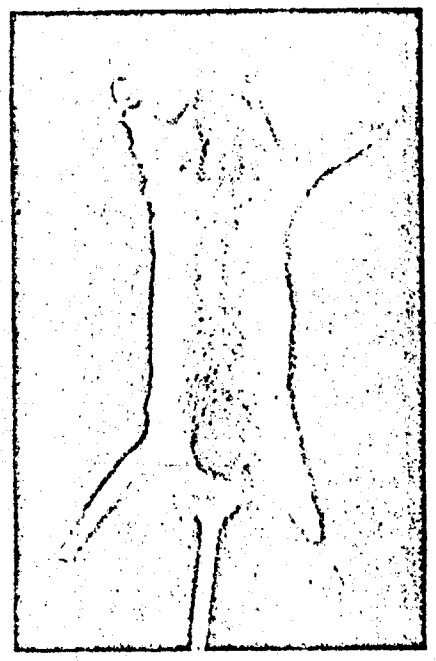

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Fig. 6. Tumor. Seven months after beginning and six weeks after cessation of painting with HCl. Histological diagnosis: cancer

The first visible effects of the painting of the skin with KOH or HCl was the falling out of hair. The initial lesion after alopecia which appeared in about 2 to 4 weeks, was pachydermia which was marked in ca. 10 weeks. The painted areas gradually developed into sessile papillomatous growths of a warty appear-

Lye-Dipping for the Removal of Objectionable Skin Color From Various Grades of Shelled Spanish Peanuts

J. POMINSKI, E. J. McCOURTNEY, M. F. STANSBURY, E. L. D'AQUIN, and H. L. E. VIX,
Southern Regional Research Laboratory,[1] New Orleans, Louisiana

MUCH interest has been shown in the production of solvent-extracted meals and light-colored proteins from peanuts (2, 3) for nutritional and industrial applications. By their presence red peanut skins discolor meals, and the pigments in such skins impart a dark brown color to protein during processing. These color difficulties may be avoided by using white-skinned peanuts (5), but their availability is limited. Light-colored proteins may be produced by controlling the pH at which they are precipitated (7), and still lighter proteins (cream color) may be produced by treating the kernels with a dilute solution of sodium hydroxide for removal of objectionable skin color (3, 4).

Pilot-plant work on peanut kernels and oil and protein losses, using the sodium hydroxide treatment on 100-g. portions of kernels, have been reported by Burnett (3). The present paper reports on the application of the sodium hydroxide treatment, commonly referred to as lye dipping, to large amounts of the three commercial grades of shelled Spanish peanuts. Data are given on oil and protein losses of these kernels after treatment, and on the protein solubility of materials from various steps involved in the preparation of solvent-extracted meal from the original shelled kernels. Information is included on the color of proteins prepared from meals produced by solvent extraction of treated kernels.

Materials

Commercial U. S. No. 1 shelled Spanish peanuts were used in an initial series of tests of several dilute sodium hydroxide treatments. Upon completion of these tests another lot of U. S. No. 1 plus lots of U. S. No. 2 and oil mill stock shelled Spanish peanuts were evaluated using a common method of lye treatment. Spanish peanuts were used in both series since this type is the most widely distributed in this country.

[1]One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

TABLE I

Grading Composition of the Shelled Spanish Peanuts

Fractions	Shelled Peanut Grades			Standards for U. S. No. 1 (9)
	U. S. No. 1	U. S. No. 2	Oil Mill Stock	
	%	%	%	%
Whole	94.6	29.0	37.3	92.9
Splits	2.7	62.2	20.6	2.0
Off color	1.9	0.9	0.8	1.25
Damaged	0	2.5	11.7	0.75
Unshelled	0.6	0	0	
Foreign matter	0.2	0	1.1	0.10
Shrivels	0	4.1	20.0	2.00
Pieces	0	1.3	8.5	0
Other varieties	0	0	0	1.0

The composition of the three commercial grades[2] of kernels used in the second series of tests in this investigation is shown in Table I, in which standard requirements for U. S. No. 1 grade are also given. The lipids and nitrogen contents of these shelled peanuts are given in Table II.

TABLE II

Analyses of Shelled Spanish Peanuts, Moisture-Free Basis

Material	Lipids	Nitrogen
	%	%
U. S. No. 1	52.49	4.96
U. S. No. 2	50.29	5.10
Oil mill stock	44.26	5.15

Method of Treatment

Batches of peanut kernels were placed in baskets made from 16- by 16-mesh galvanized wire-screen cloth backed by 4- by 4-mesh galvanized hardware cloth, which was supported by an iron frame (3).

[2] Commercially shelled Spanish peanuts are usually available in three grades, U. S. No. 1, U. S. No. 2, and oil mill stock grades. Standards (9) have been set up by the Department of Agriculture for U. S. No. 1 and No. 2 peanuts, which are essentially whole and split peanut kernels, respectively. Oil mill stock consists of small kernels, shrivels, pieces, and damaged kernels rejected during grading operations. The amounts of the three commercial grades of shelled peanuts produced by conventional shelling and drying operations from a ton of peanuts depends upon the original grade of the unshelled peanuts (Farmers Stock U. S. No. 1, 2, or 3) and the moisture content of the kernel. The yield from a ton of peanuts of U. S. No. 1 shelled Spanish peanuts ranges from 1,130 to 1,480 lb.; U. S. No. 2, 130 to 350 lb.; and oil mill stock 80 to 240 lb. (6).

The basket of kernels was dipped into an agitated solution of sodium hydroxide. The basket was then drained of the lye solution and the batch washed while in the basket successively in several tanks of water; the wash water also was agitated with a stirrer. The batch was then dried at 120° to 125°F. in a tray dryer, using circulating air.

Effects of Different Concentrations and Temperatures (U. S. No. 1 Peanut Kernels)

Experiments were made on three batches, each of approximately 120 lb. of kernels from U. S. No. 1 peanut kernels, using the following conditions of dipping and alkali concentrations:

a) Dipping for 5 minutes in 0.1% sodium hydroxide solution at 125°F.

b) Dipping for 5 minutes in 0.5% sodium hydroxide solution at room temperature.

c) Dipping for 1 minute in 0.5% sodium hydroxide solution at room temperature.

All treated peanut kernels were dried to approximately 8% moisture.

The lipids and protein losses after treatment are given in Table III. The wash solutions gave the only reliable data since the losses were so low. Lipids were determined in these wash solutions by an adaptation of an acid hydrolysis method (1). The losses reported here when compared with the figures reported by Burnett (3) show that the present lipid values are the lower. His figures varied between 0.21 and 0.36% on shelled peanuts for lipids and between 0.19 and 0.32% for protein. The protein losses on Batches 1 and 3 are practically the same as those observed by Burnett. In general, the lipids and protein losses, though not high in any instance, were smaller with cold dipping than with hot dipping.

Analyses of the dried skins of the kernels before and after alkali dipping showed a loss of lipids during dipping. Whereas the average lipids content of the skins before dipping baskets 1 and 2 was 17.79%, after undergoing the lye treatment and drying, the lipids content, m.f.b., was 10.25%. The skins have a much higher moisture content than the kernels.

TABLE III

Lipids and Protein Losses of U. S. No. 1 Peanut Kernels After Treatment

Batch Nos.	Peanut Wt.	Peanut Treatment					Losses			
		Tank No.	Time	Temp.	NaOH[a] or H_2O	H_2O Gained % Original Peanuts	Lipids		Protein[c] % Peanut Wt.	Nitrogen[d]
							Peanut Wt.	Lipids[b]		
	lb.		min.	°F.			%	%		%
1[e]	119.0	1	5	72	0.5% NaOH		0.02	0.04	0.10	0.33
		2	5	72	H_2O		.....	.....	0.05	0.16
		3	5	72	H_2O		.....	.....	0.01	0.04
		4	5	72	H_2O		.....	.....	0.01	0.02
					Totals	38.7			0.17	0.55
2[e]	119.0	1	5	125	0.1% NaOH		0.03	0.07	0.09	0.28
		2	5	72	H_2O		.....	.....	0.09	0.30
		3	5	72	H_2O		.....	.....	0.05	0.16
		4	5	72	H_2O		.....	.....	0.04	0.14
					Totals	44.9			0.27	0.88
3[e]	120.2	1	1	72	0.5% NaOH		0.02	0.05	0.04	0.12
		2	5	72	H_2O		.....	.....	0.09	0.30
		3	5	72	H_2O		.....	.....	0.03	0.10
		4	5	72	H_2O		.....	.....	0.03	0.09
					Totals	37.0			0.19	0.61

[a] Ratio of solution to kernels in all cases was approximately 15 to 1 in the NaOH dip and first washing and 11 to 1 in other washings.

[b] Per cent lipids = $\frac{\text{Lipids loss}}{\text{Lipids in original kernels}} \times 100$.

[c] Protein = 6.25 × nitrogen.

[d] Per cent nitrogen = $\frac{\text{Nitrogen loss}}{\text{Nitrogen in original kernels}} \times 100$.

[e] Fresh liquids were used in all dippings and two washings were carried on in Tank 1.

Preparation of proteins. The treated peanut kernels from Batches 1, 2, and 3 (Table III) were cracked and flaked and then solvent-extracted (8) with commercial hexane at an average temperature of 64°F. The solvent extracted meal was air-dried and then oven-dried at 125°F. for six hours. Table IV shows analyses of materials from Batch 3 at various steps of processing. Protein solubility of the extracted products, in every case over 90%, determined with sodium hydroxide solution at pH 8 and at room temperature, showed practically no change from the protein solubility of the unextracted treated kernels.

TABLE IV

Analyses at Various Steps of Processing

Description (Batch 3)	Analyses			N Solution at pH 8
	H_2O	Lipids	Nitrogen	
	%	%	%	%
Original peanuts	6.54	46.09	4.95	92.8
Flaked, dipped peanuts before extraction	7.45	46.35	4.86	92.2
Solvent extracted flakes after oven drying at 125°F.	5.49	0.60	10.28	91.0

Proteins from the three batches of the alkali-dipped solvent-extracted meals were made in the pilot plant (2) at room temperature by peptizing meal at pH 7.5 with sodium hydroxide and precipitating protein from clarified solutions at pH 4.5 using sulfur dioxide, then drying in an air-circulating oven at 120°F. Visual examination showed the proteins to be a light cream color; no other evaluation of color is reported.

Results Using Three Commercial Grades

The significant result for the initial experiments (Table III) was that in general the lipids and protein losses were smaller with cold than with hot dipping. The cold dipping as applied by Burnett was used with the three commercial grades as follows:

Fifty-pound batches of shelled peanuts from U. S. No. 2 and oil mill stock grades and a 40-pound batch of U. S. No. 1 shelled peanuts were dipped for 5 minutes in a 0.5% sodium hydroxide solution at 84°F., washed 3 times with water at 80°F., and oven-dried at 120°F. to approximately 4.5% moisture. The lipids and protein losses are given in Table V.

Data show that protein and lipids losses increased with lowering of grade. Lipids losses increased from 0.13 to 1.24% and protein losses increased from 0.53 to 1.99% by kernel weight. Lipids and protein losses were rather high on U. S. No. 2 and oil mill stock materials. A large portion of the losses was in the washings. Oil mill stock kernels absorbed more moisture than other grades of shelled peanuts.

TABLE V

Lipids and Protein Losses of Shelled Peanuts After Treatment

Peanut Grades	U. S. No. 1	U. S. No. 2	Oil Mill Stock
Wt. of peanut kernels, lb.	40	50	50
Moisture, %	7.37	7.42	8.51
Temperature of NaOH solution, °F.	84	84	84
Times washed with H_2O	3	3	3
Temperature of wash water, °F.	80	80	80
Moisture absorbed, % original wt.	36.8	36.2	49.3
Dipping losses			
Lipids, % peanut weight			
NaOH	0.06	0.36	0.78
Wash	0.07	0.43	0.46
Total	0.13	0.79	1.24
Lipids, % lipids			
NaOH	0.12	0.78	1.94
Wash	0.14	0.92	1.14
Total	0.26	1.70	3.08
Protein, % peanut weight			
NaOH	0.16	0.20	0.81
Wash	0.37	0.86	1.18
Total	0.53	1.06	1.99
Protein, % protein			
NaOH	0.55	0.68	2.76
Wash	1.27	2.91	4.00
Total	1.82	3.59	6.76
Drying time, hrs.	23.83	23.58	23.83
Drying temperature, °F.	120	120	120
Moisture after drying, %	4.35	4.57	4.86

To check the effect of damaged and shriveled peanut kernels, small amounts of 100% shrivels and 100% damaged kernels (visibly decayed) were hand-separated from the oil mill stock grade and treated with 0.5% sodium hydroxide solution.

Preparation and color of proteins. Portions of 500 to 1,000 g. of alkali-treated kernels from the various grades and the hand-separated lots were flaked and solvent-extracted for oil removal in large Soxhlets using commercial hexane and were air-dried.

Proteins were prepared in the laboratory from the solvent-extracted meals, using the same chemical conditions as those used in preparing proteins in the pilot plant; i.e., peptization at pH 7.5 using sodium hydroxide, precipitation at pH 4.5 using sulfur dioxide, and drying of the protein at 120°F. in an air circulating oven. The dried proteins prepared from meals produced from U. S. No. 1 and U. S. No. 2 kernels had a creamy color with no discernible differences between them. The protein prepared from meal produced from 100% damaged kernels had a brown color while that from the shrivels and oil mill stock grade gave an amber and tan color, respectively, showing that damaged kernels cause discoloration in protein. Analyses for extractable skin pigments by a colorimetric method being developed at the Southern Regional Research Laboratory indicated that the dark color of protein prepared from damaged kernels is not due to extractable skin pigments but rather to other constituents.

Use of the Treatment

These studies were part of extensive pilot-plant applications of Burnett's alkali treatment for objectionable peanut skin color removal at the Southern Regional Research Laboratory. Over eight tons of shelled peanuts have been so treated. Six tons of the treated kernels were extracted with commercial hexane in a continuous extraction pilot plant (10), and approximately two tons were solvent-extracted in the batch-extraction pilot plant (8). The meal products were used in a wide variety of industrial and nutritional investigations.

Summary

Experimental data have been obtained on the lipids and protein losses in the lye treatment of the various grades of shelled Spanish peanuts. It has been shown a) that lipid and protein losses on U. S. No. 1 shelled peanuts are lower for the cold than for the hot treatment though both are of a low level: b) that these losses in the cold treatment increased with the use of lower grade shelled peanuts, U. S. No. 2 and oil mill stock: c) that protein solubility of peanut kernels was negligibly affected by lye solution treatment, drying at 125°F., cold solvent extraction with hexane, air-drying, and oven-drying at 125°F.; and d) that damaged peanut kernels imparted color to protein.

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CHRONIC TOXICITY STUDIES OF DIETHYLSTILBESTROL

I. ORAL ADMINISTRATION BY STOMACH TUBE TO RATS *

H. [illegible] RUSSELL, R. C. PAGE, C. S. MATTHEWS,
C. L. SCHWABE AND F. E. EMERY

From the Laboratories of Pathology, St. Agnes Hospital, and the Departments of Physiology and Materia Medica, University of Buffalo

WHITE PLAINS AND BUFFALO, NEW YORK

[illegible] of the conflicting reports in the literature regarding the toxicity of stilbestrol in normal animals. In many of these reports [illegible] evidence of controlled studies. The doses of stilbestrol are frequently [illegible] and so far above the established therapeutic dose range that the pathological changes reported are of little significance when considering the drug for clinical [illegible]. For these reasons this chronic toxicity study of the effects of stilbestrol in various dose ranges and in two different media was conducted.

METHOD OF STUDY

[illegible] dose levels of stilbestrol were established for this study on rats as follows:

(a) [illegible] times weekly (75 [illegible] weekly/kg. body weight of rat) which is more than [illegible] as great as the average human therapeutic dose (1 mg. daily for a 50 kg. [illegible] a weekly dose of [illegible] mg. of stilbestrol for the human being.

(b) [illegible] hundred and fifty [illegible] stilbestrol [illegible] times weekly in rats (3.75 mg. weekly/kg.) which is more than 25 times as great as the average human therapeutic dose or corresponds to a weekly dose of 187.5 mg. of stilbestrol for the human being.

(c) [illegible] stilbestrol 3 times weekly [illegible] (15 mg. weekly/kg.) which is about [illegible] times as great as the average human therapeutic dose or corresponds to a weekly dose of [illegible] stilbestrol for the human being.

(d) [illegible] stilbestrol 3 times weekly, [illegible] (75 mg. weekly/kg.) which is more than 500 [illegible] as great as the average human therapeutic dose or corresponds to a weekly dose of 3750 mg. of stilbestrol for the human being.

The [illegible] in these experiments were of Wistar Stock inbred for many generations. Healthy albino females and males, and castrated females were used. The diet used for the rats was Purina Dog Chow which has maintained the colony in good health, [illegible].

The [illegible] stilbestrol used throughout was chemically pure.[1] As stilbestrol [illegible] 95% ethyl alcohol or sodium hydroxide solution, [illegible] the sodium hydroxide solution was [illegible] stilbestrol that was to be used for a group of [illegible] normal NaOH solution (0.5 cc. N NaOH [illegible]) [illegible] diluted with distilled water until the number of [illegible] to the doses to be given, e.g., 20 animals

[illegible] Wel[illegible] York.

TABLE 1. NORMAL FEMALE RATS *

No. animals in each group	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5		
	5	[illegible]	25	5	12		
Medium	Dilute NaOH	Dilute NaOH	[illegible] NaOH	1 cc. 40% EtOH	40% ethyl alcohol		
Dose stilbestrol 3X week	Control	500 γ (2.5 mg.)	[illegible] (0.25 mg.)	Control	[illegible] 250γ (0.25 mg.)		
Duration of study, days	93	[illegible]	84	41	46	77	100
Total dose stilbestrol, mg.	0	1.95	9	0	5	8.25	10.5
No. animals used for histological studies	1	1	4	2	2	2	2
Av. wt. at beginning of experiment, gm.	187	[illegible]	184	285	193	191.5	184
Av. wt. at end of experiment, gm.	210	[illegible]	195	284	135	214	160
% change in weight	+12	[illegible]	+8.1	−0.3	−30	+11	−13
Liver: Microscopic	Normal	Normal	Normal		Normal	Normal	Normal
Liver: Weight, gm.		7.5; 3.94; 3.79	9.35; 4.79; 3.79				
Kidney: Microscopic	Normal	[illegible] Normal; 1 Cloudy [illegible]	1 Normal; 2 Cloudy swelling		Normal	1 Cloudy swelling; 1 Normal	1 Cloudy swelling; 1 Normal
Kidney: Weight, gm.		[illegible]	[illegible] 0.77; 0.80				1.73; 0.81; 0.8[illegible]
Spleen: Microscopic	Normal	[illegible]	Normal		Normal	Normal	Normal
Spleen: Weight, mg		[illegible]	[illegible] 265; 366				
Adrenal: Microscopic	Normal	Normal	Normal		1 Hemorrhagic area in cortex; 1 Normal	Normal	Normal
Adrenal: Weight, mg.	30.3; 24.6; 31.6	[illegible]	29.4; 25.3; 31.6		42.5; 31.5; 31.6	39.4; 27.7; 31.6	32.8; 33; 31.6
Pituitary: Microscopic	Normal	[illegible]	Hyperplasia ant. lobe		Hyperplasia ant. lobe	Hyperplasia ant. lobe	Hyperplasia ant. lobe
Pituitary: Weight, mg	1[illegible]; 6.6; 5.3	[illegible]	12.5; 6.8; 5.3		16; 11.8; 5.3	16.2; 7.5; 5.3	11.2; 6.9; 5.3
Stomach	Normal	[illegible]	Normal	Normal	1 Atrophy of mucosa; 1 Normal	Normal	Normal
Esophagus	Normal	[illegible]	Normal	Normal	1 Normal; 1 Traumatic ulcer	Normal	Normal
Thyroid: Microscopic	Normal		Normal		Normal		Normal
Thyroid: Weight, mg			[illegible] 39; 20.6				
Uterus: Microscopic	Normal	[illegible]	[illegible] hyperplasia	Normal	1 Normal; 1 Endometrial hyperplasia	Endometrial hyperplasia	Endometrial hyperplasia
Uterus: Weight, mg.	829; 395; 217	[illegible]	[illegible] 289; 217			803; 375; 217	266; 166; 217
Ovary			[illegible] glandular epithelium	Normal			
Mammary gland	Normal		Hypertrophy of glands, cystic dilatation of ducts			Hyperplasia of ducts with cyst formation	
Ovary: Microscopic	Normal	[illegible]	[illegible] atrophy		Marked atrophy	Marked atrophy	Marked atrophy
Ovary: Weight, mg.	49.1; 23.3; 32.1	[illegible]	15.4; 7.0; 33.1		11.2; 8.5; 33.1	18.7; 8.7; 33.1	15.8; 9.8; 33.1

* [illegible] organ weights from [illegible] weight per 100 gm. body weight, control figures obtained from table 2 of Organ Weight Study by Emery, [illegible] (a) [illegible] colony.

to [illegible] 1 mg. each, then [illegible] of stilbestrol was dissolved in 0.5 cc. normal NaOH and diluted to 20 cc. with distilled water.

[illegible] the stilbestrol in the [illegible] was given by stomach tube (the details of [illegible] described in a separate paper). The stilbestrol was given to each [illegible] of animals 3 times [illegible]. All animals were weighed every 5 days and [illegible] on the female [illegible] examined and recorded daily (the results of these data [illegible] reported in a separate paper). At the end of the experiment the animals were [illegible] and a complete [illegible] performed on each. If any tissue or organ appeared [illegible] normal it was saved [illegible] study. In each group vital organs and endocrine glands of several animals were saved, regardless of gross appearance, for complete histologic study, and [illegible] were [illegible]. In addition bone marrow and [illegible] blood [illegible] were [illegible] of the animals. Ten normal animals were autopsied and tissues were studied histologically so that comparisons between the treated and untreated animals could be made for each tissue studied.

RESULTS OF STUDY

1. Normal Female Rats

Sodium hydroxide controls. [illegible] group of 5 animals received 1 cc. 1/200 N sodium hydroxide solution by stomach tube [illegible] times weekly over a period of 93 days (*Group 1*, table 1). These animals served as the sodium hydroxide control group. The sodium hydroxide solution was about [illegible] of the solute in which stilbestrol was dissolved for the groups that [illegible] stilbestrol in NaOH. The average weight gain [illegible] histologic study of vital organs (liver, kidney, spleen, stomach, esophagus), endocrine glands (adrenals, pituitary, thymus, ovary), and other organs (uterus, mammary [illegible]), showed no abnormalities and the individual organ weights per 100 gm. of body weight were within normal limits (8).

Alcohol control. A group of [illegible] animals received 1 cc. 40% ethyl alcohol by stomach tube every 3 days for [illegible] (*Group 4*, table 1). These animals failed to gain weight. The esophagus, stomach and endocrine glands showed no abnormalities on histologic examination.

Stilbestrol (50γ) in NaOH solution. Twenty animals received 50γ stilbestrol in a dilute [illegible] hydroxide solution by stomach tube 3 times weekly (750γ weekly/kg.) for [illegible] (*Group 2*, table 1). The total dose of stilbestrol for each animal was 1.95 mg. The animals failed to gain weight. At autopsy no gross pathologic lesions were found. Four of these animals were studied histologically. None of the vital organs or glands showed any significant change in weight except the ovaries which were about one-fifth of the average normal weight. Histologic examination showed no significant abnormalities in vital organs or glands except cloudy swelling in the kidney of one animal. The only histologic difference between this group and the control group were marked atrophy of the ovaries and moderate hyperplasia of the endometrium.

Stilbestrol (250γ) in NaOH solution. Twenty-five animals received 250γ stilbestrol in [illegible] sodium hydroxide [illegible] each tube 3 times weekly (3.75 mg. weekly/kg.) for [illegible] (*Group 3*, table 1). Each animal received a total of 9 mg. of stilbestrol [illegible] during the period of study was 8.1%. At autopsy [illegible] gross pathologic changes or any significant change [illegible] which were about one-fourth of the average [illegible] which [illegible] about one-half the weight of the [illegible] studied histologically and it was found [illegible] any abnormalities except cloudy swelling [illegible] variation from the normal was as follows:

TABLE 2. CASTRATE FEMALE RATS

No. animals in each group	GROUP 1	GROUP 2				GROUP 3				GROUP 4
	12	10				6				5
Menstruum	Dilute NaOH	Dilute NaOH				Dilute NaOH				40% E+OH
Dose stilbestrol, 3X weekly	2500 (0.05 mg.)	5 mg.				5 mg.				5 mg.
Duration of study, days	52	14	21	28	42	14	21	28	42	28
Total dose of stilbestrol, mg.	5.25	6	9	12	18	30	45	60	90	[illegible]
[illegible]	1	1	1	1	1	1	1	1	1	
[illegible]	134	133	183	237	184	184	136	166	211	
Av. wt. at end of experiment, gm.	219	151	184	187	184	147	136	183	183	
% change in weight	−6	−7	+2	−9	0	−4	0	−6.6	−16	
Liver: Microscopic	Normal	Normal	Early toxic necrosis	Normal	Early fatty degeneration	Areas of focal necrosis	Areas of focal necrosis	Normal	Fatty degeneration or central lobule region	Normal
Liver: Weight, gm.	10.59; 4.84; 3.70								10.08; 5.51; 3.79	
Kidney: Microscopic	Normal	Cloudy swelling	Cloudy swelling	Normal	Normal	Normal	Normal	Normal	Cloudy swelling	Cloudy Swelling
Kidney: Weight, gm.	1.48; 0.68; 0.80								1.58; 0.86; 0.80	
Spleen: Microscopic	Normal	Normal		Normal	Normal	Normal	Normal	Normal	Normal	Normal
Spleen: Weight, mg.									743; 406; 565	
Adrenals: Microscopic	Normal	Normal	Normal	Normal	Normal	Early cortical cell necrosis	Areas of hemorrhage in cortex	Normal	Normal	Early cortical cell necrosis
Adrenals: Weight, mg.	54.6; 24.9; 31.6		62.4; 33.9; 31.6	71.7; 38.2; 32.6	82.5; 44.8; 31.6		54.8; 40.3; 31.6	57.7; 31.4; 31.6	68.0; 36.6; 31.6	

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Stomach	Normal	Normal	Normal	Normal	Normal	Low grade gastritis	Normal	Normal	Normal	Normal
[illegible]	Normal	Normal	Normal	Normal	Normal	Normal	Normal		Normal	Normal
Uterus, Weight, mg.	[illegible]	[illegible]		[illegible]	[illegible]			[illegible]	[illegible]	
Vagina	Increased thickness of epithelial tissue								[illegible]	
Mammary glands	Generalized adenomatosis				Cystic dilatation of ducts			Cystic dilatation of ducts		
Thyroid, Microscopic									Normal	
Thyroid, Weight,[1] mg.									[illegible]	

[1] The organ weights from left to right are: actual weight, weight per 100 gm. body weight, control figures obtained from table 2 of Organ Weight Study by Emery, Emery, and Schwabe (8) on same rat colony.

marked atrophy of the ovaries, hyperplasia of the mammary glands, moderate hyperplasia of the endometrium and moderate hyperplasia of the anterior lobe of the pituitary gland.

Stillbestrol (250γ) *in 40% alcohol.* Twelve animals received 250γ stillbestrol in 40% ethyl alcohol by stomach tube 3 times weekly (*Group* 5, table 1). These animals were divided in 3 groups of 4 each and killed after 46, 77, and 100 days, respectively. The total dose of stillbestrol for each of these 3 groups was 5 mg., 8.25 mg., and 10.5 mg., respectively. The group as a whole showed a weight loss of 10.3%. At autopsy it was seen that one animal had had a hemorrhage into the adrenals, one had had a hemorrhage into the stomach and another had an ulcer of the esophagus. The individual organs showed no significant changes in weight except for a marked atrophy of the ovaries which were one-fourth the average weight of normal ovaries. Histologic examination of 6 animals (2 in each group) showed cloudy swelling of kidneys of 2 animals, adrenal cortical hemorrhage in one animal, moderate hyperplasia of the endometrium and of the anterior lobe of the pituitary gland in all animals. All mammary glands showed hyperplasia of ducts with cyst formation. Atrophy of the mucosa of the stomach was found in one animal and another animal had a traumatic ulcer of the esophagus.

2. *Castrate Female Rats*

Stillbestrol (250γ) *in NaOH solution.* Twelve animals received 250γ of stillbestrol in dilute NaOH by stomach tube 3 times weekly for a period of 51 days (*Group* 1, table 2). Each animal received a total of 5.25 mg. of stillbestrol. The animals lost an average of 6% in weight during the study. At autopsy none of the organs showed pathologic changes or any significant changes in weight from the normals. Two animals were studied histologically and none of the vital organs showed any pathologic changes. Endocrine glands and other organs showed moderate hyperplasia of the anterior lobe of pituitary gland, hyperplasia of the endometrium, and generalized adenomatosis of the mammary glands.

Stillbestrol (1 *mg.*) *in NaOH solution.* Ten animals received 1 mg. stillbestrol by stomach tube 3 times weekly for a period of 14, 21, 28 and 42 days (*Group* 2, table 2). These groups received during the period of study 6, 9, 12, and 18 mg. of stillbestrol, respectively. The weight changes in these 4 groups were −7, +2, −9 and 0%, respectively. At autopsy no pathologic changes were seen grossly. In the 14- and 42-day groups the pituitary gland weight was about twice normal weight. Five animals were studied histologically. One animal showed early toxic degeneration of the liver and another showed early fatty degeneration of the liver. Kidneys of 3 animals showed cloudy swelling. Hyperplasia of the anterior lobe of the pituitary and hyperplasia of the endometrium was evident in all animals.

Stillbestrol (5 *mg.*) *in NaOH solution.* Six animals received 5 mg. stillbestrol by stomach tube 3 times weekly for periods of 14, 21, 28 and 42 days (*Group* 3, table 2). These animals received a total of 30, 45, 60 and 90 mg. stillbestrol, respectively. The weight changes during the period of study were −4, 0, −6.0 and −16%, respectively. At autopsy the adrenals of 3 animals appeared hemorrhagic; the gastric mucosa was hemorrhagic in one animal. The liver of the animal that received 90 mg. of stillbestrol was found to be increased about 50% in weight over that of normals. The pituitary glands in general were increased in weight up to three times normal in the animal that received 90 mg. stillbestrol; in this latter animal the thymus was about one-half the weight of the normal gland. Four animals were studied histologically. Livers of 2 animals (1 per [illegible] day animals) showed focal areas of necrosis and one showed fatty degeneration of central lobule region (42-day animal). The liver of

the other animal was microscopically essentially normal. Kidneys were normal in all but the 42-day animal which showed cloudy swelling. Adrenals showed early [illegible] of cortical cells in one animal (14-day) and areas of hemorrhage in cortex of another (21-day) while the adrenals of the 28- and 42-day animals were entirely normal. The stomach of one animal (14-day) showed a low grade gastritis while the gastrointestinal tracts of the other animals were normal. All of the animals showed a marked hyperplasia of the anterior lobe of the pituitary and hyperplasia of the endometrium with one animal (28-day) showing squamous metaplasia of the endometrium.

Stilbestrol (5 mg.) in 40% alcohol. Five castrate female rats received 5 mg. in 40% ethyl alcohol by stomach tube 3 times weekly for 49 days (*Group 4*, table 2). The total dose of stilbestrol received by each animal was 105 mg. At autopsy no gross pathologic lesions were found. Body and organ weights were not obtained for animals of this group. One animal was studied histologically. The liver was normal. The kidney showed cloudy swelling. There was fatty infiltration of the cells of the adrenal cortex. Marked hyperplasia of the anterior lobe of the pituitary and of the endometrium was present.

3. *Normal Male Rats*

Twenty animals received 1 mg. stilbestrol in dilute NaOH by stomach tube 3 times weekly for 34 days, receiving during that time 11 mg. of stilbestrol (table 3). During the course of study the animals gained 6% in weight. Two of these animals were studied histologically. The pituitary gland showed hyperplasia of the anterior lobe. The testes were markedly atrophied weighing about one third of the normal. The cells in the spermatic tubules were thinned out with no spermia evident and spermatids were markedly reduced in number. Primary spermatogonia were the most prominent cells in the tubules. The interstitial cells also were greatly thinned out so that only an occasional island of cells was present. The prostate showed hyperplasia of the glands. The seminal vesicles showed a generalized chronic inflammatory reaction.

Blood Studies

Careful study of peripheral blood and bone marrow smears did not show any evidence of injury to the blood forming organs until the dose received was 15 mg. weekly. Two animals receiving this large dose showed hypoplasia of the bone marrow with leucopenia, thrombocytopenia, neutropenia, achromia and polychromatophilia.

DISCUSSION

In this study on the chronic toxicity of stilbestrol by the oral route, 130 animals were used. One hundred and ten of them received stilbestrol in varying doses; 10 treatment controls received only 50% ethyl alcohol or a dilute solution of sodium hydroxide and 10 were normal animals used for comparative histology. An autopsy was performed on all animals with careful inspection of their tissues and organs, with body and organ weights recorded. Peripheral blood and bone marrow smears were made on most of the animals. In the autopsy protocols there were recorded a description of any gross anatomic lesions that were encountered and histological preparations were made. In all groups regardless of gross findings representative animals were selected for complete histologic study. The tissues of 4[illegible] animals were studied microscopically.

The changes produced in these animals by stilbestrol may be divided into physiologic effects of the drug and pathologic changes.

Physiologic effects of stilbestrol. The most consistent physiologic effect on female rats was [illegible] atrophy which caused [illegible] in weight of the organ. His-

tologically there was a marked reduction in number and size of the follicles with a relative increase in the stroma. The pituitary showed mild hyperplasia when smaller doses of stilbestrol were administered. When the dose ranged from 1 to 5 mg. three times a week there was an increase in the weight of the gland from 40 to 200%. Histologically all of the sections showed hyperplasia of the anterior lobe.

Grossly many of the uteri were distended with fluid and cheesy material, and histologically there was endometrial hyperplasia. There was an increase in the thickness

TABLE 3. NORMAL MALE RATS

No. of animals		20
Menstruum		Dilute NaOH
Dose stilbestrol 3×weekly, mg.		1
Duration of study, days		34
Total dose of stilbestrol, mg.		11
No. animals used for histological studies		2
Av. wt. at beginning of experiment, gm.		183
Av. wt. at end of experiment, gm.		195
% change in weight		+6
Pituitary	Microscopic	Ant. lobe hyperplasia
	Weight,[1] mg.	10.6; 5.4; 3.2
Testes	Microscopic	Marked atrophy
	Weight,[1] mg.	729.1; 373.9; 1147.0
Prostate microscopic		Glandular hyperplasia
Seminal vesicles	Microscopic	Inflammatory reaction and congestion
	Weight,[1] mg.	60.7; 31.1
Stomach		Normal

[1] The organ weights from left to right are: actual weight, weight per 100 gm. body weight, control figures obtained from table 3 of Organ Weight Study by Emery, Emery, and Schwabe (8) on same rat colony.

of the vaginal epithelium. The ends of the uterine horns were enlarged and congested in many animals.

The mammary gland showed marked glandular hyperplasia and in many instances there was in addition cystic dilatation of the ducts, some of which contained retained secretions.

In those animals on which thymus weights were taken there was a considerable reduction in weight. Stilbestrol in male rats produced marked atrophy of all of the cellular elements of the testes and there was a 70% reduction in the weight of these organs.

Pathological effects of stilbestrol. Of the animals that received 50γ of stilbestrol 3 times weekly the only histological lesion found was cloudy swelling of the kidney of one animal of the 4 studied. No gross pathologic lesions were found at autopsy of any of the 20 animals that received this dose of the drug.

Of the [illegible] that received [illegible] stilbestrol 3 times weekly, cloudy swelling was found in [illegible] of the 12 animals studied histologically. Hemorrhagic areas were found [illegible] of the adrenal of one animal out of 49 animals autopsied.

The [illegible] that received [illegible] of stilbestrol 3 times weekly showed cloudy swelling of [illegible] of 6 of the [illegible] animals studied. In addition the livers of 4 animals showed [illegible] degeneration, one liver showed early toxic necrosis while the livers of the [illegible] animals were entirely normal.

The [illegible] that received [illegible] stilbestrol 3 times weekly showed cloudy swelling of the [illegible] of the 5 animals studied histologically. The livers showed focal necrosis in [illegible] fatty degeneration in one animal and were normal in the remaining 2 animals. [illegible] showed moderate hyperplasia (table 2) and histologically [illegible] cortical cell [illegible] in 2 animals, areas of hemorrhage in cortex of one animal [illegible] the adrenals of the remaining 2 animals were normal. One animal showed [illegible] gastritis.

In [illegible] animals that received stilbestrol in 40% alcohol ate poorly and lost considerable [illegible] weight than the animals in the corresponding sodium hydroxide group.

It [illegible] drawn conclusions from the reports of Loeser (1, 2) because they lack details [illegible] to the method of study, number of animals used, and whether control animals were studied. He [illegible] subconjunctival and nasal hemorrhage but the number [illegible] showing these phenomena was not stated. No studies of the blood were [illegible] and therefore the bleeding cannot be ascribed to injury to the blood-forming [illegible] by stilbestrol.

[illegible] that 0.1 mg. stilbestrol daily for 42 days produced congestion of the [illegible] that 1 mg. daily produced definite necrotic changes in the liver, spleen [illegible]. We found no such congestion in any of the animals receiving stilbestrol [illegible] of the [illegible] or kidney was found in any of our animals regardless [illegible] length of time stilbestrol was given.

The [illegible] effect of stilbestrol that has been described by some authors (4, 5) [illegible] to be present in only [illegible] of our animals; one had an atrophy of the gastric [illegible] (Group 5, table 1) and the other had a low grade gastritis (Group 3, table [illegible]). Our findings suggest that in the rats used in this study the large doses of stilbestrol [illegible] only a very low incidence of local irritation of the gastrointestinal tract. One [illegible] had a traumatic ulcer of the esophagus (Group 5, table 1) which can probably be ascribed to mechanical irritation caused by the passing of the stomach tube. [illegible] that our stomach tubing technique is a relatively safe procedure for rats [illegible] only one esophageal lesion although many of the animals were tubed as [illegible].

[illegible] in regard to blood forming organs agree essentially with those reported by [illegible] et al. (6) and Arnold (7). Inasmuch as no detailed figures were presented [illegible] on the changes in the blood it is difficult to make comparisons [illegible] indicate that injury to the blood-forming organs does not take place until [illegible] (approximately to rats) of stilbestrol are given that are approximately 500 times [illegible] human therapeutic [illegible]. Then the injury appears to cause a hypoplasia [illegible] with a depression of all the cellular elements of the peripheral blood [illegible] we interpret this as indicating aplastic anemia. In view of our findings of [illegible] to the blood-forming organs [illegible] massive doses of stilbestrol we will [illegible] study of this effect of the drug which will be reported at a later date.

[illegible] was [illegible] nor [illegible] evidence of tumor formation in any of the animals.

Three of the ten normal rats studied showed cloudy swelling of the kidneys on microscopic examination. We attribute this to postmortem changes that occurred before fixation took place. We therefore feel that our findings of cloudy swelling of some of the kidneys of the animals that received stilbestrol cannot entirely be attributed to the drug.

SUMMARY

No gross pathologic lesions were observed in the rats that received 750γ/kg. weekly for 14 weeks (approximately 5 times the average human therapeutic dose). No pathologic changes were found on microscopic examination except for a single instance of cloudy swelling of the kidney.

Gross pathologic lesions observed at autopsy in the 49 rats that received 3.75 mg./kg. weekly for 7 to 14 weeks (approximately 26 times the average human therapeutic dose) were hemorrhagic gastric mucosa in one animal, traumatic ulcer of the esophagus in one animal and hemorrhage into the adrenals of one animal. Pathologic changes on microscopic examination were limited to cloudy swelling of the kidneys of 4 animals.

No gross pathologic lesions were observed in the rats that received 15 mg./kg. weekly for 2 to 6 weeks (approximately 107 times the average human therapeutic dose). Microscopic examination showed pathologic changes consisting of cloudy swelling of the kidneys of 3 animals, and liver damage in 2 animals.

Gross pathologic changes at autopsy of the 11 animals that received 75 mg./kg. weekly for 2 to 7 weeks (approximately 535 times the average human therapeutic dose) were adrenal hemorrhage in 3 animals and enlarged liver in one animal. Microscopic examination showed the following: liver damage in 3 animals, cloudy swelling of kidneys of 2 animals, adrenals of 3 animals showed toxic changes, gastritis was present in one animal, squamous metaplasia of the endometrium was present in one animal and blood studies of 2 animals showed hypoplasia of the bone marrow, leucopenia, neutropenia, thrombocytopenia, achromia and polychromatophilia. We interpret these blood findings as aplastic anemia.

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3,231,392
METHOD OF CURING MEAT

Louis Sair, Evergreen Park, Ill., assignor to The Griffith Laboratories, Inc., Chicago, Ill., a corporation of Illinois

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The present application is a continuation-in-part of Serial No. 330,618, filed December 12, 1963, now abandoned, as a continuation-in-part of Serial No. 246,539, filed December 21, 1962, now abandoned.

The present invention relates generally to treating meat to improve its water-binding capacity. In particular, it relates to the cured meats.

In processing meat, particularly whole meat or canned meat products which are cooked in containing molds or in sealed cans, there is a tendency for the meat to shrink and purge aqueous material which collects as a gelatinous mass between the shrunken meat mass and the walls of the mold or can. To minimize this tendency, one or more mildly alkaline salts of phosphorus acids, such as pyrophosphoric, metaphosphoric and polyphosphoric acids, have been incorporated with the sodium chloride used in the processing with or without curing salts. When curing salts are used it is also customary to include an ene-diol compound, such as an isomer of ascorbic acid or a water-soluble salt thereof. The ene-diol compound stabilizes the color of cured meat. The term "phosphate" as used herein and in the meat industry, contemplates salts of orthophosphoric acid and salts of molecularly dehydrated phosphoric acid.

Fresh meat contains lactic acid which develops after slaughter, giving the meat a pH normally in the range from 5.5 to 6.2. The said mildly alkaline salts of phosphoric acid are so chosen that they are effective to neutralize at least some of the lactic acid and elevate the pH toward pH of 7. This elevation of the pH increases the water-binding capacity of the meat, thus minimizing purging when cooked.

One disadvantage of the phosphates is that residual phosphate salt within the meat has an effect on the taste of the meat, thus limiting the amount that should be used. Another and related disadvantage is the migration of said content from the meat to the surface on drying, as in the smokehouse, the migrated salt including the phosphate residue, thus imparting an undesirable surface taste and appearance. Certain desirable phosphate salts cannot be used in a pickle liquor in the desired amounts because the salinity of the brine presents dissolution of the desired amount. For example, in a case where 27 pounds of a certain phosphate are permitted by control authority, in 50 gallons of pickle to be used for pumping to 10% increased weight of the meat, the salting-out effect can be so great that not more than 16 pounds of the phosphate may be used.

In my copending application Serial No. 415,228, I have described incompatibilities between a meat-treating brine and alkaline materials useful in meat in such brine. One type of incompatibility is the inability to dissolve desired quantities of certain alkaline phosphate salts in a brine of a desired concentration of sodium chloride. Another type of incompatibility is the result of using a commercial grade of sodium chloride, as from rock salt, which introduces calcium ions into the brine, which from turbidity and which are precipitated by one or more of other de-

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sirable ingredients of a pickle liquor, namely, orthophosphate salts, pyrophosphate salts, carbonate salts, and alkali-metal hydroxides. According to that application the pickle liquor is provided in two solutions, one containing substantially all of the calcium-providing sodium chloride, and the other containing substantially all of the calcium precipitant.

When meat is to be cured by use of sodium nitrite or nitrate or both, provided in the pickle liquor, it is also a practice to provide an ene-diol compound which is an antioxidant effective to accelerate and improve the cure. Such ene-diol compound can be dissolved in either one of the two solutions, subject to certain limitations, the discovery of which is the basis of the present invention.

As stated above, there are numerous alkaline agents available for use to minimize purging. These may be divided into two classifications, namely, the high-pH agents, and the low-pH agents.

It has been discovered that ene-diol compounds, such as ascorbic acid, isoascorbic acid (erythorbic acid), and their water-soluble salts, are slowly decomposed in solutions containing high-pH alkaline agents, especially, tri-alkali-metal orthophosphate, normal alkali-metal carbonate, and alkali-metal hydroxide. It has also been discovered that such ene-diol compounds are stable in solution in the presence of low-pH alakine agents, especially di-alkaline-metal orthophosphate, alkali-metal metaphosphate, alkali-metal pyrophosphate, alkali-metal polyphosphates, and alkali-metal bicarbonate, all of which are useful as alkaline agents to minimize purging.

In consequence, when a high-pH alkali is to be used in a pickle liquor in meat, it should not be dissolved in advance in a solution containing an ene-diol compound subject to slow decomposition by it. The high-pH alkali and the ene-diol compound should be separately dissolved in different solutions to be combined substantially at the time of entering a piece of meat.

In separately dissolving the ene-diol compound and the high-pH alkali, the compatibility with other ingredients should be considered. For example, when precipitable calcium ions are present in a sodium chloride brine, the described high-pH alkalis should not be all dissolved in such brine, but all of the ene-diol compound may be so dissolved. When a purer grade of sodium chloride is used which does not provide precipitable calcium ions, then the high-pH alkaline agents may be present in the brine, provided the solubility of the desired amount is not repressed by the salt concentration.

Of the high-pH alkaline agents above described, the hydroxides and normal carbonates are preferred over the tri-alkali-metal phosphate, on account of the taste of the phosphate residue, and the hydroxide is preferred over the others on account of its leaving no salt residue.

It is the general object of the invention to provide two solutions to be combined to form a pickle liquor, one containing an ene-diol compound subject to slow decomposition in the presence of certain high-pH alkaline agents present in the other, said two solutions to be combined substantially at the time of introducing both of them into a piece of meat.

It is another object of the present invention to increase the water-binding capacity of meat not only with lesser amounts of salts of phosphorus acids, but even without any such salts.

It is a particular object of the invention to minimize shrinkage and purging by use of hydroxides and normal

carbonates of alkali-metal instead of only small amounts of salts of phosphorus acids.

The alkalis used are those with edible cations, namely, sodium and potassium. The essential alkaline materials used are one or more of the normal ortho-phosphates, the hydroxides and normal carbonates, and it is preferred to use the hydroxides alone or mixed with a normal carbonate when in the same solution. The alkaline agents of the present invention may not in all cases be merely substituted in a pickle liquor for the various phosphate salts. When the solution contains an ene-diol compound to stabilize the color of nitrite-cured meat, such as a water-soluble salt of an isomer of ascorbic acid, for example, sodium erythorbate, the hydroxide or normal carbonate of the present invention may not be present in such solution. However, it may be present in a solution lacking such an ene-diol compound. The ene-diol compounds so used are unstable at pH levels normally resulting from the presence of said alkali-metal normal orthophosphates or carbonates or hydroxides, in the presence of which they are readily destroyed. The destruction is not instantaneous but is gradual over a short period of time which does not permit holding a supply of the liquor containing the high-pH alkali and an ene-diol compound in a tank from which to draw a constant composition from time to time as is done in commercial practice.

When meat is injected with a pickle liquor, as in the arterial pumping of hams, or in the multiple-needle-impregnation of pork bellies, the liquor on entering the meat may contain incompatible ingredients, such as sodium ascorbate and sodium hydroxide, by combining two separate solutions in measured proportion substantially at the time of introducing the liquor to the meat. This may be done by combining two liquors just prior to entering a single-passage-needle, or just prior to entering or within a manifold delivering to a bank of needles, such as are used for pork bellies, or in the meat by use of a two-passage-needle, or by using two side-by-side single-passage-needles, or by stitch-pumping at separate multiple-needle injection stations, along a conveyor carrying a stream of meat pieces to be treated. In so doing, one solution may contain sodium ascorbate or its equivalent ene-diol compound, and the other may contain the strong alkali such as the normal phosphate, carbonate or hydroxide of sodium or potassium or a mixture of them. The alkali is neutralized by the meat before it has time to destroy the ene-diol compound.

Carbonates function for the neutralization purposes mentioned, but in some cases are undesirable because generation of carbon dioxide forms pinholes in the meat. When the water or solution in which the salt composition of the present invention is dissolved contains precipitable calcium ions deriving from commercial sodium chloride, and containing a phosphate, or carbonate or hydroxide, the composition may precipitate calcium thus rendering the liquid undesirably turbid, but not impaired in function. Precipitation and loss of the ene-diol compound may be avoided by placing the alkaline precipitant in one solution and the sodium chloride and the ene-diol compound in the other solution.

The two solutions referred to are drawn from storage tanks, each containing stable materials. These solutions are made by operators combining measured volumes of water with weighed amounts of supplied solid compositions. For example, one solution may contain all the ingredients used for curing, such as sodium chloride, sodium nitrite, sodium nitrate, sodium erythorbate, buffering agent, and any optional content of sugar or seasoning material, and the other may contain sodium hydroxide alone, or with all or part of all the ingredients except the ene-diol compound. The latter may be alone in its liquor or combined with all or part of all the ingredients except the high-pH alkalis.

Although the sodium ion is preferred for the alkaline agents as well as for any other agents used with meat, it is known that potassium compounds have long been used. It is known that potassium compounds tend to introduce bitterness to the meat. In fact, in practicing the present invention, the use of potassium hydroxide as all of the alkaline agent makes the meat undesirably bitter. In view of these facts, potassium compounds are not barred, but when used, are judiciously mixed with sodium compounds in proportions to avoid undesirable bitterness. Such mixtures apply not only to the alkaline agents but may involve proper selection of alkali nitrite and alkali nitrate.

To illustrate the effect of strong alkali on the ascorbic value of a curing pickle containing sodium erythorbate, Liquor No. 1 was made in proportion as follows.

Ingredient:	Percent
Water	78.5
Sodium chloride	15.85
Cane sugar	4.69
Sodium nitrite	0.151
Sodium nitrate	0.112
Sodium hydroxide	.56
Sodium erythorbate	.235

pH=11.6.

In preparing the above at 40° F. the last ingredient added was the sodium erythorbate. After 15 minutes, the content of the sodium erythorbate was immediately determined in one gram of pickle to be 1.03 mg. as against 2.43 mg. initially added, showing a loss of 58%.

The extent of destruction of the sodium erythorbate in a given time increases with the increased content of sodium hydroxide. Pickles were made similar to that described above with varying amounts of sodium hydroxide. After one hour, the ascorbic content was determined as shown below.

Lbs. of NaOH in 50 gallons of pickle	pH of pickle	Percent of sodium erythorbate remaining
3.0[1]	11.6	85
1.50	11.6	62
.75	11.4	50
.3	11.0	91

[1] The amount present in Liquor No. 1.

Tests made with a pickle having 3.5 lbs. of sodium hydroxide per 50 gallons show less purging of hams than is produced using a corresponding liquor with 16 lbs. of sodium tripolyphosphate per 50 gallons of pickle pumped to 10% increase of weight of the meat.

The following dry composition was prepared using sodium hydroxide and sodium bicarbonate as the alkali.

Ingredient:	Percent
Sodium nitrite	4.6
Sodium nitrate	3.4
Sodium chloride[1]	69.2
Sodium hydroxide	8.5
Sodium bicarbonate	14.3
	100.0

[1] Calcium-free.

A pickle having a pH of 10.25 was prepared using this dry composition according to Liquor No. 1, containing 35 pounds per 100 gallons, except that the sodium erythorbate was added last in proportion of 2.5 pounds per 100 gallons of pickle. After one hour, 82% of the erythorbate remained.

The destructive effect of a combination of an ascorbate salt and a strong alkali may be disregarded by maintaining each separately in a stable solution, alone or with other ingredients commonly used in pickle liquors. Sodium erythorbate is suitably stable when it is the only

solute in water. The following Example 1 shows a liquor in two parts which, when combined as described, constitute a complete curing pickle.

EXAMPLE 1

	Part 1 lbs.	Part 2 lbs.
Water	765	82.4
Sodium chloride [1]	106	
Sodium nitrite	1.9	
Sodium nitrate	1.2	
Cane sugar	34.0	
Sodium hydroxide	6	
Sodium erythorbate		2.5
Total	977.7	83.9
	pH=12.9	pH=7.75

[1] Calcium-free.

Part 1 makes 100 gallons and Part 2 makes 10.2 gallons.

The stability of Part 2 was checked and found to be as follows:

Hours later:	Percent remaining
0	100
1	100
2	96
3	96

To test the short-time stability of the pickle liquor combining Parts 1 and 2, the following procedure was used:

At zero time, 50 volumes of Part 1 and 5 volumes of Part 2 were mixed, then at given intervals thereafter in separate portions of the mixture, acid was added to effect a pH of 8 to 8.5 for arresting the destruction by strong alkali. The loss of ascorbate value was determined. The results were as follows:

Seconds later:	Percent loss of ascorbic value
15	30
30	60
60	85
180	95

From the foregoing it was found that two solutions which together make a pickle liquor, can be combined for short periods without appreciable destruction of ascorbic value contained in one liquor by strong alkali contained in the other liquor. Thus, two such liquors may be combined at the inlet end of a single-orifice injection needle, or even in a conduit ahead of said inlet. Since needles are idle for short periods between use on large quantities of meat bodies such as hams, the liquor standing in the conduit after combination may lose value. Accordingly, the path traveled after combination should be made as short as practicable to minimize loss of ascorbic value in combined liquor, which may be stagnant after combination. By the use of injection needles with two passageways therethrough, or double needles, no destruction can occur. In single-passage-needles, the union is preferably effected in a conduit or manifold shortly before reaching the needle, or at the entrance to the needle. The passage for the combined liquids in the conduit or needle should be small to limit a combined volume which may be stagnant.

However, the invention is applicable to the stitch-pumping practice either at one station or at two adjacent stations. A piece of meat on a conveyer is stich-pumped to a predetermined increase in weight by a controlled injection of one solution of the present invention, and then again pumped to a second predetermined increase in weight by controlled injection of the second solution of the present invention. The two liquids diffuse and mix within the meat to function as a single pickle liquor.

The term "substantially simultaneously" as used herein contemplates the time differentials of the commercial processing above referred to. In the two-station practice the two solutions cannot mix immediately upon injecting the second solution, since the two solutions must diffuse and mix within the tissues of the meat. Since the combined solution does not exert its function instantaneously, a moderate time interval between injections is considered to fall within the intended meaning of the term.

Two methods of injection are referred to in the drawing, in which:

FIG. 1 illustrates diagrammatically the proportioning of two solutions for use with a needle for artery pumping.

FIG. 2 represents two-station multiple-needle injection of hams with different solutions in a mass production line.

FIG. 3 is a plan view of the apparatus of FIG. 2, taken on line 3—3 of FIG. 2.

Numerals 10 and 11 illustrate, respectively, separate supplies of the two liquors from which supplies the liquors are drawn to proportioning pumps 12 and 13 operated in synchronism as indicated by a common motive source 14. Pump 12 delivers into conduit 15 successive increments 16 of liquor from supply 10. These increments are illustrated by relatively long and heavy lines.

Pump 13 delivers into conduit 17 increments 18 from supply 11. The increments 18 are illustrated by lines which are shorter and lighter than the lines indicating increments 16, thus to indicate that the content of increments 16 is greater than the content of the increments 18.

The proportioning pumps 12 and 13 are standard commercial items which may be operated so that the increments 16 alternate in succession with increments 18. They also may be operated so that the alternating increments 16 and 18 overlap to predetermined extents.

Conduits 15 and 17 are illustrated as combining their contents in a conduit 20 in which the increments 16 are illustrated as overlapping with the increments 18. The confluence of the two liquors indicated by the numeral 22 is located very close to the end of a discharge needle 24 such as is conventionally used in artery pumping of hams. The travel distance from the confluence 22 to the discharge end 26 of the needle 24 is controlled and made short as predetermined by the slow reaction time between the two liquors, so that substantially no change takes place in the combined liquor within the conduit 20 and the needle 24. Within the meat the alkali is neutralized by the meat before it is effective to reduce the content of the ene-diol compound.

To point out the versatility of the present invention, it is reiterated that it is the alkali contacting the meat that is effective. Alkalis useful for the purpose include some of lower pH not detrimental to the presence of the ene-diol material, and others of higher pH which are detrimental. Among the low-pH alkalis are alkali-metal bicarbonate, the metaphosphates, the pyrophosphates, the polyphosphates, and the di-alkali-metal orthophosphates. Among the higher pH ones are the hydroxides and normal carbonates of alkali-metal, trisodium phosphate and tripotassium phosphate. Since the tri-alkali-metal phosphates hydrolyze in water to the corresponding di-salt and the corresponding hydroxide, the hydroxide and the di-salt are preferred over the tri-salt since their proportions may be easily varied from the fixed proportions of the tri-alkali-metal phosphates alone.

In providing alkali to the meat, it is preferred to use no salts of a phosphorus acid, but these are not ruled out so long as one or more of the said higher-pH alkalis is supplied in one solution and the ene-diol compound in another. Thereby, when and if one or more of the phosphorus acid salts is used, it is not necessary to use an undesirable quantity thereof.

Example 2 gives a composition of a liquor for introducing alkali which contains disodium phosphate. Disodium phosphate is a low-pH alkali which can exist in a pickle with sodium erythorbate. However, disodium phosphate has a limited solubility in a curing pickle and

cannot be used in sufficient concentration to provide all the alkali. By substituting a portion of the disodium phosphate with a strong alkali such as sodium hydroxide, a commercial pickle can be obtained with all of the phosphate in solution.

EXAMPLE 2

	Parts by weight
Sodium chloride [1]	39.7
Sodium nitrite	4.6
Sodium nitrate	3.4
Sodium hydroxide	8.5
Disodium phosphate duohydrate	43.8
Total	100.0

pH=10.6.

[1] Calcium-free.

The 35 lbs. of the above composition made up to 100 gallons of pickle resulting in 3 pounds of sodium hydroxide and 15.6 pounds of the phosphate salt, which latter is completely in solution at 40° F., using in addition 166.4 pounds of sodium chloride, 25 pounds of cane sugar and 748 pounds of water. This is called Part 1. A second solution Part 2 was prepared containing 83.4 pounds of water and 5 pounds of sodium erythorbate.

In use by dual pumping according to FIG. 1, liquors from Parts 1 and 2 are used in proportion, respectively, of 10 and 1 volumes per 100 pounds of meat, when pumping to 10% increase in weight.

Few packers use calcium-free sodium chloride, but use a lixiviated brine from rock salt which contains calcium ions. The formulation of Example 2 above using such sodium chloride produces an undesirable precipitate, especially troublesome in multiple-needle injection. When such a precipitate is effected, Example 2 is changed as follows:

EXAMPLE 3

	Parts by Weight	
	Part 1	Part 2
Sodium chloride	180	
Sodium nitrite	1.61	
Sodium nitrate	1.11	
Sodium hydroxide		29.8
Disodium phosphate duohydrate		153
Cane sugar	25	
Water	748	83.4
Sodium erythorbate	0.5	

In use for 100 pounds of meat, 10 volumes of Part 1 is combined with one volume of Part 2.

The present invention is directed to packers who use calcium-free sodium chloride and also to those who use rock salt which introduces calcium ions. In essense, the present invention requires the ene-diol compound to be in one solution and the high-pH alkaline agents in a second solution. Since the high-pH alkaline agents and the low-pH alkaline agents include many which are calcium precipitants, it is also important to keep the calcium precipitants in one solution and the calcium-containing sodium chloride in a second solution.

The following Table I shows some of the combinations which are possible. In the center are listed most of the ingredients from which selections are made for pickle liquors. At the right are columns for Solution No. 1 and Solution No. 2, in which column X indicates that the alined ingredient can be present and compatible when the sodium chloride is calcium-free. At the left, the compatibilities are less because the sodium chloride introduces calcium ions in Solution No. 1, which makes the solution incompatible with all of the alkaline agents listed, except sodium tripolyphosphate ($Na_5P_3O_{10}$).

Table I

Solution No. 2	Solution No. 1		Solution No. 1	Solution No. 2
		$NaCl$	X	X
o	X	$NaCl(Ca)$		
X	o	$NaHCO_3$	X	X
X	o	Na_2CO_3	o	X
X	o	$NaOH$	o	X
X	o	Na_3PO_4	o	X
X	o	Na_2HPO_4	X	X
X	o	$Na_4P_2O_7$	X	X
X	X	$Na_5P_3O_{10}$	X	X
o	X	Ene-diol	X	o

In large establishments, in particular where hams are canned, the processing using one solution is quite different from artery pumping. Hereinbelow, the conventional processing is described in reference to a part of FIGS. 2 and 3.

In general, a conveyer, say two feet wide, is packed tightly from side to side with flexible boned raw hams, completely to cover the conveyer surface. The conveyer carries the hams through two stitch-pumping stations, at each of which they are injected with the pickle. The number of insertions of the needles is timed with the advance of the conveyer so that a known amount of pickle is introduced. In one form of apparatus there are two staggered rows of needles across the conveyer, each row having twenty-five needles. The needles are carried on a movable cross-head so that after they penetrate the mass of hams, they move with the conveyed hams while they are in the hams, then return to the initial position for the following insertion.

In this process, the hams are greately expanded by the forced insertion of the pickle under high pressure, with the result that some liquid leaks out on leaving the first station. At the second station, the injection is duplicated with the same pickle. Then the hams are immersed in another pickle for 24 to 48 hours after which they are drained, forced into a can, sealed and cooked.

In this processing there are limitations on the composition of the pickle, as described above, due to repressed solubility in some cases, and due to the formation of precipitate when using the said impure salt grades.

The present invention overcomes these limitations without need to alter the apparatus except as to supplies of solution to the two stations, with adjustment of the usual controls which measure the quantity of solution injected at each injection stroke.

Apparatus such as referred to above is commercially available and is represented by U.S. Patents Nos. 2,641,990, 2,674,179, 2,645,172, and others.

In FIG. 2 numeral 30 represents a conveyer moving over driving and supporting rolls 32. Boned hams H are packed on the conveyer at the left in FIG. 2 one ham thick, and carried through the first station.

The first station is represented graphically. Numeral 34 represents a cross-head carrying a bank of needles 36 in two rows (indicated in FIG. 3). The dotted line position 35 represents the advanced position of the cross-head as it is moved by the hams. The valves and volume controls are not shown, as these form no part of the present invention. Numeral 38 represents a tank at Station No. 1 for one solution with conduit means 40 to a pressure pump 42, and conduit means 44 from the pump to the needles 36.

Station No. 2 is identical with the first the parts being numbered the same, but as prime numbers. Tank 38' contains the second solution.

In operation of the present invention for two-station stitch-pumping, the success in proportioning depends not only upon the controlled proportioning of the two solution, but also on the uniformity of layer of hams H

on the conveyer. When the hams after injection are later immersed in a brine which may be comparable to the two solutions mixed, minor departures from exact proportioning of the two solutions for a particular ham are not important, as these are overcome by mutual diffusion of the injected solutions and the bath solution within the ham.

EXAMPLE 4

In the case of stitch-pumping at two stations spaced apart over a moving conveyer, different solutions are used at each station as follows:

	Station No. 1, pounds	Station No. 2, pounds
Water (100 gallons)	835	
Sodium hydroxide	10	
Sodium nitrite	2.0	
Sodium nitrate	1.62	
Water [1]		835
Sodium chloride [1] (rock salt)		75
Sodium erythorbate		4.5

[1] Together provided as 61.5° Salometer brine.

At Station No. 1, the hams are pumped to 10% increase in weight. At Station No. 2, the hams are again pumped to 12% increase in weight based on the raw ham. After pumping the hams are stored in a brine bath for 24 to 48 hours of the following composition:

Water (100 gallons)	lbs.	835
Sodium chloride	lbs.	75
Sodium nitrite	oz.	12
Sodium nitrate	oz.	8

In the foregoing it is to be observed that the strong alkali is in the meat first, and is in part neutralized by the meat before it is effective to destroy the sodium erythorbate later introduced at the second station. Thus, the prior practice of storing all the pickle content as one solution is avoided, during which storage the sodium erythorbate would be destroyed.

It is also noted in the above that the sodium hydroxide, which is a calcium precipitant, is also in the meat before calcium ions are introduced with the sodium chloride derived from rock salt. Thus, there is no common liquid to be stored, containing the calcium ions and the calcium precipitant, so as to form a precipitate which could plug needles, and affect valves in the two-station procedure.

The immersion of the pumped hams in pickle liquor tends to equalize the fluid pressures inside and outside the ham resulting in final retention of a desired amount of pickle liquor. On removal of the hams they are sealed in cans and cooked in a conventional procedure.

It is to be understood that the invention is not limited to and by the examples as will be clear by the difference in the examples given.

I claim:

1. In the method of treating meat by injecting pickle liquor containing sodium chloride, alkali-metal nitrite, alkaline material in quantity to neutralize lactic acid in the meat and elevate the pH of the meat toward 7 and to increase the water-binding capacity of the resulting cured meat, and ene-diol material subject to gradual destruction when in said liquor having an elevated pH due to the presence of the hereinafter-mentioned selected alkaline material as at least a part of said neutralizing material, the improvement in which the alkaline material includes edible high-pH alkaline material selected from the group consisting of tri-alkali-metal phosphate, alkali-metal hydroxide, normal alkali-metal carbonate, and mixtures thereof, in which said liquor is produced by combining two separate solutions, of which one of said solutions contains all of the ene-diol material and the other of said two solutions contains all of the selected alkali material, and in which the two solutions are substantially simultaneously injected into the piece of meat, whereby upon mixing the two solutions in the meat the acidity of the meat prevents the destructive reaction of the ene-diol material by the high-pH alkaline material.

2. The method of claim 1 in which the selected alkaline agent is alkali-metal hydroxide.

3. The method of claim 1 in which the ene-diol compound is a water-soluble salt of an isomer of ascorbic acid.

4. The method of claim 1 in which the two solutions combine in a conduit which is discharging the combined materials within the meat.

5. The method of claim 1 in which one solution is injected into the piece of meat and in which the second solution is injected into said piece substantially immediately after injecting said first solution.

6. In the method of treating meat by substantially simultaneously injecting two aqueous solutions together forming an alkaline curing liquor containing alkali-metal nitrite and ene-diol material subject to gradual destruction when in solution at elevated pH values and including edible alkaline material in quantity to elevate the pH of the meat toward 7 and to increase the water-binding capacity of the resulting cured meat, the improvement which comprises discharging into the meat successive increments of a first solution containing said ene-diol material and alternately injecting into the meat for combination with said increments substantially at the time of entering the meat successive increments of a second solution containing high-pH alkaline material selected from the group consisting of tri-alkali-metal phosphate, alkali-metal hydroxide, normal alkali-metal carbonate, and mixtures thereof, the increments of said two solutions being in predetermined proportions, whereby upon mixing the two solutions in the meat the acidity of the meat prevents the destructive reaction of the ene-diol material by the high-pH alkaline material.

7. In the method of treating meat by injecting pickle liquor containing sodium chloride, alkali-metal nitrite, alkaline material in quantity to neutralize lactic acid in the meat and elevate the pH of the meat toward 7 and to increase the water-binding capacity of the resulting cured meat, and ene-diol material subject to gradual destruction when in said liquor having an elevated pH due to the presence of the hereinafter-mentioned selected alkaline material as at least a part of said neutralizing material, the improvement in which the alkaline material includes edible high-pH alkaline material selected from the group consisting of alkali-metal hydroxide, normal alkali-metal carbonate, and mixtures thereof, in which said liquor is produced by combining two separate solutions, in which one of said solutions contains all of the ene-diol material and the other of said two solutions contains all of the selected alkali material, and in which the two solutions are substantially simultaneously injected into the piece of meat, whereby upon mixing the two solutions in the meat the acidity of the meat prevents the destructive reaction of the ene-diol material by the high-pH alkaline material.

8. The method of treating a piece of meat which comprises injecting into said piece at a plurality of locations a predetermined quantity, based on the weight of the piece, of one of two solutions, and substantially simultaneously and separately injecting into said piece at a plurality of locations a predetermined quantity, based on the weight of the piece, of a second solution, one of said solutions containing an ene-diol material subject to slow destruction when in solution with the hereinafter-mentioned high-pH alkaline material, the other of said two solutions containing high-pH alkaline material selected from the group consisting of tri-alkali-metal phosphate, normal alkali-metal carbonate, alkali-metal hydroxide, and mixtures thereof, said two solutions in solute and in quantity together in the piece forming a pickle liquor containing sodium chloride, curing salt selected from the group consisting of alkali-metal nitrite, alkali-metal ni-

trate, and mixtures thereof, said ene-diol compound, and edible alkaline material in amount to neutralize lactic acid in the meat and elevate the pH toward 7, said alkaline material essentially including said high-pH alkaline material, whereby upon mixing the two solutions in the meat the acidity of the meat prevents the destructive reaction of the ene-diol material by the high-pH alkaline material.

9. The method of claim 8 in which the ene-diol compound is sodium erythorbate.

10. The method of claim 9 in which the sodium chloride supplied introduces calcium ions and is present substantially all in one solution, and in which substantially all of the alkaline material precipitates calcium ions from solution and is substantially all present in the other of the two soutions.

11. The method of treating a piece of meat in a traveling succession of pieces of meat which comprises injecting into said piece at a plurality of locations a predetermined quantity, based on the weight of the piece, of one of two solutions, moving the piece to an adjacent station and injecting into said piece at a plurality of locations a predetermined quantity, based on the weight of the piece of a second solution, one of said solutions containing an ene-diol material subject to slow destruction when in solution with the hereinafter-mentioned high-pH alkaline material, the other of said two solutions containing high-pH alkaline material selected from the group consisting of tri-alkali-metal phosphate, normal alkali-metal carbonate, alkali-metal hydroxide, and mixtures thereof, said two solutions in solute and in quantity together in the piece forming a pickle liquor containing sodium chloride, curing salt selected from the group consisting of alkali-metal nitrite, alkali-metal nitrate, and mixtures thereof, said ene-diol material, and edible alkaline material in amount to neutralize lactic acid in the meat and elevate the pH toward 7, said alkaline material essentially including said high-pH alkaline material, whereby upon mixing of the solutions in the meat the acidity of the meat prevents the destructive reaction of the ene-diol material by the high-pH alkaline material.

12. The method of treating a boned ham in a traveling succession of hams which comprises injecting into the ham at a plurality of locations a predetermined quantity, based on the weight of the ham, of one of two solutions, moving the ham to an adjacent station and injecting into said ham at a plurality of locations a predetermined quantity, based on the weight of the ham, of a second solution, one of said solutions containing an ene-diol material subject to slow destruction when in solution with the hereinafter-mentioned high-pH alkaline material, the other of said two solutions containing high-pH alkaline material selected from the group consisting of tri-alkali-metal phosphate, normal alkali-metal carbonate, alkali-metal hydroxide, and mixtures thereof, said two solutions in solute and in quantity together in the ham forming a pickle liquor containing sodium chloride, curing salt selected from the group consisting of alkali-metal nitrite, alkali-metal nitrate, and mixtures thereof, said ene-diol material, and edible alkaline material in amount to neutralize lactic acid in the ham and elevate the pH toward 7, said alkaline material essentially including said high-pH alkaline material, whereby upon mixing of the solutions in the ham the acidity of the meat prevents the destructive reaction of the ene-diol compound by the high-pH alkaline material, sealing the ham in a can, and cooking the ham in the sealed can.

13. The method of claim 12 in which the ene-diol compound is sodium erythorbate.

14. The method of claim 12 in which the sodium chloride supplied introduces calcium ions and is present substantially all in one solution, and in which substantially all of the alkaline material precipitates calcium and is substantially all present in the other of the two solutions.

15. The method of treating a boned ham in a travelling succession of hams which comprises injecting into the ham at a plurality of locations a predetermined quantity, based on the weight of the ham, of one of two solutions, moving the ham to an adjacent station and injecting into said ham at a plurality of locations a predetermined quantity, based on the weight of the ham, of a second solution, one of said solutions containing an ene-diol material subject to slow destruction when in solution with the hereinafter-mentioned high-pH alkaline material, the other of said two solutions containing high-pH alkaline material selected from the group consisting of tri-alkali-metal phosphate, normal alkali-metal carbonate, alkali-metal hydroxide, and mixtures thereof, said two solutions in solute and in quantity together in the ham forming a pickle liquor containing sodium chloride, curing salt selected from the group consisting of alkali-metal nitrite, alkali-metal nitrate, and mixtures thereof, said ene-diol compound, and edible alkaline material in amount to neutralize lactic acid in the ham and elevate the pH toward 7, said alkaline material essentially including said high-pH alkaline material, whereby upon mixing of the solutions in the ham the acidity of the meat prevents the destructive reaction of the ene-diol material by the high-pH alkaline material, immersing the resulting ham in a pickle liquor of sodium chloride and curing salt for 24 to 48 hours, sealing the ham in a can, and cooking the ham in the sealed can.

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A. LOUIS MONACELL, *Primary Examiner.*

HYMAN LORD, *Examiner.*

Jan. 25, 1966 L. SAIR 3,231,392

METEOD OF CURING MEAT

Filed Dec. 2, 1964 2 Sheets-Sheet 1

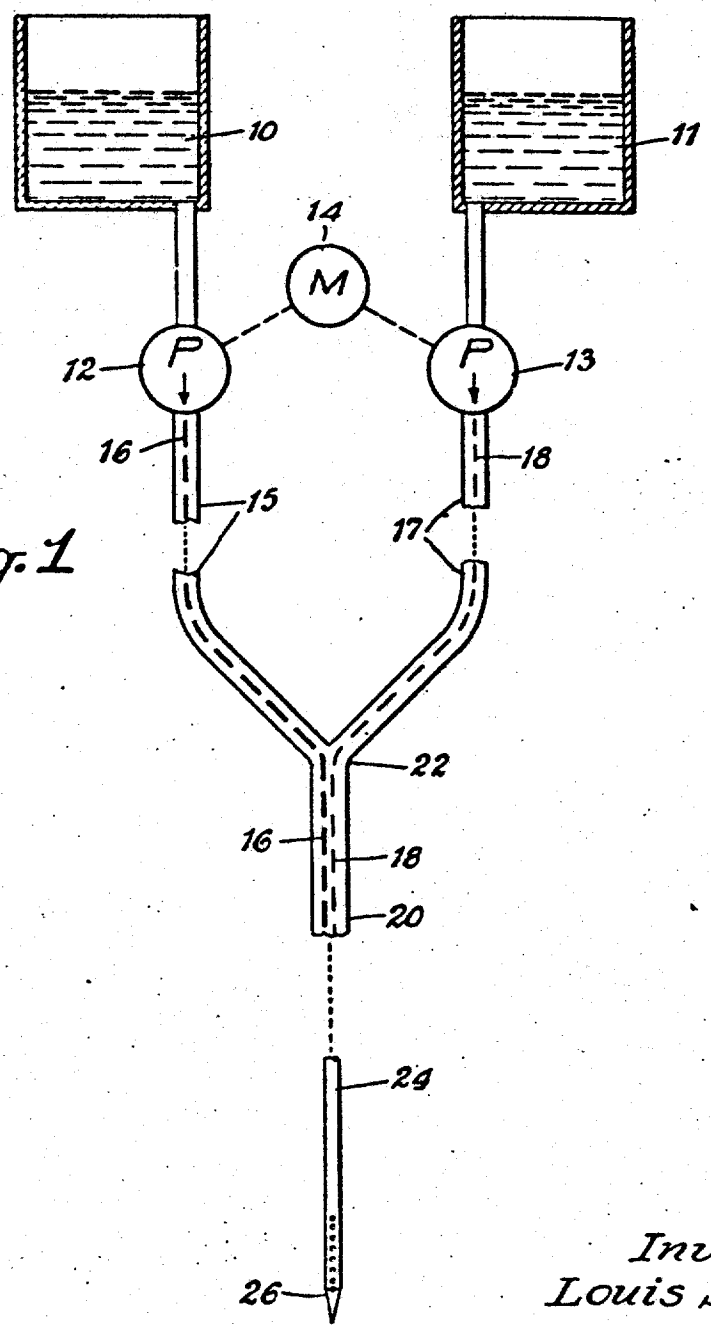

Fig. 1

Inventor
Louis Sair
by W. Bartlett Jones,
Attorney

Jan. 25, 1966 L. SAIR 3,231,392

METHOD OF CURING MEAT

Filed Dec. 2, 1964 2 Sheets-Sheet 2

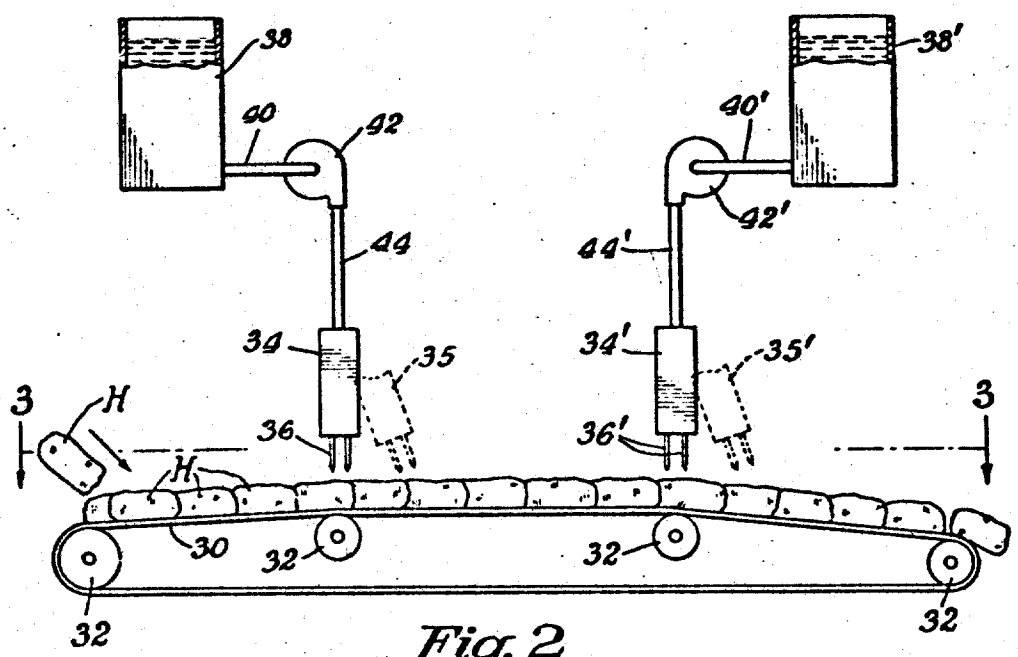

Fig. 2

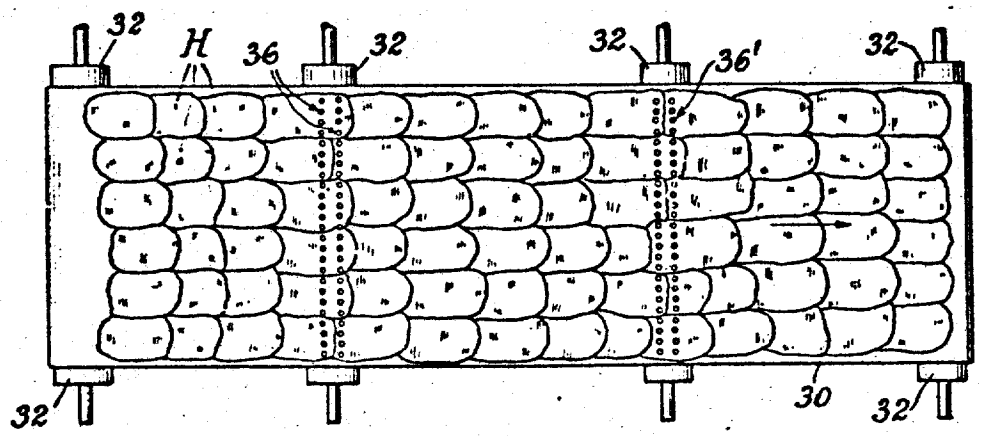

Fig. 3

Inventor
Louis Sair

by W. Bartlett Jones,
Attorney

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UNITED STATES PATENT OFFICE
CERTIFICATE OF CORRECTION

Patent No. 3,231,392 January 25, 1966

Louis Sair

It is hereby certified that error appears in the above numbered patent requiring correction and that the said Letters Patent should read as corrected below.

Column 1, lines 35 and 36, for "phosphoric" read -- phosphorus --; line 50, for "presents" read -- prevents --; line 57, for "copending" read -- cofiled --; line 66, for "from" read -- form --; column 8, line 75, for "solution" read -- solutions --.

Signed and sealed this 27th day of September 1966.

(SEAL)
Attest:

ERNEST W. SWIDER
Attesting Officer

EDWARD J. BRENNER
Commissioner of Patents

United States Patent Office

3,193,396

Patented July 6, 1965

1

3,193,396
MANUFACTURE OF ALKALINE CURING SALT COMPOSITION

Louis Sair, Evergreen Park, Ill., assignor to The Griffith Laboratories, Inc., Chicago, Ill., a corporation of Illinois

No Drawing. Filed Feb. 12, 1964, Ser. No. 342,838
10 Claims. (Cl. 99—222)

The present invention relates to a non-caking meat curing salt composition for providing a nitrogen-containing salt selected from the group consisting of alkali-metal nitrite and alkali-metal nitrate, and containing alkaline material for elevating the pH of the meat to be acted upon by said nitrogen-containing salt. In particular, it relates to such a composition containing sodium hydroxide as the alkaline content, especially in the presence of alkali-metal nitrite.

Fresh meat has a normal pH in the range from 5.5 to 6.2, due largely to a content of lactic acid formed at slaughter due to break-down of the blood and muscle sugar, namely, glycogen. The present invention is not only directed to providing a curing salt composition capable of fixing the cured color of the meat, but also capable of adjusting the pH upward to improve the water-binding capacity of whole meat, and the emulsifying properties of comminuted meat.

Curing salt compositions conventionally are mixtures having a major portion of sodium chloride, which permits the meat packer more accurately to measure the ingredients of minor proportion.

Curing salt compositions are commonly shipped in drums from a supplier to a meat packer, who removes portions from the drums for immediate use. When such compositions were made by mechanically mixing crystals of the separate salt ingredients, complaints by Government control authorities arose as a result of using such a mixture. The content of the nitrite salt used in curing is limited by such authorities. It was ascertained that in shipping and in distributing such bulk quantities of the mechanical mixture, gravitational separation frequently took place with the result that there were formed portions relatively deficient and portions relatively richer in nitrite salt. Use of the richer portions frequently resulted in excess use of nitrite in the curing process.

It is known that when such a mixture is very finely ground, the danger of separation is minimized, but other difficulty is encountered. Very fine particles tend to cake merely due to their size. This tendency makes the particles less mobile, thus minimizing separation. The longer such a fine-ground mass stands, the harder it cakes, making it more difficult to remove a portion of the cake for use.

In addition to using curing salt compositions, some packers employ one or more of edible phosphate salts for improving water-binding properties. These are commonly supplied separately from the curing salt composition. In attempts to supply phosphate salts as a component of the curing salt composition, difficulties are encountered, such as mechanical separation or settling, dustiness, and tendency to cake in finely ground mixtures. Because the U.S. Government sets limits on the amount of nitrite and phosphates which can be used, under its jurisdiction, it is not permitted to use mechanical mixtures containing individual phosphate salt crystals.

U.S. Federal regulations permit the use of pyrophosphates, polyphosphates and orthophosphates, other than the normal alkali-metal orthophosphates, because of their mild alkalinity. Stronger alkalis such as trisodium phosphate, sodium carbonate, sodium hydroxide and their potassium equivalents function as desired, but are not permitted. Such strong alkalis are too corrosive and dangerous to handle and tend to dustiness.

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In improving the water-binding properties of meat, the elevated pH resulting from use of alkalis is the important factor. In doing this with phosphates, the phosphate radical remains as a residue in the meat. If alkali-metal carbonates and hydroxides were permitted, no such undesired residue would be formed. Also, cost-wise, the alkaline agent would be less expensive by providing alkalinity with carbonate or hydroxide.

Among the alkaline agents available for the present invention are the alkali-metal carbonates and hydroxides. These are preferred because they introduce no acid residue as do, for example, certain salts derived from phosphoric acid. The hydroxides are more desirable for all purposes, since there is no possible generation of gas bubbles in a liquor or within a meat mass, as can result from the use of carbonates.

Sodium or potassium hydroxide as a single entity ingredient of a curing salt composition is most undesirable. In a comminuted form when freshly prepared and maintained dry, it is dusty and thereby is an irritant to those handling the composition. On standing in humid conditions, its hygroscopicity leads to caking of a mass containing it.

By means of the present invention, an alkali-metal hydroxide may be incorporated into a curing salt composition without exhibiting dustiness, and with a reduction of irritation to the skin in contact with it and with a greatly reduced hygroscopicity.

It is a general object of the invention to prepare a granular curing salt composition with a particle-size distribution such that all the size fractions thereof are substantially uniform in chemical composition with respect to at least one of the chemical ingredients.

It is a particular object to produce such a composition which is free-flowing, non-caking, and also non-hygroscopic when alkali-metal hydroxide is present.

The process generally is to prepare a finely divided mixture of the ingredients which is uniform in composition, mechanically to apply pressure in a manner to produce bonded caked forms of the mixture, and then to fracture the cakes to provide a composition such that the size-fractions are aggregates of all the original particles and fractions thereof have substantially the same composition as the original mixture with respect to at least one of the ingredients.

To achieve the desired results a uniform mixture of suitably finely-divided crystals is purposely bonded to caked or agglomerated form by mechanical compaction. To produce individual particles of substantially the same composition the caked or agglomerated forms are ground or comminuted to a desired fineness, but of a minimum coarseness predetermined as indicated hereinafter. Comminution is best done and controlled by passing the cake through the nip of grinding rolls set apart to control the size. Several passes are preferred, as by passing the material through a bank of such set rolls, for example, three in number, each following set being more closely spaced.

Caking may be effected in one way by mechanically squeezing together the particles of the original mixture. This may be done by mechanically compressing the individual original crystals into sheet, cake, pellet or briquette form.

One way is to extrude a rod-like form and to chop off pellets as extruded. Pellets must not be smaller than a minimum size predetermined as indicated below. Another way is to pass a mass of the original crystals through the nip of heavy compression rolls, exerting heavy pressure, so that sheet-like forms are produced of thickness, for example, 1/16 to 1/8-inch. Such forms may be further fragmented to facilitate further comminution as described.

The final product need not have particles of uniform size and its particle-size distribution must yield size-fractions all having substantially the same composition as the original mixture with respect to at least one critical ingredient, and preferably to all. These are criteria for limiting the fineness of the ultimate product. These criteria are not necessarily those of the original mixture, because the very act of compacting the original mixture can fracture larger sizes to fill spaces in producing the caked forms. Therefore, the ultimate smallest particle-size distribution of formed pellets, or of size-reduced caked forms is determined by analytical procedure for a given original mixture and the processing thereof. The size fractions of the ultimate product must have an acceptable uniform composition with respect to one or more components which are critically controlled in use of the product, especially the nitrite content.

The larger the sizes of the ultimate product up to the original caked forms, the more certain is the composition to be uniform. The finer the ultimate product is to be, the more important it is to check the composition by analytical control.

Although large cake sizes may be shipped or stored as the ultimate product, that is not commercially practicable. They provide too much void and wasted space in a container, and they permit movement in shipping, thereby wearing away of larger pieces to fines or dust of variable composition. An average size is preferred which packs tightly, but remains in free-flowing form.

Although sodium hydroxide is the preferred alkaline agent for the present invention, other agents having edible ions, alone or in combinations, may be used, such as trisodium phosphate, disodium phosphate, sodium tripolyphosphate, tetrasodium pyrophosphate, sodium carbonate, sodium bicarbonate, and the potassium salts corresponding to said sodium salts.

Where phosphates are used, it is observed that the U.S. regulations permit as much as 27 lbs. of "phosphate" per 50 gallons of pickle when pumped to 10% of added weight in curing meat such as hams. Where sodium nitrite is used in making a pickling brine, not over two pounds of sodium nitrite may be used per 100 gallons, when pumped to 10% increase in weight.

Typical compositions for a curing salt are given in Table I for the purpose only of illustration. These may be used in amount of 35 pounds per 100 gallons of pickle.

Table I

Composition	Percentage by weight				
	A	B	C	D	E
Sodium nitrite	4.6	4.6	4.6	4.6	4.6
Sodium nitrate	3.4	3.4	3.4	3.4	3.4
Sodium chloride	0	0	0	40.5	52.0
Disodium phosphate duohydrate	92.0	0	0	0	0
Sodium tripolyphosphate	0	92.0	0	0	0
Sodium pyrophosphate	0	0	92.0	0	0
Sodium carbonate	0	0	0	51.5	0
Sodium bicarbonate	0	0	0	0	40.0

Throughout this specification, the term "alkalinity" of a composition is employed. It is the measure of ml. of 0.1 N hydrochloric acid required to titrate to pH of 6 a solution of one gram of the composition in 100 ml. of water.

In producing Compositions A through E, 250 pounds of the raw material ingredients were blended. These were sampled for analysis for particle-size distribution, for the alkalinity of the size fractions, and for their nitrite content.

The remainder of each batch was ground fine so that substantially all of it passed a 200-mesh screen. The fine-ground compositions were then compacted, both dry, and with 2% of added water. After compaction at various pressures, the compacted material was dried, then ground.

COMPOSITION A.—DISODIUM PHOSPHATE DUOHYDRATE

The raw materials used had a particle-size distribution as follows:

Table II

Mesh	Percent by weight		
	$Na_2HPO_4.2H_2O$	$NaNO_2$	$NaNO_3$
On 50	68	37	14
On 100	24	45	15
On 200	2	14.7	11.5
Through 200	6	3	59

The mechanical mixture of the raw ingredients was analyzed for particle-size distribution, and the fractions were analyzed for nitrite content and for alkalinity, as set forth in Table III.

Table III

	Mesh			
	On 50	On 100	On 200	Through 200
The mixture (percent)	54	40	4.7	1.3
Sodium nitrite (percent)	2.20	4.9	17.25	13.5
Alkalinity	45.6	44.2	31.4	9.4

Table III demonstrates that the original mixture easily separates into fractions not uniform in nitrite content and in alkalinity.

After grinding the original mixture so that nearly all of it passes a 200-mesh screen, the particle-size distribution was determined, and the size-fractions analyzed for nitrite content and alkalinity. The results are shown in Table IV.

Table IV

	Mesh			
	On 180	On 200	On 270	Through 270
The mixture (percent)	11	32	22	35
Sodium nitrite (percent)	3.25	3.90	4.65	4.55
Alkalinity	56	54.2	54	53.5

The uniformity of composition is improved, but the fineness leads to dustiness, difficulty of dispersion in making a pickle, and possible caking.

The fine-ground mixture was then compacted between rolls using various pressures, measured as pounds per linear inch of roll, both dry and with 2% added water.

The compacted bodies were then ground and the same analyses made, as shown in Table V.

Table V

Example	Pressure, lbs./in.		On 50	On 100	On 200	Through 200
A-1 Dry	3,600	Too soft—Crushes with fingers.				
A-2 Dry	7,200	Too soft—Cannot be ground.				
A-3 Dry	10,800	Mixture (percent)	41	16	21	12
		Nitrite (percent)	4.2	4.8	4.5	3.95
		Alkalinity	53.2	53.0	53.2	52.5
A-4 2% H_2O	7,200	Too soft—Cannot be ground.				
A-5 2% H_2O	10,800	Mixture (percent)	39	27	18	16
		Nitrite (percent)	4.55	4.8	5.3	5.55
		Alkalinity	54.1	53.5	53.5	53.2

The uniformity of alkalinity is excellent. The uniformity of sodium nitrite content falls within 10% deviation from the median value between the two extremes, such deviation being permitted in this circumstance in Federal regulations.

The same procedure has been followed with compositions B, C, D and E, and the same series of tables are given for each composition. To facilitate comparison, the corresponding tables are identified by the same Roman number preceded by a letter designating the composition.

COMPOSITION B.—SODIUM TRIPOLYPHOSPHATE

Table B-II

Mesh	Percent by weight of fractions of initial raw materials		
	$Na_5P_3O_{10}$	$NaNO_2$	$NaNO_3$
On 50	0	37	14
On 100	2.5	45	15
On 200	7.5	14.7	11.8
Through 200	90.0	3	59

Table B-III

ANALYSIS OF INITIAL MIXTURE

	Mesh			
	On 50	On 100	On 200	Through 200
The mixture (percent)	2	5	21.0	72.0
Sodium nitrite (percent)	61.5	31.25	3.75	0.3
Alkalinity	4.0	21.8	44.8	39.6

The preceding initial Composition B was ground fine and analyzed as follows:

Table B-IV

	Mesh			
	On 150	On 200	On 270	Through 270
The mixture (percent)	11	32	22	35
Sodium nitrite (percent)	3.25	3.9	4.65	4.55
Alkalinity	56.0	54.2	54	53.5

The uniformity is satisfactory, but the composition is so fine it is dusty, difficult to disperse in a pickle, and likely to cake on storage. By compaction and grinding to a coarser particle-size distribution, the product can be made free-flowing, non-caking, non-dusting, easy to disperse in a pickle and uniform in composition as shown in the following table.

Table B-V

Example	Pressure, lbs./in.		Mesh			
			On 50	On 100	On 200	Through 200
B-1 Dry	7,200	Mixture (percent)	36	14	7.5	42
		Nitrite (percent)	4.55	4.40	5.10	4.30
		Alkalinity	40.5	40.3	40.3	39.9
B-2 Dry	10,800	Mixture (percent)	38	19	19	24
		Nitrite (percent)	4.10	4.10	4.8	4.00
		Alkalinity	40.2	38.4	38.4	38.4
B-3 2% H_2O	1,800	Mixture (percent)	62.5	27.5	2.5	7.5
		Nitrate (percent)	4.3	4.2	4.6	4.2
		Alkalinity	(1)	(1)	(1)	(1)
B-4 2% H_2O	9,000	Mixture (percent)	45	19	16	20
		Nitrite (percent)	4.2	4.2	4.2	4.10
		Alkalinity	39.7	39.8	39.8	39.4

[1] Not determined.

All of the foregoing are satisfactory in unformity and are non-caking, free-flowing, free of dustiness, and disperse readily in making a pickle.

COMPOSITION C.—TETRASODIUM PYROPHOSPHATE

Table C-II

Mesh	Percent by weight of fractions of initial raw materials		
	$Na_4P_2O_7$	$NaNO_2$	$NaNO_3$
On 50	0	37	14
On 100	1	45	15
On 200	19	14.7	11.8
Through 200	80	3	59

Table C–III

ANALYSIS OF INITIAL MIXTURE

	Mesh			
	On 50	On 100	On 200	Through 200
The mixture (percent)	1.6	6.5	27.0	65
Sodium nitrate (percent)	58.0	31.25	2.8	0
Alkalinity	11.5	25.8	46.8	64

The initial mixture was ground fine and analyzed as follows:

Table C–IV

	Mesh			
	On 150	On 200	On 270	Through 270
The mixture (percent)	0.5	6.2	10.0	83.3
Sodium nitrate (percent)	----------	5.25	29.75	3.75
Alkalinity	----------	49.2	45.2	42.0

The non-uniformity of alkalinity is lessened by the fine grinding but the nitrite contents vary.

By compaction and grinding to a coarser mesh, acceptable uniformity is obtainable as set forth in the following table.

Table C–V

Example	Pressure, lbs./in.		Mesh			
			On 50	On 100	On 200	Through 200
C-1 Dry	14,000	Mixture (percent)	44	18	19	18
		Nitrite (percent)	6.25	4.06	4.75	4.20
		Alkalinity	42.8	43	43.8	43.8
C-2 2% H_2O	3,600	Mixture (percent)	58	16	4.5	21
		Nitrite (percent)	4.20	4.20	4.4	4.2
		Alkalinity	43.2	42.8	45	42.3
C-3 2% H_2O	12,600	Mixture (percent)	49.0	18.0	17	17
		Nitrite (percent)	4.4	4.3	4.3	4.2
		Alkalinity	43.8	43.2	42.8	42.8

The compaction of Composition C at low pressures yields fragile cakes, which do not grind well. Best results are at higher pressures.

The compositions of the above table are of excellent uniformity, free-flowing, non-caking, free from dustiness, and dispersed readily in making a pickle.

COMPOSITION D.—SODIUM CARBONATE

Table D–II

Mesh	Percent by weight of fractions of initial raw materials			
	Na_2CO_3	$NaNO_3$	$NaNO_2$	NaCl
On 50	1.2	37	14	11.2
On 100	7.2	45	15	70.2
On 200	50.5	14.7	11.8	7.5
Through 200	41.1	3	59	10.1

Table D–III

ANALYSIS OF INITIAL MIXTURE

	Mesh			
	On 50	On 500	On 200	Through 200
The mixture (percent)	5.5	36.0	38.0	20
Sodium nitrite (percent)	27.5	5.55	1.05	0.3
Alkalinity	14.2	21.2	100	125.8

The initial mixture was ground fine and analyzed as follows:

Table D–IV

	Mesh			
	On 150	On 200	On 270	Through 270
The mixture (percent)	----------	6.3	13.3	80.4
Sodium nitrite (percent)	----------	11.0	7.25	3.8
Alkalinity	----------	34.0	49.4	93.4

The product is not uniform in composition. By compaction and grinding to a coarser mesh, acceptable uniformity is achieved, as shown in the following table.

Table D–V

Example	Pressure, lbs./in.		Mesh			
			On 50	On 100	On 200	Through 200
D-1 Dry	7,200	Mixture (percent)	53.0	18.0	8.0	22.0
		Nitrite (percent)	4.4	4.4	5.2	4.8
		Alkalinity	78	79.0	74.5	62.7
D-2 Dry	12,500	Mixture (percent)	49.0	24.0	16.0	11.0
		Nitrite (percent)	4.65	4.75	5.1	4.75
		Alkalinity	75.6	75.0	71.0	67.2
D-3 2% H_2O	14,400	Mixture (percent)	47.0	24.0	19.0	10.0
		Nitrite (percent)	4.55	4.65	4.8	5.0
		Alkalinity	73.7	78.2	76.2	67.0

The nitrite uniformity is acceptable but the alkaline uniformity is slightly more varied than in Table C–V for example. The variation in alkalinity comes in the fine fraction passing through the 200-mesh screen, which in Example D–3, comprises 10% of the mix. In commercial operations such fine fractions could be recycled as explained hereinafter. The products are non-caking, free-flowing, and free of dustiness, making them less corrosive and dangerous in handling.

COMPOSITION E.—SODIUM HYDROXIDE

Table E–II

Mesh	Percent by weight of fractions of initial raw materials			
	NaOH	$NaNO_3$	$NaNO_2$	NaCl
On 50	[illegible]	[illegible]	[illegible]	[illegible]
On 100	----------	[illegible]	[illegible]	[illegible]
On 200	----------	14.7	11.8	2.5
Through 200	----------	3	59	10.1

Table E-III

ANALYSIS OF INITIAL MIXTURE

	Mesh			
	On 50	On 100	On 200	Through 200
The mixture (percent)	40.0	41	17.0	2.0
Sodium nitrite (percent)	1.70	5.45	6.25	6.0
Alkalinity	216	3.2	4.7	4.5

The variations evident in the above table are due to the original coarseness of the sodium hydroxide. By fine grinding the above initial mixture, the product analyzes as follows:

Table E-IV

	Mesh			
	On 150	On 200	On 270	Through 270
The mixture (percent)	12.5	12.5	22.5	72.5
Sodium nitrite (percent)	4.3	4.05	5.15	3.75
Alkalinity	96.3	95.5	86.7	90.2

The nitrite variation is substantial. The product tends to be dusty, corrosive, hygroscopic and commercially difficult to handle. On storage it cakes.

By compaction and grinding to a coarser mesh, these drawbacks are overcome, as shown in the following table:

Table E-V

Example	Pressure, lbs./in.		Mesh			
			On 50	On 100	On 200	Through 200
E-1 Dry	9,000	Mixture (percent)	40.0	16.0	12.0	25.0
		Nitrite (percent)	4.4	4.56	5.3	3.80
		Alkalinity	98.1	97.3	91.0	87.6
E-2 2% H_2O	9,000	Mixture (percent)	57.0	15.0	10.0	18.0
		Nitrite (percent)	4.45	4.56	4.85	3.85
		Alkalinity	99.4	98.8	91.6	88.4

The compaction appears to lock the sodium hydroxide into the agglomerates in such a way that the hygroscopicity is not exerted to the degree it is in the original mechanical mix. The product is free-flowing, and not adversely hygroscopic. The difficulties arising from a content of sodium hydroxide are greatly lessened because of locking the sodium hydroxide into an agglomerate.

All of the foregoing examples were made by fine-grinding an original mixture, compacting and then grinding to a coarser mesh than the fine-ground mixture. It is not always necessary to fine-grind the original mixture, as illustrated below.

A new series of compositions was made from mechanical mixes of different lots of raw material ingredients, but without comminuting the mechanical mixtures before compaction.

The following table gives the particle-size distribution of three new lots.

Table VI

Mesh	$NaNO_2$	$NaNO_3$	NaCl
On 50	2.5	14.0	11.0
On 100	35.0	15.0	70.0
On 200	30.0	12.0	2.0
Through 200	32.0	59.0	10.0

The following table gives the particle-size distribution of the alkaline agents, these being the same as given hereinabove.

Table VII

Mesh	Figures in percent				
	A Na_2HPO_4	B $Na_4P_2O_9$	C $Na_4P_2O_7$	D Na_2CO_3	E NaOH
On 50	68	0	0	1.2	100
On 100	24	2.5	1	7.2	
On 200	2	7.5	19	50.5	
Through 200	6	90	80	41.1	

The following Compositions AA through EE were made in the same proportions of ingredients as the Compositions A through E, respectively, as given in Table I above.

COMPOSITION AA.—DISODIUM PHOSPHATE DUOHYDRATE

The mechanical mixture of its ingredients which are characterized in Tables VI and VII was analyzed as follows:

Table AA-VIII

	Mesh			
	On 50	On 100	On 200	Through 200
The mixture (percent)	64.0	25.0	7.0	4.0
Sodium nitrite (percent)	0.3	7.15	25.6	32.4
Alkalinity	46.2	42.0	31.3	18.0

Said Composition AA was compacted at 9,000 lbs./in. with 2% of added water. After drying the resulting agglomerates, the compacted material was ground to the following particle-size distribution.

Table AA-IX

	Mesh			
	On 50	On 100	On 200	Through 200
The mixture (percent)	43.0	23.0	24.0	10.0
Sodium nitrite (percent)	4.55	4.55	4.5	5.50
Alkalinity	43.6	41.6	44.0	43.2

The nitrite uniformity is not over 10% deviation from the mean nitrite content of 5.00. The fraction highest in nitrite is the smallest one passing through 200-mesh, which in commercial practice would be recycled. The alkalinity is quite uniform. This table shows great improvement over the mechanical mix characterized in Table AA-VIII.

COMPOSITION BB.—SODIUM TRIPOLYPHOSPHATE

The mechanical mixture of its ingredients which are

characterized in Tables VI and VII was analyzed as follows:

Table BB–VIII

	Mesh			
	On 50	On 100	On 200	Through 200
The mixture (percent)	1.0	3.0	11.0	85.0
Sodium nitrite (percent)	22.0	32.0	32.0	0.85
Alkalinity	2.8	60.0	26.6	32.8

The non-uniformity of the mechanical mix is evident, but can be remedied by compaction and grinding to a coarser mesh than given in the above table. The mechanical mix BB with 2% added water was compacted with 10,800 lbs./in. pressure, then dried and ground to the following mesh:

Table BB–IX

	Mesh			
	On 50	On 100	On 200	Through 200
The mixture (percent)	45.0	16.0	16.0	22.0
Sodium nitrite (percent)	4.1	5.4	5.10	3.15
Alkalinity	30.0	29.2	29.4	30.4

The result compared to that of Composition B in Table B–V shows that the particle-size distribution before compaction should not be too large, and the finer the better. Compromise for a practical limit may be determined by experience with each formulation.

COMPOSITION CC.—TETRASODIUM PYROPHOSPHATE

The mechanical mixture of its ingredients which are characterized in Tables VI and VII was analyzed as follows:

Table CC–VIII

	Mesh			
	On 50	On 100	On 200	Through 200
The mixture (percent)	1.0	7.0	25.0	66.0
Sodium Nitrite (percent)	11.3	40.0	8.2	0.85
Alkalinity	2.8	26.9	52.7	62.1

After compaction at 10,800 lbs./in. the agglomerates were ground to the following mesh:

Table CC–IX

	Mesh			
	On 50	On 100	On 200	Through 200
The mixture (percent)	42.0	18.0	12.0	29.0
Sodium nitrite (percent)	4.4	5.85	5.4	[illegible]
Alkalinity	50.0	49.2	49.4	[illegible]

The results in the preceding table compared to those in Table C–V again establish that before compaction the particle-size must not be too large, and the finer the better.

COMPOSITION DD.—SODIUM CARBONATE

The mechanical mixture of its ingredients which are characterized in Tables VI and VII was analyzed as follows:

Table DD–VIII

	Mesh			
	On 50	On 100	On 200	Through 200
The mixture (percent)	4.0	38.0	31.0	27.0
Sodium nitrite (percent)	3.6	5.0	28.5	4.7
Alkalinity	10.9	14.4	112.0	128.0

The mixture with 2% added water was compacted with 10,800 lbs./in. pressure. Then, the agglomerates were dried and ground to the following mesh:

Table DD–IX

	Mesh			
	On 50	On 100	On 200	Through 200
The mixture (percent)	36.0	25.0	19.0	20.0
Sodium nitrite (percent)	4.4	5.05	4.7	3.75
Alkalinity	80.3	68.7	65.1	96.0

The nitrite uniformity is only slightly over 10% deviation from the mean between the extremes, showing great improvement by compaction and grinding to a coarser mesh. The variations in alkalinity are also greatly improved.

COMPOSITION EE.—SODIUM HYDROXIDE

The mechanical mixture of its ingredients which are characterized in Tables VI and VII was analyzed as follows:

Table EE–VIII

	Mesh			
	On 50	On 100	On 200	Through 200
The mixture (percent)	35.0	49.0	15	1.0
Sodium Nitrite (percent)	0.4	3.6	15.6	16.0
Alkalinity	224.0	20.0	4.4	3.0

The mechanical mixture after adding 2% of water was compacted at 7,200 lbs./in. pressure. Then, the agglomerates were dried and ground to a mesh as follows:

Table EE–IX

	Mesh			
	On 50	On 100	On 200	Through 200
The mixture (percent)	56.0	22.0	8.0	14.0
Sodium nitrite (percent)	3.75	4.5	4.0	4.25
Alkalinity	104.0	67.4	57.5	69.6

The nitrite value is within 10% of the mean value. The alkalinity has been distributed. Compared to the original mixture, the product is less corrosive, less dusty, less hygroscopic, and easier to handle. It dissolves more slowly than the sodium hydroxide ingredient and can be added to water without excessively rapid generation of heat. This shows that it is more desirable to use a finer grind for the original mixture.

The following description shows the effect of using compositions of this invention in curing hams. Table X shows five compositions as designated M, N, O, P and Q, made according to the compacting procedure herein described.

Table X

	Composition				
	M	N	O	P	Q
Sodium nitrite (percent)	4.6	4.6	4.6	4.6	4.6
Sodium nitrate (percent)	3.4	3.4	3.4	3.4	3.4
Sodium hydroxide	35	30	25	20	10
Sodium chloride	57	62	67	72	82
Alkalinity	81	69	56	46	23

35 pounds of the Compositions M through P were made up to 100 gallons of pickle, which was used to pump hams to 10% added weight.

In pumping hams to 10% added weight having initially a pH of 6.1, it is desired to raise the pH by 0.2 to 0.3 unit of pH. The compositions made into pickles as described above have been so used and effect final pH in the hams as set forth in Table XI.

Table XI

Composition:	pH of cured hams
M	6.7
N	6.6
O	6.5
P	6.4
Q	6.3

From the foregoing, it appears that the content of sodium hydroxide in Composition Q is near the minimum required for pickles containing 35 pounds per 100 gallons, when pumping hams to 10% added weight.

The nitrite and nitrate salts may be combined into a physically homogeneous fusion product, and that product used to supply the two salts.

The following Table XII gives the fusion points of such salts, and of certain mixtures thereof.

Table XII

Substance—	Approximate melting point, °C.
Potassium nitrate	337
Potassium nitrite	297.5
Sodium nitrate	308
Sodium nitrite	271
Sodium chloride	804
Mixed sodium nitrite, 60%–sodium nitrate, 40%	230
Mixed sodium nitrite, 60%–potassium nitrate, 40%:	
Begins to melt at	126
And is completely liquid and transparent at	156
Mixed potassium nitrite, 60%–sodium nitrate, 40%:	
Begins to melt at	139
And is completely liquid and transparent at	172

There are some peculiar facts about the fusion points of mixtures. When dry nitrites and nitrates are mixed for fusion, the melting point is sharp when the same metal base, such as sodium, is used in both salts. When the metal bases differ, the melting point is spread over a wide range. This is believed due to a shifting of the equilibrium between the two salts forming perhaps four salts. It is also noted that where the metal bases differ the melting point is lower than when the metal base is the same for both salts.

Although the examples given above disclose the grinding of a mixture of the constituent particles, it is to be understood that supplies of the separate constituents may be ground, and then mixed.

In carrying out the invention, the caked forms are reduced in size varying in smallness from a coarseness which packs tightly for shipment, which does not cake because of fineness and which has a substantially uniform content in its size-fractions with respect to at least one critical ingredient. For example, the caked forms may be ground to coarse sizes, even such as rock salt, to serve the mechanical purposes of the user. It is noted, however, that the invention has been explained and exemplified by reference to an ultimate fineness much greater than need be practiced. The preferred size for general use is one of which substantially all the particles pass a 16-mesh screen and remain on a 100-mesh screen.

The invention is not limited in saleable products to those which contain the entire product of grinding a compacted mass. Since the final product has fractions of graded sizes, any grind may be fractionated to select from its fractions a composition of one or more fractions within a predetermined range of particle sizes. Such fractions as are rejected in doing this may be used as raw material for recycling through the compaction procedure, whether they be at the coarser end or at the finer end. In consequence, the original mass may be, or may include, particles which are aggregates of the component salts.

A granular form of curing salt of moderate fineness in the form desired by the meat packers, not only for mixing it directly in products for ground meat and for rubbing onto heat, but also for ready weighing and dissolution in forming brine.

The salt compositions which are the subjects of the present invention may contain additives, such as cane sugar, corn sugar, ene-diol compounds, such as sodium isoascorbate, and seasonings, which are compatible with the alkaline content and which function in the treatment of meat. It is to be understood that increase in the kinds of particles which constitute the original uniform mixture has a controlling effect on smallest permitted sizes of the ultimate product to maintain the desired uniformity of composition.

Although small amounts of alkaline material have been used in dry solid curing salt compositions as a buffering agent to protect one or more ingredients of the composition, the buffering amount is small and far below the 40 to 92 parts of alkali used per 8 parts of the selected curing salt, as given in Table I above.

The invention is not limited to or by the details and examples given above to illustrate and explain the invention, and numerous changes and modifications are contemplated as falling within the scope of the invention as expressed in the appended claims.

This application is a continuation-in-part of my earlier application Serial No. 223,269, filed September 12, 1962, now abandoned.

I claim:

1. The method of producing an alkaline curing salt composition, which composition comprises bonded ag-

gregates of physically homogeneous particles of curing salt selected from the group consisting of alkali-metal nitrite, alkali-metal nitrate and mixtures thereof, and chemically homogeneous particles of edible-ion alkaline material in kind and quantity to elevate the pH of meat for which said composition is used, which method comprises bonding to caked forms a granular mixture of finely divided particles of the constituents of said composition, which mixture is substantially uniform throughout in composition, by compacting said granular mixture with an effective mechanical pressure sufficient to lock the particles to each other so that when the said caked forms are reduced in size the size-fractions have a substantially uniform composition, and then reducing the size of said caked forms which are substantially dry to provide a mass of such particle-size distribution that all the size-fractions thereof have a substantially uniform composition.

2. The method of claim 1 wherein the caked forms are ground to a free-flowing non-caking fineness.

3. The method of claim 1 wherein said granular mixture contains sodium chloride crystals.

4. The method of producing an alkaline curing salt composition, which composition comprises bonded aggregates of physically homogeneous particles of curing salt selected from the group consisting of alkali-metal nitrite, alkali-metal nitrate and mixtures thereof, and chemically homogeneous particles of edible-ion alkaline material in kind and quantity to elevate the pH of meat for which said composition is used, which method comprises mechanically mixing supplies of the constituent particles, grinding the resulting mixture to a granular mass which is substantially uniform throughout in composition, bonding to caked forms said resulting granular mass by compacting said granular mass with an effective mechanical pressure sufficient to lock the particles to each other so that when the said caked forms are reduced in size the size-fractions have a substantially uniform composition, and then reducing the size of said caked forms which are substantially dry to provide a mass of such particle-size-distribution that all the size-fractions thereof have a substantially uniform composition.

5. The method of producing an alkaline curing salt composition, which composition comprises bonded aggregates of physically homogeneous particles of curing salt selected from the group consisting of alkali-metal nitrite, alkali-metal nitrate and mixtures thereof, and chemically homogeneous particles of alkaline material selected from the group consisting of alkaline alkali-metal orthophosphates, alkali-metal tripolyphosphate, tetrasodium and tetrapotassium pyrophosphate, alkali-metal carbonates and alkali-metal hydroxides, which method comprises mechanically mixing supplies of the constituent particles, grinding the resulting mixture to a granular mass which is substantially uniform throughout in composition, bonding to caked forms said resulting granular mass by compacting said granular mass with an effective mechanical pressure sufficient to lock the particles to each other so that when the said caked forms are reduced in size the size-fractions have a substantially uniform composition, and then reducing the size of the resulting caked forms which are substantially dry to a free-flowing granular mass of such particle-size-distribution that all the size fractions thereof have a substantially uniform composition.

6. The method of claim 5 wherein said constituent particles contain sodium chloride crystals.

7. The method of producing an alkaline curing salt composition, which composition comprises bonded aggregates of physically homogeneous particles of curing salt selected from the group consisting of alkali-metal nitrite, alkali-metal nitrate and mixtures thereof, and chemically homogeneous particles of alkaline material selected from the group consisting of alkaline alkali-metal orthophosphates, alkali-metal tripolyphosphate, tetrasodium and tetrapotassium pyrophosphate, alkali-metal carbonates and alkali-metal hydroxides, which method comprises bonding to caked forms a granular mixture of particles of the constituents of said composition which mixture is substantially uniform throughout in composition, by compacting said original mass with an effective mechanical pressure sufficient to lock the particles to each other so that when the said caked forms are reduced in size the size-fractions have a substantially uniform composition, and then reducing the size of said caked forms which are substantially dry to provide a mass of such particle-size-distribution that all the size-fractions thereof have a substantially uniform composition.

8. The method of producing an alkaline curing salt composition, which composition comprises bonded aggregates of physically homogeneous particles of curing salt selected from the group consisting of alkali-metal nitrite, alkali-metal nitrate and mixtures thereof, and chemically homogeneous particles of alkaline material selected from the group consisting of alkaline alkali-metal orthophosphates, alkali-metal tripolyphosphates, tetrasodium and tetrapotassium pyrophosphate, alkali-metal carbonates and alkali-metal hydroxides, which method comprises bonding to caked forms a granular mixture of particles of the constituents of said composition which mixture is substantially uniform throughout in composition, by compacting said granular mixture with an effective mechanical pressure sufficient to lock the particles to each other so that when the said caked forms are reduced in size the size-fractions having a substantially uniform composition, and then reducing the size of the resulting caked forms which are substantially dry to a particle-size-distribution coarser than that of said mixture and characterized by size-fractions of substantially uniform composition.

9. The method of producing an alkaline curing salt composition, which composition comprises bonded aggregates of physically homogeneous particles of curing salt selected from the group consisting of alkali-metal nitrite, alkali-metal nitrate and mixtures thereof, and chemically homogeneous particles of edible-ion alkaline material in kind and quantity to elevate the pH of meat for which said composition is used, which method comprises bonding to caked forms a granular mixture of finely ground particles of constituents of said composition which mixture is substantially uniform throughout in composition and which contains a small amount of added water, by compacting said granular mixture with an effective mechanical pressure sufficient to lock the particles to each other so that when the said caked forms are reduced in size the size-fractions have a substantially uniform composition, drying the caked forms, and then reducing the size of said caked forms which are substantially dry to provide a mass of such particle-size-distribution that all the size-fractions thereof have a substantially uniform composition.

10. The method of producing an alkaline curing salt composition, which composition comprises bonded aggregates of physically homogeneous particles of curing salt selected from the group consisting of alkali-metal nitrite, alkali-metal nitrate and mixtures thereof, and chemically homogeneous particles of edible-ion alkaline material in kind and quantity to elevate the pH of meat for which said composition is used, which method comprises bonding to caked forms a granular mixture of finely ground particles of constituents of said composition which mixture is substantially uniform throughout in composition and which contains a small amount of added water, by compacting said granular mixture with an effective mechanical pressure sufficient to lock the particles to each other so that when the said caked forms are reduced in size the size-fractions have a substantially uniform composition, and then reducing the size of the resulting caked forms which are substantially dry to a particle-size-distribution coarser than that of said mixture and char-

acterized by size-fractions of substantially uniform composition.

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A. LOUIS MONACELL, *Primary Examiner.*

HYMAN LORD, *Examiner.*

Induction and Differentiation of an Epithelial Tumour in the Newt (*Triturus cristatus*)

by F. SEILERN-ASPANG *and* K. KRATOCHWIL[1]

From the Österreichische Krebsforschungsinstitut, Abteilung für experimentelle Biologie Wien (Leiter: Doc. Dr. F. Seilern-Aspang),

WITH SEVEN PLATES

INTRODUCTION

WADDINGTON (1935) and later Needham (1936) have suggested that the autonomy of tumours originates in a 'morphological escape' of tumour cells from the controlling influence of an individuation field which has locally become weak or has [illegible]. The persistence and strength of such an individuation field can be [illegible] its ability to induce its parts to regeneration (Ruben, 1955). This theory [illegible] be supported by the observation that in animals which are capable of regeneration, spontaneous tumours are rarely observed. [illegible] (1951) compiled a [illegible] all [illegible] of spontaneous tumours throughout the whole animal kingdom [illegible] that in animals which regenerate well spontaneous tumour [illegible] low. Waddington and Needham suggested that degraded, that is [illegible] might be brought under control again if they were exposed to the [illegible] a particularly strong individuation field, e.g. the regeneration [illegible] rating urodele limb.

The first experiment [illegible] to examine these speculations were performed by Rose & Wallin[illegible] (1948). They implanted anuran cancer (Lucké carcinoma of *Rana pipiens*) into regeneration blastemata of limbs of adult *Triturus viridescens*. The authors [illegible] differentiation of the tumour cells into cartilage, muscle, and connective tissue cells. Although these results seem to support the speculation of Waddington and Needham, no definite evidence could be seen that the differentiated cells were actually the *Rana* cells. The only available difference used to distinguish *Rana* and *Triturus* cells was the smaller size of *Rana* nuclei.

Ruben (1955, 19[illegible]) implanted the same carcinoma into limb regeneration blastemata of *Amblystoma* larvae and adult *T. viridescens* and could not observe any alteration of the tumour tissue except degeneration.

In both of these [illegible] experimental attempts the method used does not seem to be adequate to permit definite statements. There was always a foreign

[1] *Authors' [illegible] Krebsforschungsinstitut, Vienna, Austria.*

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tumour implanted into the regeneration blastemata of *Triturus* or *Amblystoma*. In all experiments these foreign implants showed only a survival without noteworthy growth, even when implanted into non-regenerating limbs. If the tumour cells failed to be affected by the strong individuation field, this could be due to heterogeneity between individuation field and implant as well as to the degeneration of the implant. Furthermore, the Lucké carcinoma is caused by a virus. The permanent carcinogenic influence of the virus might prevail in spite of the controlling influence of the regeneration field.

We have been interested in the concepts suggested by Waddington and Needham, and in the studies reported on here we have induced epithelial tumours in newts by chemical carcinogens. These induced tumours avoid the previous problems of permanent carcinogenic effect of a virus. Furthermore, we applied these carcinogens to animals which were capable of regeneration so that problems of tumour transplantation were entirely avoided. Using this chemically induced tumour system we examined the problem whether differentiation processes occur in the tumours of such animals.

The results of these studies appear to support the earliest speculative opinion of Waddington (1935) and Needham (1936).

PART 1

The development of a chemically induced epithelial tumour in T. cristatus

Our first problem was to produce a typical tumour, comparable with tumours in mammals, which can clearly be distinguished from other proliferative processes such as inflammation and regeneration.

Only in exceptional cases have tumours been produced in urodeles by application of chemical carcinogens (Koch, Schrieber, & Schrieber, 1939; Breedis, 1951); Leone (1957) succeeded in producing metastatic neoplasms in *T. cristatus* by subcutaneous implantation of methylcholanthrene crystals. Numerous other attempts have failed. Nevertheless, a few spontaneous epithelial tumours in urodeles have been reported: a melanoma of the axolotl (Scheremetjeva-Brunst & Brunst, 1948), a transplantable epithelial carcinoma of *T. alpestris* (Champy, & Champy, 1935), and a carcinoma of the epithelial mucous glands in *T. cristatus* (Murray, 1908).

Since a spontaneous mucous gland carcinoma has been reported in *T. cristatus* we attempted to induce tumours at this same site by the application of carcinogens.

MATERIAL AND METHODS

Investigations have been made on the newt *T. cristatus*, which were all collected in the same district (Neusiedler See, Burgenland). A total of 1,800 newts were used in these studies. The animals received subcutaneous injections as shown in Table 1.

Since it will be shown that the formation of epithelial growths depends upon

the entrance of the [illegible] into the mucous glands, care was taken to be sure that the carcinog[illegible] solution permeated from the surrounding tissues into the base of the glands, [illegible] could be sure of this if the olive oil could be seen extruding from the mou[illegible] the gland pores.

If not stated oth[illegible] all results in this paper refer to subcutaneous injections. Other groups of [illegible] were treated by painting different parts of their bodies with a 2 per cent. [illegible] of benzpyrene in benzene. The injections and painting were made [illegible] parts of the body of the newts. Groups received injections into [illegible] on: the dorsal trunk, the lateral trunk, the ventral trunk, the tail, and [illegible] pouch.

After injection [illegible], all the animals were observed for the appearance of epithelial tumo[illegible] first appeared as small depigmented white spots in the area of injecti[illegible] were killed at various times between one week and one year after [illegible]ion. Most sacrificed animals were fixed in formalin and serial tissue slid[illegible]. The slides were stained with haematoxylin-erythrosin, [illegible] allory, or mucicarmin. A total of approximately 600 slides from 85 ne[illegible] studied. Since animals had on average three tumours each the behaviour [illegible] total of 250 tumours was studied histologically.

First the normal [illegible] of *Triturus* will briefly be described. It consists of an epithelium [illegible] layers thick. On the outside it is limited by a thin cornified layer of [illegible] epithelial cells which is desquamated at regular intervals. The epith[illegible] limited proximally by a basement membrane. Under this basement [illegible] lies a layer of pigment cells containing the blood supply (arteria [illegible]). Beneath this layer, holocrine mucous glands with their outlet [illegible] regularly distributed. A thin layer of subcutaneous connective tissue [illegible] lies innermost (Plate 1, fig. 1).

RESULTS

Tumour induction [illegible] of carcinogenic substances

It was found th[illegible] epithelial reactions were quite identical following the application of any [illegible] carcinogens used in our studies. The description of tumour developm[illegible] valid for all carcinogens listed in Table 1. Whereas the histological p[illegible] were identical for all carcinogens we found that the mixture of 2 per [illegible] 1·0-2 per cent. BP yielded the highest frequency of tumours. Therefor[illegible] this solution in our studies.

All tumours ob[illegible] began as multicentric growths, each originating in a separate mucous [illegible]. These growths then coalesced to form a large tumour area. Cell changes [illegible] started in the basal part of the gland pocket with an excessive proliferati[illegible] the cells of the germinative layer of these holocrine glands.

The genesis of [illegible] growths can be subdivided into five stages: (1) proliferation inside of [illegible] pocket; (2) expansively growing tumour; (3) infiltrating tumour; (4) [illegible]ation into the peritoneal cavity; (5) metastasis.

Stage 1. Cells of the basal germinative layer of the mucous g[illegible]s begin to proliferate (Plate 1, figs. 5, 6). While the gland pocket progressively fills with small cells, its distal part and the duct remain open and functional (Plate 1, fig. [illegible]). Compare with a normal mucous gland, shown in Plate 1, fig. 2.

Simultaneously the epithelium also shows an increased mitotic activity, as revealed by experiments with tritiated thymidine.

Stage 2. Not only does the gland pocket fill with tumour cells, but the growths perforate the gland wall and coalesce with the epithelium leaving no boundary (Plate 1, fig. 7, right gland pocket). In this way many small growths originating from mucous glands fused into a continuous mass of cells. This tumour grew expansively without perforation of the basement membrane (Plate 2, fig. 8) even though it penetrated into the musculature, forming long projections. In the region of the tumour, all mucous glands, vessels, and the pigment cell layer are eventually destroyed (Plate 2, fig. 8). Later, connective tissue and muscles are also destroyed. In some cases the destruction of the musculature was more intensive than the growth of the tumour, so that the whole tumour area sank in. In extreme cases tail tumours reached the spine after destruction of all the muscles of one lateral half of the tail. In some instances, such expansively growing tumours formed [illegible] projections into the musculature (Plate 2, fig. 9).

Stage 3. In some cases the tumour became an infiltrating growth (Plate 2, figs. 10-13). Infiltration took place either in large areas of the tumour's periphery or infiltrative rod-like projections sprouted from the epithelial tumour (Plate 2, fig. 12).

Stage 4. Some infiltrating tumours (65 per cent.) of the dorsal and lateral trunk were seen to have penetrated into the peritoneal cavity and formed there an epithelial layer which covered large areas of the peritoneum (Plate 3, figs. 14, 15) and continuously desquamated cornified cell layers into the peritoneal cavity.

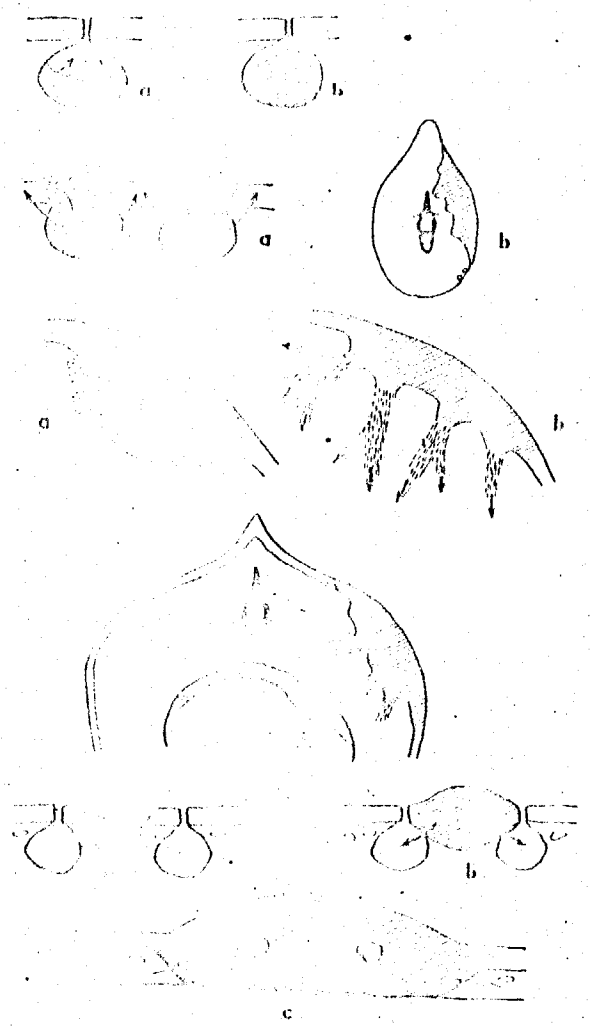
Stage 5. Metastasis. All primary tumours induced in the sacral region metastasized extensively. A few (slowly growing) tumours induced in the tail

TABLE 1

Compounds injected subcutaneously into newts

	Compounds injected	*No. of animals injected*
Carcinogens (in olive oil)	benzpyrene (BP) (0·1–2·0%)	160
	methylcholanthrene (MCA) (0·1–2·0%)	40
	dibenzanthracene (DBA) (2%)	500
	benzpyrene (2%) and methylcholanthrene (1%)	100
	dibenzanthracene (2%) and benzpyrene (0·2%)	1,000
Controls: non-carcinogenic, irritating materials (aqueous solutions)	trichloroacetic acid (2%)	20
	hydrochloric acid (0·4%)	20
	[illegible] acid (10%)	20
	[illegible] hydroxide (0·4%)	20

Each animal [illegible] approximately 0·01 ml.–0·02 ml. of the solution.



[illegible] ...tic representation of tumour stages. *Stage 1*: *a*, the basal germinative layer [illegible] to prolifer[illegible] excessively (Plate 1, figs. 5, 6). *b*, the gland pocket has been [illegible] (Plate 1, fig. [illegible], left gland). *Stage 2*: *Expansive growth*. *a*, in two tumourous [illegible] wall h[illegible] perfor[illegible] and the multicentric growths coalesce together [illegible] Plate 1, fig. [illegible] gland). *b*, transverse section through the tail with an expan[illegible] which are [illegible] numerous mucous glands. Two normal mucous glands are [illegible] of the [illegible] (Plate [illegible] 8). *Stage 3*: *Infiltrative growth*. *a*, large tumour [illegible] infiltrati[illegible] and like tumour projections sprouting from the [illegible] fig. 1[illegible]). [illegible] The [illegible] has penetrated into the peritoneal cavity and [illegible] 2, fig. [illegible] 15). *Stage 4*: *Metastases*. *a*, a metastasis between [illegible] to the [illegible] cutanea (Plate 3, fig. 17). *b*, the metastasis has [illegible] (Plate [illegible] 18). [illegible] metastasis which had coalesced with the outer [illegible] and musculature but does not penetrate into the [illegible] (Plate 3, fig. 19).

also were metastatic. Numerous metastases were formed beneath the epithelium in the region of the arteria cutanea. Metastases were seen on the dorsal head, neck, and trunk, also on the tail, and on the extremities (Plate 3, fig. 16). In contrast to the primary tumour, these arose without relation to the mucous glands (Plate 3, figs. 17, 18). The metastases always [illegible] with the epithelium and grew only expansively; they did not penetrate through the subepithelial layer into the musculature (Plate 3, fig. 19), as did the primary tumour. The musculature appeared to be impenetrable for these epithelial metastases, and the metastases spread only between the musculature and the epithelium, sometimes attaining considerable size. Here, too, mucous glands, vessels, and the pigment cell layer were destroyed (Plate 3, fig. 19) as was seen in the growth of the primary tumour.[1]

In a few cases numerous metastases were found in the lung, in the musculature, and in the kidney, but the epithelium always remained the most involved region. Apparently the tumour cells were spread in the newt epithelium by their highly developed and extensive network of arteria and vena cutanea, which represents the most important respiratory system of the newts. In these vessels and also in the vessels of the lung, numerous tumour cell emboli were found.

These five stages of tumour development are also shown in Text-fig. 1.

It was remarkable that these five stages of tumour development were not represented at the same frequency in different parts of the body; 93 per cent. of tail tumours did not pass the stage of expansive growth, and only 7 per cent. became infiltrative. This percentage has been determined on 100 tail tumours studied histologically. On the other hand, 90 per cent. of the tumours on the trunk soon became infiltrative and only 10 per cent. remained at the stage of expansive growth (here 50 tumours were examined histologically). Metastases arose from a very high percentage (100 per cent.) of primary tumours situated in the sacral region (200 animals). Only 6 per cent. of tail tumours metastasized (300 animals). These differences might be explained by the decreased tendency to infiltration of the tail tumours as compared to those of the sacral region, i.e. only 7 per cent. of tail tumours, but all sacral tumours were infiltrative. Only a few tumours of the trunk metastasized, although 90 per cent. of these tumours were infiltrative. Metastasis always occurred only after a rather long period of tumour growth, and, since the tumours on the trunk were lethal after only a few days, the most probable reason for the very low frequency of metastasis from trunk tumours was the premature death of the animals.

Cytologically, the tumour cells were very similar to epithelial cells, but they had rather spherical or oval shapes while the epithelial cells were more flattened. Tumour nuclei appeared slightly enlarged, the scanty cytoplasm stained strongly with haematoxylin and surrounded the nucleus as a rain border. Numerous mitoses were found.

[1] In later stages a secondary parasitic infection of these epithelial metastases sometimes seemed to be involved.

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All the phenomena described here have only been observed in animals treated with one or other of the carcinogens used in these studies. Such changes were never [illegible] application of the irritating, non-carcinogenic substances listed in [illegible].

Concentrations of 0·1 per cent. and 0·2 per cent. of BP or MCA did not produce tumours [illegible] carcinogenic solutions above 0·[illegible] per cent. were successful. For this [illegible] control injections with olive oil alone were omitted.

Tumour [illegible] was most successful on the dorsal and lateral trunk and on the tail [illegible] pouch, only 1 of 40 injections yielded tumour formation, and on the [illegible] trunk 50 subcutaneous injections of 2 per cent. BP did not produce [illegible] at all. These results might be due to the sparse distribution of mucous [illegible] these areas.

[illegible] been painted with benzpyrene. This manner of application [illegible] on the tail (in about 50 per cent. of cases). No other body region [illegible] tumourous reaction following painting of carcinogens. The [illegible] to the abundant distribution of mucous glands on the tail.

It [illegible] that hibernating animals were most susceptible to tumour formation [illegible] animals treated in the spring were most resistant.

Control [illegible] with non-carcinogenic, irritating materials

[illegible] were performed in exactly the same manner as the injections of [illegible] substances. We used trichloroacetic acid, hydrochloric acid, lactic [illegible] sodium hydroxide as irritating materials. Trichloroacetic acid was found [illegible] especially irritating (Table 2).

TABLE 2

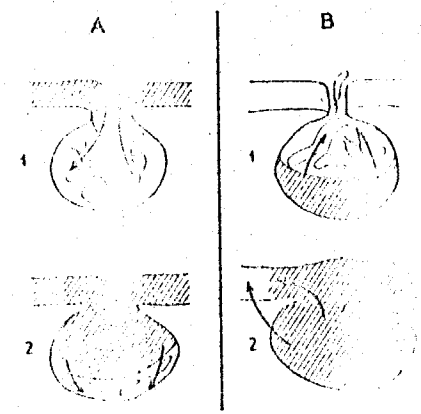
Injections of non-carcinogenic, irritating materials

Material	Number of animals injected	Animals with inflammatory reactions	Died
[illegible]% trichloroacetic acid	20	20	16
[illegible]% hydrochloric acid	20	14	2
[illegible]% lactic acid	20	8	0
[illegible]% sodium hydroxide	20	8	2

M[illegible] animals showed inflammatory processes. These inflammations decl[illegible] appeared after 5–10 days. In the histological sections from these animals [illegible] epithelial growth could be seen in the centre of inflammation except a thi[illegible] the epithelium (Plate 1, fig. 3) like that found during normal regen[illegible] the basement membrane was observed to be temporarily disintegr[illegible] the epithelium has coalesced with the subepithelial tissues. This same [illegible] behaviour has also been observed in normal limb regeneration in *Tri*[illegible], 1948).

In [illegible] of the inflamed zone epithelial proliferation was observed. The

epithelium was seen to have migrated through the outlet ducts into the damaged mucous glands. The gland pockets were filled from without by epithelial cells and only occasionally remnants of the basilar gland epithelium remained (Plate 1, fig. 4). The proliferation stopped when the whole gland pocket was filled; a perforation of the gland pocket never occurred. [illegible], these growths degenerated by necrotic disintegration beginning in the centre.



TEXT-FIG. 2. Diagrammatic representation showing the differences between an inflammation process and early tumour formation. A. *Inflammatory process.* 1, the epithelium begins to encroach from without into the damaged gland pocket. 2, almost the entire gland [illegible] filled with epithelial cells (Plate 1, fig. 4, left gland). B. *Tumour formation.* 1, the basal germinal layer of the mucous gland begins to proliferate and starts to fill the gland pocket from below with tumour cells (Plate 1, figs. 5, 6). 2, the entire gland pocket had been filled with tumour cells. Now the wall of the gland pocket is perforated and the growth coalesces with the epithelium (Plate 1, fig. [illegible] gland). Altered areas are depicted by parallel lines.

It should be noted that in the case of inflammatory reactions the epithelium migrates from without into the gland pocket where the basal parts of the gland maintain their normal histology for a long time. In contrast, a proliferation following an injection of carcinogenic solutions [illegible] in the basal part of the gland pocket and then migrates through the outlet ducts and coalesces with the epithelium (Text-fig. 2). The further stages of tumour development or similar formations could never be observed after injection of non-carcinogenic substances.

DISCUSSION

Part 1

The major question which arises from the results shown in Part 1 is the following: can we be sure that the growths which were produced in our experiments are real tumours, comparable with the cancer of mammals?

The relatively short time between injection and the appearance of tumours, a minimum of 8 days, may be considered unusual. However, some of these same tumours arose 2 or 3 months after injection. In [illegible] a 0·1 per cent. solu-

[illegible]tion of benz[illegible] [illegible]ibited a[illegible] [illegible] days; 0·5 per cent. benzpyrene resulted in [illegible] [illegible]ys (Winte[illegible]ner, 1936). After application of 1 per cent. [illegible] to [illegible] the first [illegible]oma arose as early as 30 days afterwards ([illegible] [illegible]ger[illegible] 1933). But [illegible] concentration of benzpyrene was highly toxic [illegible] [illegible] of the mice [illegible]. Newts, on the other hand, can tolerate as [illegible] [illegible] or even 3 per cent. similar carcinogenic solutions. Since we [illegible] [illegible]ic solutions of such high concentration in our studies, the average [illegible] [illegible] time [illegible] 15–20 days for tumour formation can reasonably be compared [illegible] the incubation time in mice treated with lower concentration of carcinog[illegible] [illegible]. Furthermore, it should be noted that the same carcinogen [illegible] [illegible]ours to appe[illegible] [illegible]ice after a few weeks, produces tumours in [illegible] [illegible]ly after one or two years. Thus, there appears to be a high variab[illegible] [illegible] tumour appearance between species.

The epith[illegible] which were produced in these experiments in *Triturus* share certain [illegible] characteristics with [illegible]malian tumours.

Destructi[illegible] [illegible] even in several [illegible]s. In the tail, especially, the whole [illegible] [illegible] connective tissue [illegible] lateral half was often totally destroyed. [illegible] [illegible]tion was also [illegible] in the trunk. Infiltration was [illegible] in the [illegible] growths, and [illegible] histological picture resembled infiltrating [illegible] [illegible]ive carcinoma in man.

Above all [illegible] of metastases [illegible] these tumours was a characteristic which [illegible] [illegible] of leaving us any doubts [illegible] the cancerous nature of this epithelial g[illegible] *Triturus*.

It perhaps [illegible] be mentioned that [illegible] tumours arose only after the application [illegible]nic substances and [illegible] they were identical for all the carcinog[illegible] [illegible] tumours arose even [illegible]ing painting where all effects of injury w[illegible] [illegible].

A similar [illegible] tumour, i.e. multicent[illegible] origin from glands of the skin, has also be[illegible] [illegible] in man. Boyd (1943) described a precancerous stage in the[illegible] [illegible] foci arising in a limited area, apparently deriving from [illegible].

In con[illegible] [illegible]ents with non-carcinogenic but irritating substances an epithelial [illegible] [illegible]ed which could easily be distinguished from the post-carcino[illegible] [illegible] processes. The detachment of single epithelial cells into the subepith[illegible] [illegible] in the inflamed zone is very different from infiltration, seen in the fo[illegible] [illegible] of tumour development was found in any inflammatory lesion.

Regen[illegible] [illegible] might also be difficult to distinguish from tumourous growth. [illegible] [illegible] in models starts with dedifferentiation and increased proliferation [illegible] [illegible] cells. These cells form a blastema by interaction with the sub[illegible] [illegible] If this interaction is disturbed (e.g. by hypophysectomy) the blas[illegible] [illegible] proliferate, and tongues penetrate into the tissue beneath [illegible] [illegible]ll, 1952). However, the small size of such regenerative

tongues is very different from the formations of large [illegible] expansively growing carcinogen-tumour of *Triturus* [illegible] tion, the proliferation never starts in mucous glands [illegible] and metastases are [illegible] observed.

For these reasons [illegible] have no doubt that this epithelial t[illegible]our of [illegible] reasonably compar[illegible] with malignant carcinoma [illegible]s.

During preparation of this manuscript we receiv[illegible] [illegible] Christeansen (1961) who performed similar injec[illegible] [illegible] *cristatus*. The authors observed epithelial proli[illegible] [illegible] frequent following injection of DBA, as in our stu[illegible] [illegible] not be produced, pro[illegible]ly because the injections w[illegible] into the mu[illegible] not, as in our mat[illegible]beutaneously, so that the carcinogenic solution could permeate into the [illegible] pocket. Moreover, the aut[illegible] the injection [illegible] the tail, a very unf[illegible]le location, since we cou[illegible] on the tail only in [illegible]. The epithelial hyperpl[illegible] of [illegible] sen regressed after [illegible] 30 days. No definite hi[illegible] [illegible]scription of [illegible] origin and the regr[illegible] of these hyperplasias has [illegible] giv[illegible].

PART 2

Differenti[illegible] processes in the epithelial t[illegible] of [illegible]cristatus

It has been dem[illegible]ed in Part 1 that the [illegible] growth which was produced in *T. cri[illegible]* is a real tumour and that [illegible] may be compared with carcinoma in man[illegible]. With the ability to produce tumours in newts we were now able to investig[illegible] this tumour would show differentiation phenomena as predicted by our [illegible] speculations (see introduction).

In fact the tumo[illegible] [illegible]ed a pronounced tendency to spo[illegible]neous regression, even without any [illegible]ental influence. The frequency of tumour re[illegible]ssion appeared to be hi[illegible] [illegible]pendent upon the anatomi[illegible] site of the tumour and various biological [illegible]ns of the animals such as age, s[illegible]nal rhythm, [illegible]. These differences in the frequency of tumour regression will be presented in a later paper. In ev[illegible] in which no such spontaneous h[illegible]ing occurred the tumours led to the [illegible] of the animals.

Our attention w[illegible]ssed to the manner in which this spontaneous h[illegible]ing took place. We [illegible] to see if differentiation phenomena occurre[illegible] and if tumour cells were p[illegible] incorporated into the normal tissues of the organism. Furthermore, we w[illegible] to investigate if the different types or stages of tumours (expansive, infiltrati[illegible]mours, and metastases) showed differences in their manner of spontan[illegible]ling.

RESULTS

Numerous serial h[illegible]gical sections of healing or already healed tumours showed that differenti[illegible] had occurred during spontaneous healing. Tumour cells were seen to h[illegible]ifferentiated into apparently normal cells of *Tritu[illegible]*

and [illegible] [illegible]ganized into tissues, normally found in this newt. In some [illegible] [illegible]y differentiated tissues had formed abnormal but not cance[illegible]

Th[illegible] [illegible]tia[illegible] of the cancer into normal [illegible] depended upon the initial type [illegible] [illegible]ur [illegible] observed. Expansively growing tumours which had different [illegible] [illegible]elded [illegible]ctures quite different from infiltrative tumours or metastases [illegible] [illegible]ffer[illegible]ion fate of each of these tumour types will be considered separ[illegible]

Diffe[illegible]n of [illegible]sively growing tumours

Th[illegible] [illegible]ntia[illegible] such expansively growing tumours took place in various ways [illegible] [illegible]ffer[illegible]n into continuous tissues or differentiation by single cells [illegible] tissu[illegible] [illegible]inuity. Furthermore, abnormal cell types could also be obs[illegible]

D[illegible] [illegible]ation [illegible] [illegible]ntinuous tissues usually took place by pigmentation and [illegible] [illegible]ication. This differentiation began by the appearance of [illegible] [illegible]helial a[illegible] [illegible]ent of the tumour cells on the margin of the tumour [illegible] [illegible]g. 20). [illegible] [illegible]ocess did not set in simultaneously all over the tumour but [illegible] [illegible] [illegible]se parts of the tumour which had most deeply penetrat[illegible] [illegible] the [illegible] [illegible]ure. In some cases, the beginning of this differentiation [illegible] [illegible] [illegible]s of the tumour which were adjacent to blood-vessels. Th[illegible] [illegible] which [illegible] now epithelially arranged began to lengthen and were co[illegible] [illegible] [illegible]ed by additional elongated cells also derived from the tu[illegible] [illegible] Plate 4, [illegible] Subsequently, the formation of pigment granules was [illegible] in these [illegible] this pigmentation became more intense and the cells [illegible] [illegible] take on [illegible]tic shapes (Plate 5, fig. 26). Finally, a dense layer of [illegible] cells was [illegible] at the periphery of the tumour (Plate 5, fig. 27).

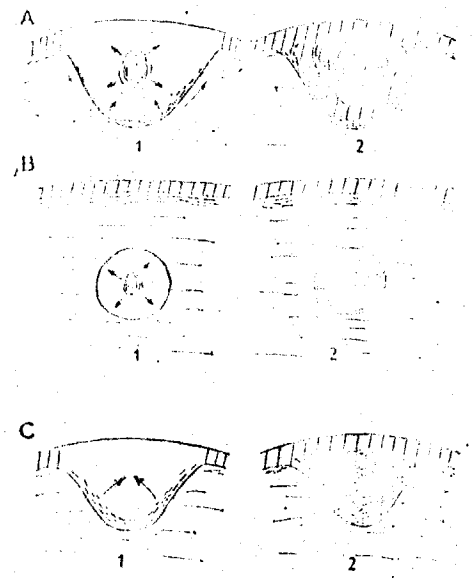
[illegible]ltaneously [illegible] centre of the growth, a cornification process set in. [illegible] cells rou[illegible] and then became enveloped by flat pavement cells [illegible] [illegible], fig. 22). [illegible] [illegible]ocess continuously new layers of squamous epithelium [illegible] formed (Pl[illegible] [illegible]. 23). These layers, built up by former tumour cells, [illegible] [illegible]istological[illegible] [illegible] with the cornified external layer of the normal [illegible] [illegible] [illegible]lly desquamated. In this way, an onion-like multi[illegible] [illegible] structure [illegible] [illegible]d which was cornified in its centre. This structure [illegible] [illegible] [illegible]d therefore approached the peripheral pigment cell [illegible] of the tum[illegible] [illegible] process of cornification stopped at a distance of two [illegible] cells fro[illegible] [illegible]ment layer.

[illegible] resulting [illegible] [illegible] became stable. This final structure then consisted of [illegible] [illegible] [illegible]tre which was enveloped by epithelium and pigment [illegible] [illegible]ers (Plate [illegible] [illegible]). This envelopment was very similar to the normal [illegible] [illegible]nt.

[illegible] similarity [illegible] [illegible]ormal integument became further evident by the dif[illegible] [illegible]tion of mu[illegible] [illegible]lands from the tumour (Plate 4, fig. 24). Numerous

small tumour-cell groupings were formed at the outer margin of the tumour (Plate 4, fig. 25). A swelling of cytoplasm could be observed in these cell groups, and, finally, the central cells of these groups disintegrated to form mucus. The resulting mucous glands were usually enveloped by the pigment cell layer (Plate 5, figs. 26, 27).

These mucous glands oriented their ducts to the centre of the cornified 'onion' where they formed thickened ends, filled with degenerated cells. This orientation of the gland ducts suggested that the cornified layers in the centre were also physiologically identical with the external layer of normal epithelium.



TEXT FIG. 3. Scheme of the differentiation types in expansively growing tumours. A. *Simultaneous cornification and pigmentation process.* 1, beginning of central cornification and peripheral pigmentation (compare with Plate 4, figs. 20, 21, 22, 23). 2, final stage. An epithelium remains between the cornified and pigmented tissues. Mucous glands are not drawn in this [illegible] (Plate 4, fig. 24). B. *Cornification without pigmentation.* 1, beginning of cornification is seen in the centre of the tumour; no pigmentation occurs at its periphery. 2, final stage: the entire tumour is cornified (Plate 5, fig. 28). C. *Pigmentation without cornification.* 1, beginning of pigmentation at the periphery of the tumour is shown; no cornification in its centre takes place. 2, final stage: the entire tumour is melanized (Plate 5, fig. 29).

In such structures, the system of vessels of the arteria and vena cutanea, as well as the subepithelial connective tissue, were notably absent and in distinct contrast to the normal integument. The pigment cell layer of these structures was always in immediate contact with the musculature (Plate 5, figs. 26, 27). Once such a structure formed it remained stable for the entire period of observation, which was more than 9 months. Ninety per cent. of the expansively growing tumours in these newts showed this type of differentiation.

Other variations in tumour differentiation were cornification without pig-

[illegible] mentation without cornification. If no pigmentation took place [illegible] of the tumour, the cornified onion-like structure enlarged until [illegible] margin of the tumour. Therefore in these cases no epithelium and [illegible] were formed and the whole tumour was cornified (Plate 5, fig. 28). [illegible] without pigmentation was found to occur in deeply [illegible] tumours. Several of these deep tumours arose following [illegible] applies [illegible] carcinogen.

On [illegible] hand, if pigmentation without cornification occurred, the entire tumour [illegible] into pigment cells. Pigmentation began at the periphery of the [illegible] in this type of response, progressed centrally until the entire [illegible] pigmented.

This [illegible] of tumour differentiation could only be observed in [illegible] cases: all of [illegible] in tumours which followed injection of dibenzanthracene + benzene [illegible] this type of differentiation there was also no epithelium formation [illegible] of the responses resulted in a pigmented tongue or nodule [illegible] the forward tumour (Plate 5, fig. 29).

The [illegible] different variations of differentiation seen in expansively growing [illegible] shown in Text-fig. 3.

If [illegible] differentiation occurred without tissue continuity, the same differentiation [illegible] were found as above but occurred in a less organized [illegible] the distal parts of the tumour differentiation of single [illegible] pigment cells took place. The differentiated cells were scattered [illegible] unchanged tumour cells (Plate 5, fig. 30). Cornification was very [illegible] to see because of simultaneous degenerative processes. Pigment [illegible] imperfect; pigment cells contained only few pigment granules. The [illegible] pigment cells did not show the oblong and dendriform shape of [illegible]; usually they maintained the spherical shape of melanoblasts. Some [illegible] several pigment cells formed irregular clusters.

In [illegible], it can be stated that expansively growing tumours of the tail (stage [illegible] tumour development) may differentiate into cornified, pigment, glands [illegible] normal epithelium cells. Differentiation occurred either into [illegible] tissues or only in single cells. In the latter case, the differentiation [illegible]. Necrosis of tumour cells could only exceptionally be observed. It [illegible] either in the centre of large expansive tumours or during the differentiation [illegible] of large tumours as necrosis of single cells scattered between [illegible] cells.

[illegible] processes in infiltrating tumours of the tail and trunk (stage 3 of [illegible])

[illegible] differences in the manner of differentiation were seen in infiltrating [illegible] continuous tumour masses which showed peripheral infiltration [illegible] in a similar manner as was seen in the expansively growing [illegible] described above. An onion-like structure was formed in the centre of

each of these masses and the margin of the tumour [illegible] pigment [illegible] epithelium then appeared between these two tissues. However, the [illegible] structure did not cornify and developed only a few layers of flat epithelium which soon disintegrated by necrosis. The pigment layer usually was [illegible] prominent and mucous glands were seldom observed.

In contrast to the above, the differentiation of infiltrating rod-like [illegible] projections (as described in Part 1) occurred in a different manner. [illegible] only a few peripheral cells became pigment cells (Plate 6, [illegible] 33). The [illegible] of the remaining tumour cells began to elongate in the longitudinal direction [illegible] the 'rod' (Plate 6, figs. 32, 33, 34). The cytoplasm of [illegible] cells increased and therefore the nuclei moved apart. Furthermore, intense longitudinal fibrillation appeared in this cytoplasm. By continuation of this tendency of differentiation, i.e. the alternation of tumour cells to elongated fibrous cells, the outer layer of the 'rods' took the appearance of fibrous connective tissue (Plate 6, fig. 35), while a centre core of tumour cells remained. Finally, the central core became thinner and even disappeared. This process was continued until only connective tissue-like cells were found at the site of the original tumour 'rod'. The [illegible] cells initially remained different from the normal adjacent connective tissue, as judged by their more intense staining and increased fibrillation. Finally they closely approached the appearance of normal connective tissue. Degeneration or necrosis was not seen in these rods.

Therefore two processes may be distinguished. (*a*) Transformation of the tumour cells into connective-tissue-like cells with rich fibrillation. The [illegible] differ from the surrounding connective tissue. (*b*) Further transformation of this fibrous connective tissue to cells which closely resemble the surrounding connective tissue. This transformation was also seen in numerous transitional stages.

The transitional stages between tumour cells and fibrous connective tissue-like cells are as follows. (1) *Tumour cell*: large spherical nucleus, scanty cytoplasm which stains intensively with haematoxylin (Plate [illegible], fig. 31). (2) *Early transitional stage*: oval or long nucleus with increased cytoplasm which stains intensively, beginning of fibrillation (Plate 6, fig. 32). (3) *Later transitional stage*: nucleus most elongated, cytoplasm intensively staining, with intense fibrillation (Plate [illegible], fig. 33). (4) *Connective-tissue-like cell*: most elongated nucleus, cytoplasm further increased and now less staining, but with intense fibrillation (Plate [illegible], fig. 35). This classification is very artificial, transition between tumour cells and connective-tissue-like cells was quite continuous.

The resulting tissue differed from the last stages of tumour cell transformation only by its significantly paler tinge and differed from other connective tissues by its large nuclei and its more intense and irregular fibrillation.

In all newts investigated 4 weeks after the appearance of a tumour, only few remnants of the tumour could be found with indefinite transition to the connective tissue of the surrounding areas.

Differentiation of metastases

Upon [illegible] healing of metastases always occurred simultaneously with the healing [illegible] primary tumour. It took place by differentiation of degenerative mucous [illegible] in a metastasis had grown to a certain size, single cells in its central [illegible] separated from the others and formed loose clusters (Plate 7, fig. 36), [illegible] with methylene blue. The cytoplasm increased and these cells became [illegible] cells. Additional cells of the metastasis continuously joined this cell [illegible] (Plate 7, fig. 37) and in this way the entire metastasis was transformed into [illegible] degenerative mucous cells (Plate 7, fig. 38). These cells were finally [illegible] duct, probably deriving from a destroyed mucous gland. The [illegible] formed which were later assimilated into the normal epithelium [illegible] regeneration.

In contrast, the large metastases which were situated in the musculature did not [illegible] to form mucous cells but differentiated exactly in the same way as [illegible] rapidly growing tumours. In the centre of these large metastases a [illegible] process took place (Plate 7, fig. 39) and an onion-like multi[illegible] was formed (Plate 7, fig. 40). The peripheral tumour cells dif[illegible] cells. Between the inner cornified structure and the [illegible] pigment cells an epithelium-like tissue was formed. The [illegible] glands at the periphery, which was found in the dif[illegible] rapidly growing tumours, was also seen in the differentiation of the metastasis.

The [illegible] structure (Plate 7, fig. 40) was identical with the structures formed by the [illegible] differentiation of expansively growing primary tumours (Plate 4, fig. 24).

DISCUSSION

Part 2

Differentiation phenomena have been observed in these growths, one [illegible] if they are actually tumours. Let us examine the question: is dif[illegible] of a growth a reason to deny its cancerous nature? Certainly the [illegible] seems to be a prevalent opinion.

It [illegible] reasonable to us that rather different circumstances prevail for the newt [illegible] tumours in animals with great regenerative powers as compared to those [illegible] in the non-regenerating mammals. It is well known that newts are [illegible] of differentiating new tissues from undifferentiated blastema cells during [illegible] regeneration. The existence of this capacity in newts strongly suggested to us [illegible] these animals would be also capable of differentiating tumour cells during [illegible] process comparable to regeneration.

[illegible] have been observations of differentiation phenomena reported [illegible] tumours. Witten & Zak (1952) reported spontaneous healing [illegible] differentiation in a prickle cell epithelioma. In some tumours of the [illegible] the uterus differentiation into cross-striated musculature has been

observed (Hamperl, 1956). Pierce *et al.* (1960) succeeded in producing differentiations in a malignant carcinoma of the testis in mice. With repeated transplantation of this malignant carcinoma they obtained a teratocarcinoma in which glands, nerves, and even cartilage and muscles were differentiated. Numerous cases of tumour differentiation following X-ray have been compiled by Zollinger (1960). These observations all show that differentiation of tumour cells can occur even in non-regenerating animals. In those animals such as newts, whose high differentiation capacity typically reveals itself during regeneration, we can even more reasonably expect that differentiation of tumour cells may occur. Therefore the differentiation of tumours in our newts can no longer be a reason to doubt the initial cancerous nature of this tumour which showed numerous malignant characteristics as described in Part 1.

If we assume that tumour differentiation shows certain similarities with a regeneration process, we should attempt to explain how spontaneous tumour differentiation becomes released. A regeneration process is normally released by amputation. As could be shown in triclads by Ortner & Seilern-Aspang (1962), any quantitative disturbance of the biological system by amputation or an addition of a certain quantity of tissue, releases a regeneration process. Thus we would suggest that in the newts the destruction of a certain quantity of normal tissue by the growing tumour is the cause for the onset of the differentiation process.

We observed that the cells of infiltrating rod-like tumour projections differentiated into connective-tissue-like cells. Since the transformation from epithelial cells to mesenchymal cells has often been described in newts during regeneration (Rose, 1938; Hay, 1952; Rose *et al.*, 1955), it seems very likely to us that these connective tissue-like cells had actually differentiated into true connective tissue.

It was striking that only infiltrating tumours differentiated to connective tissue whereas the differentiation of expansively growing tumours, i.e. the advanced tumour stages, was restricted to the formation of tissues corresponding to the epithelial origin of the tumour (cornified cells, pigment cells, mucous cells). Since we observed increased pluripotential differentiation capacities in advanced tumour stages (differentiation of connective tissue from infiltrating tumours), this suggests that tumour growth not only causes morphological dedifferentiation of the cells, but also an increase of pluripotency. In consequence of this observation we do not agree with Rose & Wakeford (1953) who suggested that a dedifferentiation of the tumour cells of the newts is still necessary before their differentiation.

The classical histological technique used here to follow the course of tumour-cell differentiation makes our observations probable but not proven. Other techniques which permit marking of individual cells are needed. The use of tritiated thymidine for this purpose is contemplated.

As already mentioned in Part 1 the carcinogen-induced hyperplasia of 7.

cri [illegible] by Arffmann & Christensen (1961) also regressed, but no his[illegible]ption of this process has been given by the authors.

SUMMARY

1. [illegible]mours have been induced in *T. cristatus* by any of several carc[illegible] tumours arise from the mucous glands of the skin.

2. [illegible] showed infiltrative and destructive growth, sometimes penetratin[illegible]ritoneal cavity. Metastasis also occurred. The tumours thus app[illegible]lignant.

3. [illegible]s malignant characteristics this carcinogen-induced epithelial tumo[illegible]ressed. This regression of an apparently malignant tumour occu[illegible]ously. During regression of the tumour, differentiation of the tumo[illegible] normal, non-malignant tissues occurred. Expansively growing tumo[illegible]ted independently of their location into pigment cell layers, co[illegible]ucous glands, and epithelium of the integument. In contrast, infilt[illegible]s differentiated in accordance with their surroundings and eve[illegible]ssue like cells were formed. Tumour metastases differentiated by th[illegible] of degenerative mucous cells or in the same ways as expansively [illegible]ours. If no differentiation occurred, the tumours were lethal.

4. [illegible] of newts to bring their tumour cells under control again by a diff[illegible]rocess is attributed to the great regeneration power of these anima[illegible]

ZUSAMMENFASSUNG

1. [illegible] (*Triturus cristatus*) wur[illegible] durch verschiedene Cancerogene epith[illegible]en erzeugt. Die Tumoren gehen von den Schleimdrüsen der Haut [illegible]

2. [illegible]oren zeigten infiltrierendes und destruierendes Wachstum, [illegible]gen sie in die Bauchhöhle ein. Es wurden auch Metastasen [illegible] Tumoren verhielten sich also wie eine maligne Geschwulst.

3. [illegible] malignen Merkmale heilte der Tumor oft spontan aus. [illegible] Ausheilung wurde die Differenzierung von Tumorzellen zu [illegible]en beobachtet. Expansiv gewachsene Tumoren differenzierten [illegible] und unabhängig von ihrer Umgebung zu Pigmentgeweben, [illegible]gen, Schleimdrüsen und zu Epithel. Infiltrierende Tumoren [illegible]zierten [illegible]gemäß und dabei wurden sogar bindegewebsartige [illegible] Bei der Differenzierung von Metastasen wurden degenerative [illegible]bildet oder ähnliche Strukturen wie bei der Differenzierung [illegible]er Tumoren. Wenn keine Differenzierung auftrat, starben die [illegible]moren.

4. [illegible]ieit der Molche, Tumorzellen wieder zu differenzieren und [illegible]ntrolle zu bringen, wird in der Diskussion in Zusammenhang mit [illegible]nerationsvermögen dieser Tiere gebracht.

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EXPLANATION OF PLATES

PLATE 1

[illegible] normal [illegible] of *T. [illegible]* [illegible] subcutaneous connective tissue; *e*, epithelium, with a [illegible] layer on [illegible] limit; [illegible] musculature; *p*, pigment cells. ×75.

[illegible] normal [illegible] of the skin, [illegible] duct; *g*, germinative layer of the gland pocket. [illegible]

[illegible] of the epithelium during an inflammation process caused by injection of lactic [illegible]

[illegible] inflammation process. The [illegible] from without into two damaged mucous [illegible] right gland [illegible] already [illegible] with [illegible] cells, the left one only in its distal part. *g*, [illegible] gland [illegible]. ×100.

[illegible] of [illegible] in the [illegible] layer of a mucous gland following injection [illegible]. ×100.

[illegible] of the [illegible] the [illegible] layer. The gland pocket has become [illegible] character. ×300.

[illegible] outlet ducts are still open and remnants of [illegible] in the left gland, [illegible] is still limited by the gland wall, in the right [illegible] the gland [illegible] with the epithelium. *m*, mucus. ×150.

PLATE 2

[illegible] gland [illegible] which [illegible] coalesced together and with the epithelium [illegible] wing to [illegible] on the [illegible]0.

A [illegible] trunk [illegible] penetrated [illegible] the musculature. Early infiltration may be [illegible]

An [illegible] tumour [illegible] already [illegible] the spine. *s*, spine. ×100.

A [illegible] tumour [illegible] which infiltrates the musculature ×150.

A [illegible] infiltration of an infiltrating tu[illegible] on the trunk. The beginning of rod-like pro[illegible]

[illegible] of infiltrating cells of [illegible] infiltrative and destructive tumour, *m*, muscula[illegible]

PLATE 3

[illegible] the tu[illegible] penetrate [illegible] peritoneal cavity where it forms an epithelium-like pave[illegible]

[illegible] a tumour [illegible] penetrated [illegible] to the peritoneal cavity. ×50.

[illegible] head, [illegible] extremities of *T. cristatus*. Metastases can be [illegible] the [illegible] 4, 5.

[illegible] Early [illegible] the [illegible] of the arteria cutanea. Contact with the [illegible] *e*, [illegible]thelium, *m*, [illegible]tosis, *v*, vessels of the arteria cutanea. ×250.

[illegible]

[illegible] with the [illegible]thelium. Connective tissue and mucous glands [illegible] penetrate [illegible] the musculature. Note that primary tumour [illegible] only in the tail [illegible] were [illegible] in the trunk. ×250.

PLATE 4

[illegible]0. [illegible] of an [illegible]ively growing tumour. One layer of tumour [illegible] the m[illegible] the tum[illegible] connective tissue; *t*, tumour. ×250.

[illegible]1. The [illegible] layer on [illegible]in of the [illegible]our has become more distinct. It becomes [illegible] part of [illegible] tumour was lost during preparation of the [illegible]00.

[illegible]2. [illegible] of the cornifi[illegible] in the [illegible]otre of a small tumour. One single spherical [illegible] in the [illegible] around which [illegible] cornified cells are arranged. Pigmentation of [illegible] cells can [illegible]. ×400.

FIG. 23. Advanced cornification in the centre of an expansively [illegible] tumour. Cornified layers form an onion-like structure. ×400.

FIG. 24. Large differentiated expansively grown tumour. In its [illegible] the onion-like structure of numerous cornified cell layers, on its margin an epithelium, a [illegible] several mucous glands all derived from the tumour. ×50.

FIG. 25. Beginning of the formation of a mucous gland from [illegible] A [illegible] peripheral cells have detached from the tumour and agglomerated. Pigment cell [illegible] differentiated. *c*, cornified cell layer in the centre of the tumour; *g*, beginning of a mucous [illegible]

PLATE 5

FIG. 26. Advanced differentiation of a mucous gland and of the [illegible] pigment cell layer from tumour cells. There is no subcutaneous connective tissue between the [illegible] tumour and the [illegible]. ×300.

FIG. 27. Further stage of the differentiation of a gland and of the [illegible] layer from tumour cells. No subepithelial connective tissue has been formed. ×100.

FIG. 28. Small and deeply penetrated masses of tumour cells which [illegible] from the tumour, [illegible] following painting with 2 per cent. benzpyrene, have [illegible] cornified. Neither pigment nor epithelial cells nor mucous glands have been differentiated. [illegible] tumour; *s*, spine. [illegible]

FIG. 29. A tongue of a tumour which had penetrated into the musculature has been totally melanized. No cornification is present. ×100.

FIG. 30. Irregularly [illegible] and imperfect differentiation of a tumour [illegible] pigment and cornified [illegible]. No tissues were formed. *c*, cornified cell; *p*, pigment cell. ×100.

PLATE 6

FIG. 31. Tumour tissue before the start of differentiation pro[illegible]

FIG. 32. Infiltrative tumour at the beginning of the differentiation pro[illegible] cells and nuclei begin to elongate. ×400.

FIGS. 33 & 34. Infiltrating rod-like tumour projection during its [illegible]. Tumour cells are still more attenuated, pigment cells are differentiated on the [illegible]. Fig. 33: ×300; Fig. 34: ×200.

FIG. 35. An infiltrating rod-like tumour projection in advanced [illegible]. Elongation of the nuclei and formation of fibrous cytoplasm is seen. The former tumour [illegible] originally deriving from gland cells, now assume a connective tissue-like appearance. ×300.

PLATE 7

FIG. 36. Metastasis whose degraded cells detach into a newly formed cavity in its centre. ×250.

FIG. 37. One degraded cell can be seen to be advancing into a [illegible] the metastasis. There tumour cells become mucous cells. *mc*, mucous cells; *tc*, tumour cell [illegible] just entering the cavity. [illegible]00.

FIG. 38. The metastases have been differentiated by progressive [illegible] of tumour cells into the enlarging cavities and by their transformation to mucous cell[illegible]

FIG. 39. Early stage of the differentiation of a metastasis in the [illegible] of the trunk (primary tumour on the tail). In the [illegible]tre of the metastasis a few layers of [illegible] and epithelium already have been formed. *cl*, cornified layers; *m*, musculature; [illegible]. ×150.

FIG. 40. Late stage of differentiation of a large metastasis situated [illegible] the musculature of the trunk (primary tumour on the tail). Cornification has set in at two centr[illegible] therefore two 'onions' of cornified layers have been formed. Differentiation of pigment cells has [illegible] the periphery of the metastasis. Furthermore, three mucous glands have been [illegible]. *cl*, cornified layers; *m*, musculature; *mg*, mucous glands; *pc*, pigment cells. ×75.

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The effect of different levels of sodium hydroxide spray treatment of wheat straw on consumption and digestibility by cattle

BY MAHENDRA SINGH AND M. G. JACKSON

College of Agriculture, U.P. Agricultural University, Pantnagar, District Nainital, U.P., India

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SUMMARY

A sodium hydroxide spray treatment of wheat straw was evaluated in experiments on twenty male calves. Sodium hydroxide solutions of 0, 3·3, 6·7 and 10 % concentration were sprayed on ground wheat straw (Exp. 1) and chaffed wheat straw (Exp. 2) at the rate of 1000 litres per tonne. The straws were not washed. The treated straw was then fortified with groundnut cake, molasses and a mineral supplement. In Exp. 1 rations were prepared in bulk and dried and in Exp. 2 the rations were prepared daily and fed wet. A 1 kg green forage supplement was fed daily to all animals. The dry-matter intake (g/kg body weight$^{0·75}$), and organic matter digestibility coefficients (%) for the four levels of alkali treatment were 86, 95, 78, 66 and 53, 63, 63 and 62 respectively in Exp. 1 and for Exp. 2 they were 95, 121, 105, 89 and 55, 70, 71 and 71. The intake of digestible organic matter thus increased by 25 and 63 % in Exp. 1 and Exp. 2, respectively. Nitrogen, calcium and phosphorus retentions were not adversely affected up to 3·3 % alkali treatment in Exp. 2. The effectiveness of this spray treatment in terms of increased nutritive value is compared with that of the older Beckmann method of alkali treatment. Treatment costs are also compared.

INTRODUCTION

The low digestibility and voluntary intake of roughages like wheat straw are mainly due to their high content to lignin. By treating them with delignifying agents digestibility and voluntary intake can be increased. This was first demonstrated many years ago by Beckmann (1921) in Germany, and then subsequently by Ferguson (1942) in England and Sen, Ray & Talapatra (1942) in India. Sodium hydroxide was the delignifying agent used and the treatment involved soaking the roughage in large vats of sodium hydroxide solution and then washing it to remove residual alkali. This method is being used commercially today in some parts of Norway (Homb, 1956). Treatment by this method is, however, expensive, mainly because of the heavy loss of nutrients (20–25 %) by leaching during the washing operation. The cost of the treated roughage is high and it is not competitive with other foodstuffs in most countries. Recently Wilson & Pigden (1964) described a process in which the dry roughage is sprayed with only a small amount of a sodium hydroxide solution and then fed directly without washing. Leaching losses are thus avoided and less labour, water and capital investment are needed than in the earlier method developed by Beckmann. This method of treatment has been tried in laboratory-scale experiments, comparing a number of different delignifying agents by Chandra & Jackson (1971). They have recommended sodium hydroxide for removing lignin and increasing digestibility on the basis of effectiveness and ease of treatment. With the NaOH treatment, rumen digestibility (dacron bag technique) increased linearly with increasing amounts of NaOH up to a level of 10 g/100 g of roughage and levelled off thereafter. Between the 0 and 10 % levels of treatment, the amount of unreacted NaOH in the roughage after treatment, as a percentage of the total sprayed on, increased from 0 to 30 %. It was concluded that, in animal feeding, the advantage of greater digestibility with increasing levels of alkali might be offset at some point by an adverse effect of residual alkali. The next step in evaluating this alkali spray treatment was thus to determine the optimum level of alkali by means of animal feeding experiments. Such evaluation was the objective of the two experiments described in this paper.

EXPERIMENTAL

Experiment 1. Wheat straw ground in a hammer mill through a 3 mm sieve was treated with solu-

tions of sodium hydroxide at the rate of 1000 litres per tonne. Four solutions were used, 0, 3·3, 6·7 and 10%. After 12 h, groundnut cake, molasses (to improve smell and taste) and a mineral mixture were made into a slurry and mixed with the treated straw. The material composition of the rations is shown in Table 1. The rations were then spread in the sun on a cement platform until dry (80–90% dry-matter content). Drying took about 3 days. Dried rations were stored in gunny bags. The chemical composition of these rations is given in Table 2. They were supplemented with 1 kg per head of green forage daily to supply carotene and long roughage. The forage was wilted lucerne for the first half of the experiment and sorghum during the second half. The experiment was done on eight Sahiwal and Sahiwal–Jersey crossbred male calves 9–18 months of age. A 4 × 4 changeover experimental design (Cochran, Autery & Cannon, 1941) was used, each of the four periods being 21 days long. The animals were offered weighed amounts of feed twice a day (9 a.m. and 5 p.m.) Fifteen per cent refusals were allowed. The digestibility of nutrients was determined by conventional digestibility trials conducted during the last 7 days of each period. Dry-matter intake was also determined during these digestibility trials. All chemical analyses were done by the methods of the Indian Standards Institution (1962). The animals were kept in metabolism cages throughout and were taken out for 3 consecutive days after every 21 days, at the end of each period, to be weighed. Water was offered *ad libitum* 3 times a day. Daily water consumption was measured during the digestibility trials.

Experiment 2. The wheat straw used in Exp. 2 came from the same lot as that used in Exp. 1. It was chaffed into 2–4 cm lengths and then made into the same four rations already described for Exp. 1. The only difference was that the rations were made in small lots daily and fed to the animals in a semi-wet condition. The chaffed straw was put into a half-tonne horizontal batch mixer. The NaOH solution was sprinkled on from a garden sprinkling can within 5 min, while the mixer blades were turning. An additional 10 min was allowed for reaction of the NaOH and the straw (see Chandra & Jackson (1971) for a discussion of reaction time). Then the other ingredients were added and mixing continued for another 5 min. The prepared rations were then offered to the animals within 1–2 h. The chemical composition of these rations is also given in Table 2. A 1 kg supplement of green forage was given as for Exp. 1. The experiment was done on twelve male calves, 9–18 months of age, divided into four groups of three in accordance with the procedure required for a completely randomized experimental design. Each group of animals was fed their respective ration continuously for 23 weeks. Seven-day digestibility and intake trials were conducted during the 7th, 10th and 22nd weeks of the experiment. Up to the 14th week, the calves were tied in simple digestion stalls and thereafter in metabolism cages. During the last digestibility trial, the animals were fitted with urine collecting funnels and urine collected as well as faeces, so that balances of nitrogen, calcium and phosphorus could be estimated

Table 1. *Material composition of the experimental rations* (%)

Ingredient	Ration, i.e. level of NaOH treatment (%) 0	3·3	6·7	10·0
Straw*	79·0	76·5	73·8	71·8
NaOH	0·0	2·5	5·2	7·2
Cake	10	10	10	10
Molasses	10	10	10	10
Minerals	1	1	1	1
Total	100	100	100	100

* Weight of dry straw before treatment.

Table 2. *Chemical composition of rations and of green forage supplements*

Exp.	Level of NaOH treatment (%)	Dry-matter content as fed (%)	Chemical composition on a dry-matter basis, (%) Crude protein	Calcium	Phosphorus
1	0	89	7·6	0·55	0·23
	3·3	85	7·4	0·65	0·25
	6·7	81	7·5	0·65	0·24
	10·0	80	6·9	0·63	0·22
2	0	47	7·0	0·50	0·40
	3·3	48	5·9	0·50	0·33
	6·7	47	6·2	0·55	0·28
	10·0	46	5·7	0·50	0·33
Lucerne (wilted)		40	16·4	0·70	0·38
Sorghum		31	4·8	0·25	0·36

The animals were weighed on three consecutive days at the end of each digestibility trial. Water consumption was measured during the last digestibility trial. The data were analysed statistically as in a split-plot field trial experimental design (Panse & Sakhatme, 1957). The level of sodium hydroxide was the main treatment and digestibility trial was the subtreatment.

The ground nut cake used in this experiment was from several lots which were lower in crude protein content than the cake used in Exp. 1. The crude protein content of the rations in Exp. 2 was thus much lower than the average of 7.3 % for the rations in Exp. 1 (Table 4). After the second digestibility trial it was decided to increase the percentage of cake in the ration to 15 to raise the crude protein content of Exp. 2 rations to the level obtaining in Exp. 1 rations (see Table 2). This increase was done at the expense of the treated straw.

RESULTS

The animals in both experiments remained in good health throughout and gained weight. In Exp. 2 the live-weight gains in g/day were 238, 311, 238 and 202 (standard error ± 52 g/day) for 0, 3·3, 6·7 and 10 % rations, respectively. They ruminated normally. The voluntary dry-matter intake and nutrient digestibility coefficients for the rations in the two experiments are given in Table 3. In Exp. 2 the sodium hydroxide treatment with the 3·3 % solution significantly increased dry-matter intake. The 6·7 % treated roughage ration was poorer in this respect than the 3·3 % rations, while the 10 %

Table 3. *Dry-matter intake, nutrient digestibility coefficients and calculated digested nutrient intake in Exp. 1 and 2 (green forage supplements included)*

Exp.	Level of NaOH treatment (%)	Dry-matter* intake, g/kg body weight$^{0.75}$	Nutrient digestibility coefficients (%)		Calculated digested organic matter, % of dry-matter	Calculated digestible organic matter intake, g/kg body weight$^{0.75}$
			Organic matter	Crude protein		
1	0	86	53	48	46	40
	3·3	95	63	55	53	50
	6·7	78	63	53	52	41
	10.0	66	62	56	51	34
	S.E. of mean	6·2	1·9	2·8	—	1·6
	Error D.F.	15	15	15	—	15
2	0	95	55	62	48	46
	3·3	121	70	63	61	75
	6·7	105	71	61	61	64
	10·0	89	71	51	60	53
	S.E. of mean	1·3	1·3	0·8	—	0·6
	Error D.F.	7	7	7	—	7

* The mean green forage dry-matter intake for all rations in both experiments was 9 g/kg body weight$^{0.75}$.

Table 4. *Dry-matter intake and nutrient digestion coefficients in different digestibility trials of Exp. 2*

Digestion trial	Dry-matter intake, g/kg body weight$^{0.75}$	Crude protein content, % of dry-matter	Nutrient digestibility coefficients (%)	
			Organic matter	Crude protein
1	117	6·1	67	59
2	96	4·6	68	58
3	95	7·9*	67	62
S.E. of mean	2.9	—	1·5	2·0
Error D.F.	16	—	16	16

* The amount of groundnut cake in the rations had to be increased to 15 % during the later part of the experiment.

ration was poorer even than the control. In Exp. 1 the same pattern obtained, but the differences among rations was smaller and they were statistically non-significant, except that the 10 % ration was significantly poorer than the control and 3·3 % rations. Digestibility coefficients for organic matter were significantly increased by the 3·3 % treatment, but then levelled off with higher levels of treatment. Crude protein digestibility coefficients were highest with the 3·3 % rations, though not always significantly so. In terms of digested organic matter intake, the 3·3 % treatment effected a 25 and 63 % increase for the ground and chaffed straw, respectively. There was a marked difference in both the initial levels of intake and the increase brought about by the 3·3 % treatment between the ground and chaffed straw, in Exps. 1 and 2.

In Exp. 2 there was a significant decrease in dry-matter intake from the first to the third digestibility trial (Table 4). The lower intake during the last trial was probably a result of the restlessness of the calves when they were fitted with the urine funnels. Digestibility coefficients did not vary significantly among trials. There was no statistically significant interaction between rations and trials for dry-matter intake or protein digestibility, but there was for organic matter digestibility. The organic matter digestibility decreased significantly for the untreated straw ration from the first to the third trials and increased significantly for the 10 % [illegible] Further, the 10 % [illegible] was significantly better even than the [illegible] 3·3 % [illegible] in the third trial. The amounts of nitrogen, calcium and phosphorus retained were greatest with the 3·3 % ration in the last trial of Exp. 2, though not always significantly so (Table 6). The water consumption by the calves was not significantly increased by the alkali treatment in either [illegible] (Table 7).

Table 5. *Organic matter digestibility coefficients in different digestibility trials of Exp. 2*

Level of NaOH treatment	Digestibility trial			
	I	II	III	Mean
0	60	56	50	55
3·3	71	73	67	70
6·7	71	71	72	71
10·0	64	72	77	71
Mean	67	68	67	—

S.E. of values in the body of the table = 3·0. Error D.F. = 16.

DISCUSSION

The objective of these experiments was to determine whether the sodium hydroxide spray treatment would improve the nutritive value of wheat straw, and, if so, what level of the alkali would give

Table 6. *Nitrogen, calcium and phosphorus balances during the third digestibility trial of Exp. 2, g/animal/day*

Nutrient	Level of NaOH treatment (%)				S.E. of mean	Error D.F.
	0	3·3	6·7	10·0		
Nitrogen						
Intake	49	61	37	31	—	—
Outgo						
Faeces	16	19	14	16	—	—
Urine	11	12	8	8	—	—
Total	27	31	22	24	—	—
Retention	22	30	15	7	4·2	8
Retention as a % of intake	45	49	41	23	—	—
Calcium						
Intake	19	25	24	16	—	—
Outgo	9	8	6	9	—	—
Retention	10	17	18	7	2·4	8
Retention as a % of intake	53	68	75	44	—	—
Phosphorus						
Intake	14	16	9	10	—	—
Outgo	8	8	5	4	—	—
Retention	6	8	4	6	1.3	8
Retention as a % of intake	45	49	41	23	—	—

Table 7. Water intake of animals, litres/100 kg body weight per day

Level of NaOH treatment	Exp. 1	Exp. 2
0	7·6	8·4
3·3	11·6	7·7
6·7	11·7	8·5
10·0	11·6	8·0
S.E. of mean	1·3	0·5
Error D.F.	8	8

Table 8. The comparative cost of the Beckmann alkali treatment and the new spray process at current market prices, rupees

Item	Beckmann process	Spray process
Wheat Straw (one tonne)	150	150
NaOH	180 (1)	60 (2)
Handling, capital investment etc. (per tonne of straw treated)	20	10
Total	350 (3)	220
Total per tonne of treated straw (dry equivalent)	470	220

(1) Cost of 100 kg of NaOH, assuming only two-thirds of the original 150 kg is used up in the process.
(2) Cost of 33 kg (3·3 %) of NaOH.
(3) The cost Rs. 350 is for only 750 kg dry equivalent as 250 kg dry-matter is lost in treatment.

the maximum effect. Animal health was to be safeguarded. The results show a marked increase in nutritive value, in terms of digestible organic-matter intake, for both the ground and chaffed wheat straw spray-treated with 3·3 % of sodium hydroxide. Higher concentrations of spray were less effective and the 10 % treated straw was no better than the untreated straw. Over a period of 6 months continuous feeding no adverse effects of the alkali treatment were observed even at the 10 % level. Nitrogen, calcium and phosphorus balances were not adversely affected up to the 3·3 % level of alkali. Water consumption was not increased.

The next step in evaluating this spray treatment is obviously to determine what the enhanced nutritive value obtained with the 3·3 % alkali spray means in terms of animal production and feed costs. One experiment already done in the U.S.A. points to the potential utility of this treatment. Klopfenstein (personal communication) treated wheat straw with a 4 % sodium hydroxide spray and supplemented it with soyabean oil meal, ground wheat and minerals. The straw comprised 70 % of the ration. Dry-matter intake, body weight gain and feed efficiency of sheep were increased 25, 100 and 30 %, respectively.

In the experiments of Chandra & Jackson (1971), the rumen digestibility of treated roughage increased linearly up to 10 % of the alkali. In the animal feeding experiments reported here, digestibility, and voluntary dry-matter intake also, increased up to only the 3·3 % level of sodium hydroxide. It is possible that the increasing amounts of residual alkali exerted an adverse effect on the rumen fermentation, which, of course, would not be expected when digestibility was determined by the nylon bag technique. Another possible adverse factor with the 6·7 and 10 % sodium hydroxide rations might have been a high intake of sodium. In passing it should be noted, however, that the results of a short-term experiment done in Canada (Egden, personal communication) do not entirely agree with those reported here. With sheep, dry-matter digestibility was 44, 50, 59 and 59 for 0, 3, 6 and 9 % sodium hydroxide treated wheat straw supplemented with soyabean oil meal. Dry-matter intake increased from 34 to 65 g/kg body weight$^{0.75}$ from the control to the 9 % level of treatment. In that experiment the 6 or 9 % levels were thus best in terms of nutrient intake. Further, in the present Exp. 2, digestibility, though not voluntary intake, of the 10 % ration was significantly greater than that of the 3·3 % ration in the third trial. It might be that the comparative digestibility values for the third trial represent the true situation under long-term feeding better than the overall experiment means, but a judgement is difficult to make.

A critical evaluation of this spray treatment should include a comparison of it with the older method of Beckmann in terms of both effectiveness and cost. In the older method, the chaffed or whole roughage is soaked for 22 h in ten times its own weight of a 1·5 % sodium hydroxide solution (see review by Homb, 1956). This amounts to an availability of 15 kg sodium hydroxide per 100 kg of roughage. According to Sen *et al.* (1942) not all the sodium hydroxide is used up and the solution may be made up to strength and used once more. In laboratory tests with the spray process, Chandra & Jackson (1971) also found that much of the sodium hydroxide (40 %) is not used when 15 % sodium hydroxide is used. The loss of dry-matter on treatment is 20–30 % (Ferguson, 1942; Sen *et al.* 1942). In a large number of experiments done in Norway (Homb, 1956) between 1940 and 1950 the digestibility of straw organic matter increased on an average from 42 to 67 %. In experiments done in England during the same period (Ferguson, 1942) wheat straw organic matter digestibility increased, on the average, from 52 to 69 %. Sen *et al.* (1942) working in India reported increases in organic

matter digestibility coefficients from 57 to 76% and from 51 to 72% for paddy straw and wheat straw, respectively. The increases observed for the older method are thus from 15 to 25 units of digestibility, clearly greater than the 15 unit increase observed with the 3·3% spray treatment in the present experiment (Exp. 2) and that of Pigden (personal communication) reported above. This is not surprising in view of the much larger amounts of sodium hydroxide used in the older method and the fact that residues of alkali are then washed out. Further, in the new method the lignin which is rendered soluble by the alkali is not removed, and it would obviously depress the apparent digestibility coefficient of the total organic matter of the ration. The older method is thus more effective in increasing digestibility. Voluntary dry-matter intake was not measured by any of the above experimenters.

The spray-treated straw, however, costs less than half as much as that treated by the old method (Table 8). The higher cost of the latter is due primarily to the greater amount of sodium hydroxide used and to the loss of dry-matter during treatment. These comparative costs would presumably be found similar in all countries. Another real advantage of the spray process is that very little water is needed. Homb (1956) writes that in Norway the spread of alkali treatment of straw on farms is limited at places by lack of sufficient water. Shortage of water is certainly a problem in large parts of India.

The effectiveness of the sodium hydroxide treatment in increasing digestibility and dry-matter intake was greater in Exp. 2 than in Exp. 1. In the experiments of Chandra & Jackson (1971) it was concluded that the effectiveness of sodium hydroxide treatment was equal with ground and chaffed straw, but here in actual feeding experiments it was not so. Grinding of roughage by itself has usually been found to increase voluntary intake, but here in conjunction with sodium hydroxide treatment, consumption increased more with the chaffed straw than with the ground. The reason for these observations might not be the physical form of the roughage at all, but may be that one ration (Exp. 2) was wet and the other dry and dusty (Exp. 1). Pelleting the 3·3% ration of Exp. 1 perhaps made it more acceptable, for in another experiment (Singh and Jackson, unpublished data) calves consumed pellets at the rate of 3·9 kg of dry-matter per 100 kg body weight. Organic matter digestibility of the pellets was no different, however, than that for the 3·3% ration in Exp. 1, i.e., 63%. Clearly the effects of grinding and pelleting in conjunction with the alkali spray treatment need to be investigated in detail, and such investigation would best be done on alkali-spray treated straw based complete rations, as used in these experiments so that deficiencies of other nutrients do not bias the results as happened in one experiment (Exp. 2) done on unsupplemented oat straw by Donefer, Adeleye & Jones (1969). Of course, supplementing straws with various nutrients in which they are deficient is itself a method of improving their digestibility and voluntary intake, but it is assumed that the effects of supplementation and chemical treatment are additive. This assumption is supported by the observations of Donefer *et al.* (1969) on the effects of urea supplementation and sodium hydroxide treatment separately and in combination. And straw, however treated, must anyway be supplemented to form a satisfactory ration for long-term experiments or commercial feeding.

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Range-Finding Toxicity Data: List VI

HENRY F. SMYTH, JR., Ph.D., CHARLES P. CARPENTER, Ph.D., CARROL S. WEIL, M.A., URBANO C. POZZANI, M.S., and JEAN. A. STRIEGEL, B.S.

Mellon Institute of Industrial Research, Pittsburgh, Pennsylvania

A table presents acute toxicity and irritation data on more than 300 compounds, accumulated in a continuing program for screening potential commercial products. The standardized experimental methods are described.

DURING the seven years since the appearance of our last communication on the range-finding test,[1] many additional data have become available for presentation. The following Table lists these data under names conforming to the nomenclature used in *Chemical Abstracts*. This may make them unrecognizable to those who know them only by their trade names. For example: ethers of ethylene glycol are sold as CELLOSOLVE solvents, of diethylene glycol as CARBITOL solvents, and of propylene and dipropylene glycol as UCAR solvents. All materials included here are either in commercial production or have been evaluated for commercial potential within the past few years.

As has been stated in earlier communications,[1-5] the range-finding test is relied upon only to allow predictions of the comparative hazards of handling new chemicals. Acute toxicity studies, no matter how precisely executed, yield no more than an indication of the degree of care necessary to protect exposed workman or lead to an opinion that certain technically feasible applications of a chemical may or may not eventually be proved safe.

Our last paper[1] included data for epoxyethylbenzene. Recently obtained data upon another sample differ somewhat from that previously published. The latest assay yields a single-oral LD_{50} value of 2.33 ml/kg. Four hours in a concentrated vapor atmosphere caused no deaths among [illegible] rats while 1000 ppm inhaled for a similar period of time killed only two of six rats.

Because of minor changes in experimental methods and to eliminate reference to earlier papers in this series,[1-5] the methods for obtaining the range-finding data are briefly described.

Single oral dose toxicity is estimated by the gastric intubation of groups of five nonfasted, Carworth-Wistar male rats, or in rare instances indicated in the Table of female rats, four to five weeks of age and 90 to 120 grams in weight which have been reared in our own colony and maintained from time of weaning on Rockland rat diet, complete. The dosages are arranged in a logarithmic series differing by a factor of two. Whenever possible, the chemical is administered undiluted. When a lesser concentration is necessary, solution in water or corn oil or suspension in semi-solid agar are the preferred expedients. Occasionally, a 1% solution of TERGITOL Penetrant 7 (essentially an aqueous solution of 25% sodium 3,9-diethyl-6-tridecanol sulfate) has been used as a dispersing agent. Based upon mortalities during a 14-day observation period, the most probable LD_{50} value and its fiducial range are estimated by the method of Thompson[6] using the Tables of Weil.[7] The figures in parentheses show limits of $\pm$ 1.96 standard deviations while the absence of parentheses indicates that no range is calculable because no dosage resulted in fractional mortality.

Penetration of rabbit skin is estimated by a technique closely akin to the one-day cuff method of Draize and associates,[8] using groups of four male albino New Zealand rabbits weighing 2.5 to 3.5 kg. The fur is removed from the entire trunk by clipping, and the dose is retained beneath an impervious plastic film. Dosages greater than 2 ml/kg cannot be retained in contact with the skin.[8] The animals are immobilized during the

24-hour contact period, after which the film is removed and the rabbits are cared for the subsequent 14-day observation period. The LD_{50} is calculated as described above.

Concentrated vapor inhalation consists of subjecting groups of six male or female albino rats to a flowing stream of vapor-ladened air. The vapor-air mixture is generated by passing 2.5 liters/minute of dried air at room temperature through a fritted glass disc immersed to a depth of at least one inch in approximately 50 ml of the test chemical contained in a gas-washing bottle. Inhalations are continued for time periods in a logarithmic series with a ratio of two extending from one-fourth to eight hours, until the inhalation period killing about half the number of rats within 14 days is defined. For inhalation periods of ten, five and two minutes in duration, a static technique is used whereby 50 to 100 grams of material, spread over a shallow tray 200 square inches in area, is placed in a 120-liter sealed chamber for at least 24 hours. Six rats are then rapidly introduced by means of a drawer-type cage designed to minimize vapor loss. This static technique, continuing for a maximum of eight hours if necessary, is also employed for mixtures of liquids and for solids. The Table records the longest inhalation period which permitted all rats to survive the two-week observation period. A symbol is used to indicate those few instances when the shortest period attempted caused 100% mortality.

Inhalation of metered vapor concentrations by rats is conducted with flowing streams of vapor prepared by various styles of proportioning pumps.[9,10] Inhalation periods are usually of four hours' duration unless slight toxicity enforces an eight-hour period. Concentrations recorded are nominal and not analytically verified. They are in an essentially logarithmic series with a factor of two, and the Table records the concentration yielding fractional mortality among six rats within 14 days. Where no fractional mortality was observed, usually both the concentration yielding no mortality and that yielding complete mortality are indicated.

Primary skin irritation on rabbits is re- [illegible] ordinal series and is [illegible] ops on the clipped skin of each of five albino rabbits within 24 hours of the uncovered application of 0.01 ml of undiluted sample or of solutions in water, propylene glycol, or acetone. Grade 1 in the Table indicates no irritation and Grade 2 the least visible capillary injection from the undiluted chemical. Grade 6 indicates necrosis when undiluted and Grade 10 indicates necrosis from a 0.01% solution.

Eye injury in rabbits is recorded in a 10-grade ordinal series and is based upon the degree of corneal necrosis that results from instillation of various volumes and concentrations of chemical, as detailed by Carpenter and Smyth.[11] Grade 1 in the Table indicates at most a very small area of necrosis resulting from 0.5 ml of undiluted chemical in the eye. Grade 5 indicates a so-called severe burn from 0.005 ml, and Grade 10 indicates a severe burn from 0.5 ml of a 1% solution in water or propylene glycol.

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RANGE-FINDING TOXICITY DATA

Material Studied	Single Oral LD_{50} for Rats Ml/Kg	Single Skin Penetration LD_{50} for Rabbits Ml/Kg	Concentrated Vapor Inhalation by Rats Maximum for No Death	Inhalation of Metered Vapor Concentration by Rats: Concentration ppm	Time, hr.	Mortality	Irritation on Uncovered Rabbit Belly	Corneal Injury in Rabbits
Hydrocarbons								
Benzene	11.4 (8.4-16.9)	—	5 min.	16,000	4	4/6	3	3
Diethylcyclohexane (mixed isomers)	64	2.5	1 hr.	2,000	4	2/6	4	1
Ethylbenzene	5.46 (5.09-5.86)*	17.8	1 hr.	4,000†	4	3/6	4	2
Fluoranthene	2.00 (1.27-3.13)*	3.18 (2.35-4.29)*	8 hr.	—	—	—	—	7
3a,4,7,7a-Tetrahydro-4, 7-methanoindene (Dicyclopentadiene)	0.41 (0.31-0.53)	4.46 (2.44-8.15)	30 min.	500†	4	1/6	5	2
m-Xylene	7.71 (6.24-9.53)	14.1	2 hr.	8,000	4	10/12	5	1
Hydroxy Compounds								
2-Butene-1-ol	0.93 (0.67-1.30)	1.27 (0.94-1.72)	15 min.	1,000†	4	0/6	1	7
Cyclohexanol	2.06 (1.95-2.18)*	—	8 hr.	—	—	—	2	7
2-Cyclopentene-1-ol	0.47 (0.34-0.66)	0.18	1 hr.	500†	4	0/6	2	5
2,2-Dimethyl-1,3-butanediol	10.0 (6.3-15.7)	>10	8 hr.	—	—	—	1	5
2,2-Dimethylbutanol	2.33 (1.74-3.12)	1.77 (1.09-2.86)	—	—	—	—	2	7
2,5-Dimethyl-1,2,6-hexanetriol	24.2 (19.7-30.1)*	>20	—	—	—	—	1	2
2,3-Dimethylpentanol	2.38 (1.46-3.87)	2.5 (1.7-3.7)	8 hr.	—	—	—	2	9
Dodecylphenol (mixed isomers)	2.14 (1.54-2.99)	5.00 (3.38-7.40)	—	—	—	—	7	2
1-Ethynylcyclohexanol	0.60 (0.43-0.84)	1.00 (0.68-1.48)	8 hr.	—	—	—	3	8
2-Ethyl-4-methylpentanol	4.29 (3.07-5.98)	>5.0	8 hr.	—	—	—	3	5
Heptadecanol (mixed primary isomers)	51.6 (44.4-59.9)	16.8 (12.0-23.6)	8 hr.	—	—	—	3	1
4-Hexene-1-yne-3-ol	0.034 (0.024-0.047)*	0.071	1 hr.‡	62.5†	4	1/6	2	7
2-Hydroxymethyl-norcamphane	1.68 (1.18-2.39)	0.71	8 hr.	—	—	—	3	5
2-Hydroxymethyltetrahydropyran, (mixed 2,4- and 3,4-dimethyl isomers)	3.10 (2.69-3.57)*	—	8 hr.	—	—	—	1	5
5-Indanol	3.25 (2.45-4.26)*	0.45 (0.24-0.82)*		—	—	—	7	9
2-Methylbutanol	4.92 (3.75-6.46)	3.91	8 hr.	—	—	—	2	8
Methylheptanol (mixed primary isomers)	5.16 (4.17-6.38)	2.52 (1.86-3.41)	4 hr.	—	—	—	2	5
4-Methylpentanol	6.50 (4.23-9.99)	3.97 (2.93-5.37)	8 hr.	—	—	—	2	5
1-Naphthol	2.59 (2.10-3.21)*	0.88 (0.33-2.35)*	8 hr.	—	—	—	—	9
1,5-Pentanediol	5.89 (5.38-6.44)*	>20	8 hr.	—	—	—	1	2
p-tert-Pentylphenol	3.08 (2.49-3.81)*	2.00 (1.35-2.96)*	8 hr.	—	—	—	6	10
2-Propylheptanol	6.73 (4.79-9.45)	>10	8 hr.	—	—	—	2	2
Tridecanol (mixed primary isomers)	17.2 (12.3-23.9)	7.07 (2.33-21.4)	8 hr.	—	—	—	4	2
2,2,4-Trimethyl-1,3-pentanediol	3.73 (2.68-5.21)*	6.30 (4.66-8.52)		—		—	2	7
2,2,4-Trimethylpentanol	3.73 (2.68-5.21)	6.30 (4.66-8.52)	8 hr.	—	—	—	—	7
Ethers								
Allyl vinyl ether	0.55 (0.30-1.02)*		5 min.§	8,000	4	2/6	1	2

RANGE-FINDING TOXICITY DATA—CONTINUED

Material Studied	Single Oral LD_{50} for Rats Ml/Kg	Single Skin Penetration LD_{50} for Rabbits Ml/Kg	Concentrated Vapor Inhalation by Rats Maximum for No Death	Inhalation of Metered Vapor Concentration by Rats: Concentration ppm	Time, hr.	Mortality	Irritation on Uncovered Rabbit Belly	Corneal Injury in Rabbits
2,3-Bis-2,3-epoxypropoxy-1,4-dioxane	1.07 (0.77-1.50)*	1.59 (1.17-2.20)	8 hr.	—	—	—	4	7
Cyclopentyl ether	0.47 (0.34-0.66)	1.41	2 hr.	250	4	5/6	4	2
1,3-Dimethoxybutane	3.73 (2.68-5.21)	10.0 (4.6-21.9)	1 hr.	8,000	4	4/6	2	2
4,4-Dimethyl-1,3-dioxane	3.73 (2.68-5.21)	3.54	4 hr.§	8,000†	4	2/6	2	7
2-Ethoxy-3,4-dihydro-1,2-pyran	6.16 (5.52-6.88)*	3.56	—	8,000†	4	1/6	3	5
2-Ethoxy-4-methyl-3,4-dihydropyran	5.63	1.34 (0.96-1.87)	2 hr.	—	—	—	3	2
Ethyl ether	3.56 (3.23-3.92)*	>20	5 min.	32,000†	4	3/6	1	2
2-Ethyl-2-methyl-1,3-dioxolane	2.83	10.0 (4.6-21.9)	—	4,000	4	4/6	2	2
Ethyl-1-propenyl ether	19.0 (13.4-27.0)	—	5 min.	8,000	4	0/6	2	2
Isobutyl vinyl ether	17.0 (12.2-23.8)	20	10 min.§	16,000	4	3/6	1	2
1-Methoxy-1,3-butadiene	2.14 (1.54-2.99)	—	15 min.§	—	—	—	4	2
4,4'-Methylenediphenol	4.95 (3.82-5.52)*	—	8 hr.	—	—	—	—	9
4-Methyl-2-vinyl-1,3-dioxolane	0.89*	0.79 (0.59-1.07)	—	1,000	4	3/6	2	4
1-Propenyl-2-butene-1-yl ether	8.00 (5.10-12.5)	>10	1 hr.	5,000†	4	2/6	4	2
1,1,3-Triethoxybutane	4.92 (3.75-5.46)	1.77	2 hr.	2,000	4	5/6	2	3
1,1,3-Triethoxyhexane	17.0 (12.2-23.8)	—	4 hr.	—	—	—	2	2
Hydroxy Ethers								
Diethylene glycol divinyl ether	3.73 (2.68-5.21)*	14.1	4 hr.	—	—	—	2	1
Diethylene glycol ethyl methyl ether	6.50 (4.95-8.53)	7.07	8 hr.	—	—	—	2	4
Diethylene glycol mono-2-methylpentyl ether	5.66	1.58 (1.16-2.13)	8 hr.	—	—	—	3	6
Diethylene glycol ethyl vinyl ether	11.3	8.41 (5.05-14.0	8 hr.	—	—	—	2	1
Dipropylene glycol monomethyl ether	5.66	10.0 (6.8-14.8)	8 hr.	—	—	—	1	2
Ethylene glycol mono-2-methylpentyl ether	3.73 (2.68-5.21)	0.44	4 hr.	—	—	—	2	6
Ethylene glycol mono-2,6,8-trimethyl-4-nonyl ether	5.36 (3.84-7.48)	3.15 (1.85-4.26)	8 hr.	—	—	—	2	5
Propylene glycol monomethyl ether	5.66	14.1 (8.8-22.9)	4 hr.	10,000	4	0/6	2	3
Triethylene glycol monobutyl ether	6.73 (4.13-11.0)	3.54 (1.06-11.8)	8 hr.	—	—	—	3	5
Triethylene glycol monomethyl ether	11.3	7.1	8 hr.	—	—	—	2	1
Aldehydes								
4-Cyclohexene-1-carboxaldehyde	2.46 (1.88-3.23)	1.77	4 hr.	2,000	4	0/6	4	3
1-Decanal (mixed isomers)	3.73 (2.43-5.74)	5.04 (3.73-6.82)	8 hr.	—	—	—	5	1
2,3-Dimethyl-4-pentenal	5.66	>10	2 hr.	10,000	4	5/6	1	2

RANGE-FINDING TOXICITY DATA—CONTINUED

Material Studied	Single Oral LD_{50} for Rats Ml/Kg	Single Skin Penetration LD_{50} for Rabbits Ml/Kg	Concentrated Vapor Inhalation by Rats Maximum for No Death	Inhalation of Metered Vapor Concentration by Rats: Concentration ppm	Time, hr.	Mortality	Irritation on Uncovered Rabbit Belly	Corneal Injury in Rabbits
2,3-Dimethyl valeraldehyde	3.54 (2.19-5.72)	7.1 (4.4-11.4)	2 hr.	6,000	4	0/6	2	2
Glutaraldehyde, 25%	2.38 (1.68-3.38)	2.56 (1.88-3.44)	8 hr.	—	—	—	—	9
Glyoxal 29.2%	7.46 (5.35-10.4)	>20	8 hr.	—	—	—	2	3
1-Hexanal (mixed isomers)	9.51 (5.84-15.5)	>10	4 hr.	—	—	—	2	5
3-Methoxy butyraldehyde	0.54 (0.35-0.82)	0.31 (0.12-0.78)	15 min.	500†	4	0/6	2	8
2-Methyl-4-cyclohexene-1-carboxaldehyde	5.66	3.15 (2.33-4.26)	8 hr.		—		5	5
4-Methyl valeraldehyde	5.66	4.46 (2.44-8.15)	30 min.	8,000†	4	0/6	2	3
1-Octanal (mixed isomers)	5.63	6.35 (4.70-8.59)	8 hr.	—	—	—	4	2
1-Pentanal (mixed isomers)	4.76 (3.35-6.75)	>20	5 min.	8,000	4	3/6	2	2
4-Pentenal	0.62 (0.40-0.95)*,**	1.59 (0.71-3.53)	5 min.	250	4	1/12	1	5
Ketones								
Acetone	10.7 (7.7-15.0)**	>20	30 min.	16,000†	4	1/6	1	5
2-Butanone	6.56 (5.09-8.45)**	>10	—	8,000†	8	3/6	2	5
2-Heptanone	1.67 (1.48-1.88)*, **	12.6 (9.3-17.0)	30 min.	2,000†	4	0/6	4	2
4-Hexene-1-yne-3-one	0.071 (0.044-0.11)*	0.10 (0.04-0.25)	5 min.§	12.5	4	2/6	2	10
5-Methyl-2-hexanone	4.76 (2.92-7.75)	10.0 (6.8-14.8)	—	2,000†	4	0/6	1	2
2-Pentanone	3.73 (2.68-5.21)*	8.00 (3.60-17.8)	30 min.	2,000†	4	1/6	1	8
Acids, Anhydrides, and Lactones								
Acrylic acid, glacial	2.59 (2.10-3.21)*	0.95 (0.67-1.30)	8 hr.	—	—	—	6	9
Allylsuccinic anhydride	1.07 (0.77-1.50)	0.32 (0.23-0.43)	—	—	—	—	3	9
Crotonic anhydride	2.83 (1.77-4.53)*	—	8 hr.	—	—	—	5	10
Decanoic acid (mixed isomers)	3.73 (2.93-4.86)	1.77 (1.09-2.86)	8 hr.	—	—	—	5	9
Dimethyl-e-caprolactone (mixed isomers)	11.3	3.54 (2.19-5.72)	8 hr.	—	—	—	2	5
2,5-Dimethyl-2-hydroxy-adipaldehyde	5.89 (5.38-6.44)*	—	8 hr.	—	—	—	1	7
Glutaric anhydride	4.46*	1.78*	—	—	—	—	—	8
Hexanoic acid (mixed isomers)	2.05 (1.66-2.53)	1.05 (0.29-3.85)	8 hr.	—	—		4	9
2-Hydroxy-3-ethyl-heptanoic acid	3.40 (3.11-3.72)*	1.78	—	—	—	—	4	9
6-Hydroxytetrahydropyran-2-carboxylic acid lactone	5.66	3.97 (2.93-5.37)	—	—	—	—	1	7
Methyl-e-caprolactone (mixed isomers)	11.2	7.1	8 hr.				2	8
2-Methyl propionic acid	0.28 (0.17-0.46)	0.50 (0.37-0.67)	8 hr.				5	9
Octanoic acid (mixed isomers)	1.41 (0.88-2.29)	0.71	4 hr.	—		—	5	9
Pentanoic acid (mixed isomers)	1.12 (0.45-2.79)	0.70 (0.38-1.28)	8 hr.	—			6	8
Propionic acid	4.29 (3.07-5.98)*	0.50 (0.37-0.67)	8 hr.	—			6	9

RANGE-FINDING TOXICITY DATA—CONTINUED

Material Studied	Single Oral LD_{50} for Rats Ml/Kg	Single Skin Penetration LD_{50} for Rabbits Ml/Kg	Concentrated Vapor Inhalation by Rats Maximum for No Death	Inhalation of Metered Vapor Concentration by Rats: Concentration ppm	Time, hr.	Mortality	Irritation on Uncovered Rabbit Belly	Corneal Injury in Rabbits
Trimethyl-ε-caprolactone (mixed isomers)	7.43 (5.32-10.4)	6.30 (4.66-8.52)	8 hr.	—			2	5
Esters								
Acetic acid, amyl ester (mixed isomers)	6.50 (4.95-8.53)*	>20	4 hr.	—	—	—	3	2
Acetic acid, 1,3-butadienyl ester	0.71*	0.42 (0.30-0.60)	10 min.§	62.5†	4	3/6	6	9
Acetic acid, 2-butoxyethyl ester	7.46 (5.35-10.4)	1.58 (1.16-2.13)	8 hr.	—	—	—	1	2
Acetic acid, ethyl ester	11.3**	>20	15 min.	8,000†	4	0/6	1	2
Acetic acid, isobutyl ester	15.4 (12.5-19.1)	>20	1 hr.	8,000†	4	4/6	1	2
Acetic acid, methyl ester	6.97 (6.32-7.69)*		—	16,000†	4	0/6	1	5
Acrylic acid, decyl ester	6.46 (4.93-8.48)*	6.30 (4.66-8.52)	4 hr.	—	—	—	5	2
Acrylic acid, tridecyl ester	44.7	6.30 (4.66-8.52)	8 hr.	—	—	—	5	2
Adipic acid, di-(decyl) ester (mixed isomers)	20.5 (16.5-25.3)	8.41 (5.00-14.1)	8 hr.	—	—	—	2	1
Adipic acid, di-2-propynyl ester	0.20 (0.13-0.31)*	0.44 (0.24-0.82)	—	—	—	—	1	2
Azelaic acid, di-(2-ethylhexyl) ester	8.72 (4.72-16.1)	20.0 (9.1-43.8)	8 hr.	—	—	—	3	1
1,2,4-Butanetricarboxylic acid, tri (2-ethylhexyl) ester	>64	20	—	—	—	—	1	2
Crotylidene dicrotonate	2.59 (2.10-3.21)*	—	4 hr.	—	—	—	4	1
Decanoic acid, vinyl ester (mixed isomers)	6.17 (4.99-7.63)	14.1 (8.8-22.9)	8 hr.	—	—	—	4	1
Dibenzoyl diethylene glycol ester	2.83 (1.93-4.15)*	20.0 (13.5-29.6)	8 hr.	—	—	—	1	1
Dibenzoyl dipropylene glycol ester	9.80 (7.47-12.9)*	>10	—	—	—	—	1	1
Di-(decanoyl) triethylene glycol ester (mixed isomers)	7.46 (5.20-10.7)	11.2 (6.1-20.5)	8 hr.	—	—	—	3	1
2,5-Endomethylene cyclohexane carboxylic acid, ethyl ester (mixed formyl isomers)	7.46 (5.35-10.4)	10	—	—	—	—	3	2
2,5-Endomethylene-3-cyclohexene carboxylic acid, ethyl ester	4.29 (3.07-5.98)	>5	—	—	—	—	3	2
2-Ethylhexanoic acid, 2-ethylhexyl ester	27*	>20	8 hr.	—	—	—	2	2
Formic acid, vinyl ester	2.92 (2.42-3.30)*	3.17 (1.56-6.44)	5 min.§	1,000†	4	0/6	1	9
b-Formyl propionic acid, ethyl ester	9.87 (7.52-13.0)	7.1	—	—	—	—	2	5
Fumaric acid, diethyl ester	1.78 (1.60-1.98)*	—	8 hr.	—	—	—	—	2
Fumaric acid, di-isobutyl ester	8.12 (6.19-10.7)	7.49 (5.87-10.7)	8 hr.	—	—	—	3	2
Hexanoic acid, vinyl ester (mixed isomers)	19.7 (15.0-25.8)	>20	1 hr.	4,000	4	5/6	2	1
Isophthalic acid, di-(decyl) ester (mixed isomers)	>64	>10	8 hr.	—	—	—	1	1

RANGE-FINDING TOXICITY DATA—CONTINUED

Material Studied	*Single Oral LD_{50} for Rats Ml/Kg	Single Skin Penetration LD_{50} for Rabbits Ml/Kg	Concentrated Vapor Inhalation by Rats Maximum for No Death	Inhalation of Metered Vapor Concentration by Rats: Concentration	Time	Mortality	Irritation on Uncovered Rabbit (%)	Corneal Injury in Rabbits
Isophthalic acid, di-(2-ethylhexyl) ester	17.3 (9.4-32.0)	7.94 (5.87-10.7)	8 hr.				1	1
Maleic acid, dihexyl ester	7.34 (6.22-8.66)**, *		8 hr.				3	1
Maleic acid, dimethyl ester	1.41*	0.53 (0.38-0.75)	4 hr.				3	3
Maleic acid, dipentyl ester	4.92 (3.75-6.46)	10	8 hr.				2	1
Mandelic acid, hexyl ester	17.0 (12.2-23.8)*	15.4 (13.3-17.8)					3	4
Methyl borate	6.14 (4.96-7.95)	1.98 (1.47-2.68)					1	3
Octanoic acid, vinyl ester (mixed isomers)	7.46 (4.86-11.5)	>10	4 hr.				[illegible]	1
Phthalic acid, di-(decyl) ester	>64	16.8 (10.0-28.2)	4 hr.				2	2
Phthalic acid, di-(tridecyl) ester	>64	>20					2	2
Propionic acid, vinyl ester	4.76 (3.35-6.75)*	10.0 (6.8-14.8)	5 min.	4,000	4	4/6	2	2
Epoxy Compounds								
Acetic acid, 3,4-epoxy-6-methylcyclohexylmethyl ester	9.8 (6.1-12.0)	7.94 (5.87-10.7)	8 hr.				2	2
Acrylic acid, 2,3-epoxypropyl ester	0.21 (0.15-0.30)*	0.40 (0.18-0.92)	30 min.	[illegible]	4	[illegible]	[illegible]	10
Bis-[2,3-epoxycyclopentyl] ether	2.14 (1.54-2.99)		8 hr.				1	3
2,2-Bis-[2,2,2-trimethylethyl] oxirane	6.69 (4.11-10.9)	14.1	8 hr.				6	1
1,4-Dichloro-2,3-epoxybutane	0.71*	2.83	2 hr.				2	2
1,2-8,9-Diepoxylimonene	5.63*	1.77	8 hr.				4	3
1,2-7,8-Diepoxyoctane	1.07 (0.77-1.50)	0.32 (0.23-0.43)	4 hr.				6	7
1,2-Epoxybutane	1.41 (0.88-2.29)	2.10 (1.50-2.95)		4,000†	4	1/6	1	4
2,3-Epoxybutyric acid, butyl ester	0.50 (0.32-0.79)	2.83	8 hr.				1	2
3,4-Epoxycyclohexanecarbonitrile	1.23 (0.94-1.62)	0.99 (0.73-1.34)	8 hr.				2	6
4,5-Epoxycyclohexane-1,2-dicarboxylic acid, di-(decyl)ester (mixed isomers)	>64	>20	8 hr.				2	2
4,5-Epoxycyclohexane-1,2-dicarboxylic acid, di-(2-ethylhexyl) ester	>64	>20	8 hr.				2	1
3,4-Epoxy-2,5-endo-methylcyclohexanecarboxylic acid, ethyl ester	4.76 (3.28-6.75)	3.54	8 hr.				2	2
2-(a,b-Epoxyethyl)-5,6-epoxybenzene	[illegible]	[illegible]	8 hr.					
2,3-Epoxy-2-ethylhexanol	[illegible] (4.49-5.68)	[illegible] (2.33-4.26)	[illegible]				1	
3,4-Epoxy-6-methylcyclohexanecarboxylic acid, allyl ester	0.50 (0.32-0.79)	2.83 (0.93-8.58)	8 hr.				[illegible]	1
3,4-Epoxy-6-methylcyclohexanecarboxylic acid, 3,4-epoxy-6-methylcyclohexylmethyl ester	4.92 (3.75-6.46)	>10	8 hr.				2	1

RANGE-FINDING TOXICITY DATA—CONTINUED

Material Studied	Single Oral LD_{50} for Rats Ml/Kg	Single Skin Penetration LD_{50} for Rabbits Ml/Kg	Concentrated Vapor Inhalation by Rats Maximum for No Death	Inhalation of Metered Vapor Concentration by Rats: Concentration ppm	Time, hr.	Mortality	Irritation on Uncovered Rabbit Belly	Corneal Injury in Rabbits
2,3-Epoxypropyl butyl ether	2.05 (1.56-2.69)*	2.52 (1.28-4.97)	2 hr.	4,000	4	1/6	1	5
N-(2,3-Epoxypropyl) diethylamine	0.42 (0.26-0.68)	0.79 (0.59-1.07)	30 min.	1,000†	4	0/6	6	9
9,10-Epoxystearic Acid, allyl ester	1.41 (0.88-2.29)	15.9 (11.7-21.5)	8 hr.	—	—	—	3	1
9,10-Epoxystearic acid, 2-ethylhexyl ester	30.8 (24.9-38.1)	>20	8 hr.	—	—	—	3	1
1,2-Epoxy-4-vinylcyclohexane	2.00 (1.28-3.13)	2.83	2 hr.	—	—	—	2	5
Ethylene glycol di-(2,3-epoxy-2-methylpropyl) ether	7.46 (5.35-10.4)	3.15 (2.33-4.26)	—	—	—	—	2	5
Aliphatic and Alicyclic Amines								
Acrylic acid, N,N-diethylaminoethyl ester	0.89 (0.56-1.41)*	0.20 (0.15-0.27)	2 hr.	—	—	—	6	9
7-Aminoheptanoic acid, isopropyl ester	4.00 (2.70-5.92)	0.89 (0.63-1.25)	8 hr.*	—	—	—	6	9
3-Aminopropanol	2.83	1.25 (0.84-1.85)	8 hr.	—	—	—	5	9
Bis-(3-aminopropyl)amine	0.81 (0.53-1.25)	0.11	8 hr.	—	—	—	7	3
N,N-Bis-(3-aminopropyl) methylamine	1.54 (1.25-1.91)	0.14	8 hr.	333	1	6/6	6	8
Bis-(2,5-endomethylene-cyclohexylmethyl)amine	1.41*	0.11 (0.06-0.21)	8 hr.	—	—	—	6	9
Bis-(a-methylbenzyl) amine	2.93 (1.52-5.67)	3.97 (3.00-5.37)	8 hr.	—	—	—	6	2
N-Butylcyclohexylamine	0.33 (0.21-0.54)*	0.53 (0.38-0.75)	8 hr.	—	—	—	6	9
N-Butyl-a-methylbenzylamine	0.36 (0.24-0.52)*	0.57 (0.35-0.91)	8 hr.	—	—	—	4	5
Decylamine	0.28 (0.14-0.53)	0.35 (0.22-0.57)	2 hr.	—	—	—	6	9
Diallylamine	0.65 (0.49-0.85)*	0.28	—	1,000†	4	0/6	6	8
1,8-Diamino-p-menthane	0.77 (0.62-0.95)	0.63 (0.47-0.85)	4 hr.	—	—	—	7	9
Diamylamine	0.27 (0.19-0.37)	0.35	30 min.	62.5†	4	4/6	6	5
3-(Dibutylamino) propylamine	0.82 (0.62-1.07)*	0.27 (0.16-0.45)	8 hr.	—	—	—	6	9
N,N-Dibutyl-(2-hydroxypropyl)amine	1.99 (1.64-2.42)*, **	—	8 hr.	—	—	—	5	2
N,N-Dibutylmethylamine	0.54 (0.38-0.75)	0.88	5 min.	125†	4	0/6	4	5
(3-Diethylaminopropyl) amine	1.41 (1.21-1.65)*	0.75 (0.54-1.06)	4 hr.	—	—	—	6	9
Di-(hydroxyethyl)-o-tolylamine	2.2*	1.0	8 hr.	—		—	1	3
(3-Dimethylaminopropyl) amine	1.87 (1.34-2.60)*		8 hr.	—	—	—	6	9
(1,3-Dimethylcyclopentene-1-yl-methyl) amine	0.81 (0.62-1.07)*	0.40 (0.30-0.54)	4 hr.	—	—	—	6	9
Dipropylamine*	0.93 (0.67-1.30)*	1.25 (0.84-1.85)	5 min.	1,000†	4	2/6	6	9
Di-(tridecyl)amine	9.85 (7.51-12.9)	3.54	8 hr.	—	—	—	7	1

RANGE-FINDING TOXICITY DATA—CONTINUED

Material Studied	Single Oral LD_{50} for Rats Ml/Kg	Single Skin Penetration LD_{50} for Rabbits Ml/Kg	Concentrated Vapor Inhalation by Rats Maximum for No Death	Inhalation of Metered Vapor Concentration by Rats: Concentration ppm	Time, hr.	Mortality	Irritation on Uncovered Rabbit Belly	Corneal Injury in Rabbits
(2,5-Endomethylene-cyclohexylmethyl) amine	1.41*	0.52 (0.35-0.76)	8 hr.	—	—	—	6	9
N-Ethyl(cyclohexyl) amine	0.59 (0.53-0.67)*	0.75 (0.54-1.08)	1 hr.	500	4	5/6	6	9
N-(2-Ethylhexyl)cyclohexylamine	0.71*	0.11 (0.08-0.15)	4 hr.	—	—	—	6	5
N-Ethyl(a-methylbenzyl) amine	0.36*	0.89	4 hr.	—	—	—	6	9
N-(2-Hydroxyethyl) phenylamine	2.23 (2.01-2.47)*	0.063 (0.043-0.094)	8 hr.	—	—	—	2	5
N-Hydroxypropyl-diethylenetriamine	3.73 (2.68-5.21)*	1.50 (0.89-2.52)	8 hr.	—	—	—	2	5
N-(3-Hydroxypropyl)-1,2-propanediamine	5.66*	2.12 (1.51-2.98)	8 hr.	—	—	—	6	5
N-Methyl(butyl)amine	0.42 (0.26-0.69)*	1.26 (0.93-1.70)	10 min.§	2,000	4	3/6	6	9
1,3-Propanediamine	0.35 (0.22-0.57)	0.20 (0.15-0.27)	8 hr.	—	—	—	7	9
Propylamine	0.57*	0.56 (0.30-1.02)	2 min.§	8,000	4	5/6	6	9
N,N,N',N'-Tetramethyl-dipropylenetriamine	1.62 (1.02-2.60)	0.31 (0.06-1.50)	8 hr.	—	—	—	6	9
Triallylamine	1.03 (0.83-1.28)*	0.40 (0.29-0.54)	15 min.	500†	4	1/6	5	2
Triisooctylamine	1.62 (1.24-2.13)	3.18 (2.35-4.29)	—	—	—	—	7	1
Aromatic Nitro and Amino Compounds								
p-Chloroaniline	0.31 (0.20-0.48)*	0.36*	4 hr.	—	—	—	—	8
a-Chloro-p-nitrostyrene	0.71*	0.39 (0.29-0.53)*	—	—	—	—	6	7
N,N-Dimethylaniline	1.41	1.77 (1.09-2.86)	8 hr.	—	—	—	3	5
2,4-Dinitrochlorobenzene	1.07 (0.77-1.50)*	0.13 (0.08-0.19)*	—	—	—	—	7	10
N-(2-Ethylhexyl)aniline	2.41 (1.88-3.23)*	7.1 (2.7-18.5)	8 hr.	—	—	—	5	1
2-Methyl-1-naphthylamine	0.62 (0.47-0.81)*	2.83	—	—	—	—	2	2
1-(p-Nitrophenyl) ethyl nitrate	2.14 (1.54-2.99)*, **	14.1 (8.8-22.9)	8 hr.	—	—	—	2	1
o-Toluidine	0.94 (0.67-1.31)*	3.25 (2.01-5.25)	8 hr.	—	—	—	2	8
Amide and Imide Compounds								
N,N-Diethylacetoacetamide	4.76 (3.39-6.68)	—	8 hr.	—	—	—	1	
N,N-Dimethylacetamide	5.63*	2.24 (1.23-4.11)	8 hr.	—	—	—	2	3
N,N-Dimethylacetoacetamide	22.6	14.1	8 hr.	—	—	—	2	2
N,N-Dimethyl carbamic acid, m-isopropyl phenyl ester	0.16 (0.12-0.21)*	0.28 (0.18-0.46)	—	—	—	—	2	2
2,4-Dimethylphenyl maleimide	0.71 (0.44-1.14)*	—	8 hr.	—	—	—	7	8
N-Ethylacetoacetamide	14.2 (8.9-22.5)	—	8 hr.	—	—	—	1	5
N-(2-Ethylbutoxyethoxypropyl)bicyclo[2.2.1]heptene-2,3-dicarboximide	9.5 (6.7-13.4)*	16.0 (10.8-23.8)	—	—	—	—	5	5

RANGE-FINDING TOXICITY DATA—CONTINUED

Material Studied	Single Oral LD_{50} for Rats Ml/Kg	Single Skin Penetration LD_{50} for Rabbits Ml/Kg	Concentrated Vapor Inhalation by Rats Maximum for No Death	Inhalation of Metered Vapor Concentration by Rats: Concentration ppm	Time, hr.	Mortality	Irritation on Uncovered Rabbit Belly	Corneal Injury in Rabbits
N-Methyl-N-vinylacetamide	2.83	1.41	4 hr.	4,000	4	0/5	1	7
p-Tolylmaleimide	0.71 (0.48-1.05)*	0.36 (0.22-0.58)*	8 hr.	—	—	—	—	9
Heterocyclic Nitrogen Compounds								
Acrylic acid, 2-(5-ethylpyrid-2-yl)-ethyl ester	4.92 (3.75-6.46)	2.23 (1.38-3.59)	8 hr.	—	—	—	5	2
1-(2-aminoethyl)piperazine	2.14 (1.54-2.99)	0.88 (0.34-2.51)	8 hr.	—	—	—	6	5
1,4-Di-(2-hydroxyethyl)piperazine	3.75 (2.68-5.21)	>10	—	—	—	—	3	5
1-(2-N,N-Dimethylaminoethyl)-4-methylpiperazine	1.42 (0.96-2.08)	0.39 (0.29-0.53)	8 hr.	—	—	—	6	8
2,6-Dimethylmorpholine	2.83*	0.71 (0.39-1.30)	4 hr.	4,000	4	0/6	2	7
5-Ethyl-2-methylpyridine-1-oxide	2.00 (1.28-3.13)	1.77	—	—	—	—	2	5
Hexahydro-1,4-diazepine	2.83*	1.05 (0.75-1.48)	8 hr.	—	—	—	6	9
2-Hydroxyethyl-5-ethylpyridine	4.29 (3.07-5.98)*	1.78 (0.97-3.26)	—	—	—	—	2	7
1-(2-Hydroxyethyl)piperazine	4.92 (3.75-6.46)	>5.0	8 hr.	—	—	—	1	5
Indole	1.00 (0.64-1.57)*	0.79 (0.59-1.07)*	8 hr.	—	—	—	—	8
1-Methylpiperazine	2.83	1.49 (0.88-2.50)	8 hr.	—	—	—	6	8
1-Phenylpiperazine	0.21 (0.13-0.34)*	0.14 (0.08-0.25)	8 hr.	—	—	—	6	9
Piperidine	0.52 (0.42-0.64)*	0.32 (0.23-0.43)	15 min.‡	2,000†	4	0/6	6	9
1-(5,5,7,7-Tetramethyl-2-octanyl)-2-methyl-5-ethylpyridinium chloride	0.54 (0.38-0.75)*	0.071 (0.044-0.11)*	—	—	—	—	7	9
Nitriles								
Acetic acid, 1-cyanovinyl ester	0.10 (0.08-0.14)*	0.14 (0.08-0.26)	5 min.	125	4	4/6	4	10
2-Acetoxyisosuccinodinitrile	0.12 (0.10-0.15)*	0.11 (0.06-0.20)*	1 hr.	—	—	—	—	9
Acrylic acid, 2-(2-cyanoethoxy)ethyl ester	1.12 (0.84-1.50)	0.75 (0.51-1.11)	8 hr.	—	—	—	2	5
Acrylic acid, 2-cyanoethyl ester	0.18 (0.13-0.24)*	0.22	8 hr.	—	—	—	5	8
Allyl cyanide	0.12 (0.10-0.14)*	1.41	5 min.	250†	4	0/6	2	3
Butyronitrile	0.14 [illegible]*	0.50 [illegible]	—	1,000	4	5/6	1	2
Chloroacetonitrile	[illegible] (0.17-0.29)*	[illegible]	15 min.‡	250†	4	1/6	2	5
4-Cyanoethoxy-2-methyl-2-pentanol	3.2*	1.5	—	—	—	—	2	5
6-Cyanohexanoic acid, ethyl ester	11.2	7.07	8 hr.	—	—	—	3	2
N-Cyanomethylmorpholine 50%	1.23 (0.94-1.61)	0.26 (0.15-0.27)	4 hr.	—	—	—	1	5
3-Cyanopropionic acid, ethyl ester	10.3 (8.3-12.8)		4 hr.	—	—	—	1	2
Diethylene glycol mono-2-cyanoethyl ether	13.4 (8.2-21.8)*, **		8 hr.	—	—	—	2	1
2,4-Dihydroxy-3,3-dimethylbutyronitrile	0.31 (0.24-0.41)*	0.13 (0.09-0.17)	8 hr.	—	—	—	1	7

RANGE-FINDING TOXICITY DATA—CONTINUED

Material Studied	Single Oral LD_{50} for Rats Ml/Kg	Single Skin Penetration LD_{50} for Rabbits Ml/Kg	Concentrated Vapor Inhalation by Rats Maximum for No Death	Inhalation of Metered Vapor Concentration by Rats: Concentration ppm	Time, hr.	Mortality	Irritation on Uncovered Rabbit Belly	Corneal Injury in Rabbits
Dimethylamino acetonitrile	0.050 (0.032-0.079)*	0.17 (0.12-0.24)	10 min.	125†	4	0/6	2	5
3-(Dimethylamino) propionitrile	2.60 (1.98-3.41)	1.41 (0.88-2.29)	8 hr.	—	—	—	1	5
Glycolonitrile, 70%	0.016 (0.013-0.020)*	0.0050 (0.0034-0.0074)*	8 hr.	250	4	4/6	2	‡
a-Hydroxyisobutyronitrile	0.017 (0.011-0.026)*	0.017 (0.012-0.024)	5 min.	62.5†	4	2/6	1	‡
Isobutyronitrile	0.10 (0.077-0.13)*	0.31 (0.21-0.46)	10 min.§	500†	4	0/6	1	‡
Methacrylonitrile	0.25 (0.19-0.32)	0.35 (0.19-0.67)	15 min.§	1,000†	4	4/6	2	1
3-Methyl-3-butenonitrile	0.54 (0.39-0.75)*	2.83	15 min.	1,000†	4	1/6	1	2
Trichloroacetonitrile	0.25 (0.19-0.32)*	0.90 (0.49-1.64)	5 min.§	125†	4	0/6	6	10
Halogen Compounds								
3-Chloro-2-fluoro-1-propene	0.28 (0.21-0.37)	0.20 (0.09-0.44)	—	1,000	4	5/6	1	‡
Chloroform	2.18 (1.14-4.22)*, **	—	5 min.§	8,000	4	5/6	2	—
6-Chlorohexanoic acid	3.08 (2.49-3.81)*	—	8 hr.	—	—	—	—	9
6-Chlorohexanoic acid, ethyl ester	4.92 (3.75-6.46)	7.07	8 hr.	—	—	—	1	1
2-(4-Chlorophenoxy) ethanol	2.83*	0.50 (0.37-0.68)	4 hr.	—	—	—	2	8
Decyl chloride (mixed primary isomers)	45.3 (14.0-146.8)	5.66 (3.25-9.85)	4 hr.	—	—	—	4	1
1,1-Diacetoxy-2,3-dichloropropane	0.32*	1.0	—	—	—	—	5	10
2-(1,2-Dichloroethyl)-4-methyl-1,3-dioxolane	0.62 (0.47-0.81)*	1.01 (0.74-1.36)	—	—	—	—	2	2
2,3-Dichloro-2-methylpropionaldehyde	1.62 (1.24-2.13)*	0.36 (0.19-0.68)*	15 min.	250†	4	1/6	2	8
1,3-Dichloro-2-propanol	0.11 (0.059-0.20)*	0.80 (0.54-1.18)	30 min.	125†	4	2/6	2	8
2,3-Dichloro-1-propene	0.32 (0.26-0.40)*	1.58 (1.16-2.13)	15 min.§	500†	4	3/6	5	5
2,3-Dichloropropionic acid	0.42 (0.26-0.69)*	0.40 (0.18-0.88)*	—	—	—	—	7	9
2-Fluoro-2-propene-1-ol	0.13 (0.071-0.24)*	0.0031 (0.0014-0.0068)	—	1,000	1	4/6	1	‡
1,1,3-Tri (2-chloroethoxy)propane	0.71*	6.3	1 hr.	—	—	—	1	1
1,2,2-Trichloro-1,1,3,3,3-pentafluoropropane	14.9 (10.7-20.8)	>20	—	8,000†	4	1/6	1	3
1,1,1-Trichloropropane	7.46 (5.35-10.4)*	>20	30 min.§	8,000	4	4/6	4	2
1,2,3-Trichloropropane	0.32 (0.26-0.40)	1.77	—	1,000	4	5/6	1	4
1,2,3-Trichloropropene	0.62 (0.47-0.81)*	0.64 (0.47-0.86)	30 min.§	500	4	5/6	5	5
2,2,3-Trichloropropionaldehyde	0.24 (0.17-0.33)	0.71	2 hr.	—	—	—	6	8
2,2,3-Trichloropropionic acid	2.46 (1.88-3.23)*	1.77 (1.09-2.86)*	—	—	—	—	7	9
Sulphur Compounds								
Acrylic acid, 2-methylthioethyl ester	1.23 (0.94-1.62)	1.49 (0.89-2.47)		—	—	—	6	4
2-Acryloxyethyl dimethylsulfonium methyl sulfate	1.87 (1.34-2.60)	2.00 (0.51-7.78)	—	—	—	—	4	5
Benzenesulfonic acid	0.89 (0.36-2.21)	—	8 hr.	—	—	—	7	9

RANGE-FINDING TOXICITY DATA—CONTINUED

Material Studied	Single Oral LD_{50} for Rats Ml/Kg	Single Skin Penetration LD_{50} for Rabbits Ml/Kg	Concentrated Vapor Inhalation by Rats Maximum for No Death	Inhalation of Metered Vapor Concentration by Rats: Concentration ppm	Time, hr.	Mortality	Irritation on Uncovered Rabbit Belly	Corneal Injury in Rabbits
2-Ethylhexenyl sulfonate, sodium salt	25.8 (19.7-33.9)	>10	8 hr.	—	—	—	3	4
Methyl sulfoxide	20.5 (16.9-25.3)*	—	4 hr.	—	—	—	2	2
Methyl vinyl sulfone	0.57*	0.032 (0.013-0.079)	8 hr.	—	—	—	2	>5
Phenyl sulfide	0.49 (0.38-0.65)	11.3	8 hr.	—	—	—	5	2
Sulfamic acid, 2-aminoethyl ester	7.46 (5.35-10.4)*	14.1	4 hr.	—	—	—	1	1
Vinyl S-(butylmercapto-ethyl) ether	2.83*	10	8 hr.	—	—	—	5	2
Vinyl S-(ethylmercapto-ethyl) ether	4.92 (3.75-6.46)*	>10	8 hr.	—	—	—	2	2
Vinyl sulfone	0.032 (0.025-0.043)*	0.022 (0.014-0.036)	8 hr.	—	—	—	7	>5
Phosphorous Compounds								
P-Chloro-5,10-dimethyl-2,4-dioxa-P-thiono-3-phosphabicyclo(4.4.0)decane	0.11 (0.071-0.18)	0.20 (0.09-0.44)	—	—	—	—	2	2
2-Chloro-4,6-dimethyl-2-oxo-1,3,2-dioxaphosphorinane	0.38 (0.18-0.61)*	0.50	—	—	—	—	4	9
P-Chloro-2,4-dioxa-5-ethyl-P-thiono-3-phosphabicyclo(4.4.0)decane	0.14*	0.40 (0.27-0.59)	—	—	—	—	4	1
P-Chloro-2,4-dioxa-5-methyl-P-thiono-3-phosphabicyclo(4.4.0)decane	0.12 (0.086-0.16)*	0.36	—	—	—	—	2	1
2-Chloro-5-ethyl-2-oxo-4-propyl-1,3,2-dioxaphosphorinane	0.025 (0.020-0.030)*	1.41	—	—	—	—	5	9
2-Chloro-5-ethyl-4-propyl-2-thiono-1,3,2-dioxaphosphorinane	0.30 (0.18-0.49)*	1.00 (0.74-1.35)	—	—	—	—	1	1
Chlorophosphoric acid, diethyl ester	0.011 (0.010-0.012)*	0.0079 (0.0059-0.011)	—	—	—	—	2	1
2-(Dibutylphosphono) succinic acid, dibutyl ester	25.2*	>10	8 hr.	—	—	—	2	1
2-(Dibutylphosphono) succinic acid, di-(2-ethylhexyl)ester	>40*	>20	8 hr.	—	—	—	2	1
O,O'-Di(2-ethylhexyl) dithiophosphoric acid	2.14 (1.54-2.99)	1.25 (0.50-3.14)	—	—	—	—	7	8
Di-(2-ethylhexyl) phosphoric acid	4.94 (3.75-6.46)	1.25 (0.57-2.74)	8 hr.	—	—	—	6	9
Phosphoric acid, di-(2-ethylhexyl) hydroxypropyl ester	11.3*	—	8 hr.	—	—	—	3	4
Phosphorothioic acid, tri(2-chloroethyl) ester	0.82 (0.62-1.07)*	1.8	8 hr.	—	—	—	2	4
Tributylphosphine sulfide	0.93 (0.67-1.30)	1.00 (0.68-1.48)	8 hr.	—	—	—	4	2
Triisooctyl phosphine	21.4 (15.4-29.9)	3.97 (2.93-5.37)	—	—	—	—	4	1
Silicon Compounds								
(4-Aminobutyl)diethoxy (methyl)silane	6.50 (4.06-10.4)*	0.045 (0.015-0.136)	8 hr.	—	—	—	3	8
(4-Aminobutyl)triethoxysilane	1.62 (1.02-2.60)	2.50 (1.69-3.70)	8 hr.	—	—	—	4	9

RANGE-FINDING TOXICITY DATA—CONCLUDED

Material Studied	Single Oral LD_{50} for Rats Ml/Kg	Single Skin Penetration LD_{50} for Rabbits Ml/Kg	Concentrated Vapor Inhalation by Rats Maximum for No Death	Inhalation of Metered Vapor Concentration by Rats: Concentration ppm	Time, hr.	Mortality	Irritation on Uncovered Rabbit Belly	Corneal Injury in Rabbits
(3-Aminopropyl) diethoxy(methyl) silane	4.76 (3.35-6.75)	2.52 (1.86-3.41)	8 hr.	—	—	—	3	8
(3-Aminopropyl)triethoxysilane	1.78 (0.93-3.41)*	—	8 hr.	—	—	—	6	8
Amyltrichlorosilane	2.34 (1.98-2.77)*	0.78 (0.59-1.02)	4 hr.	1,000†	8	0/6	6	9
(2-Carbethoxyethyl) diethoxy(methyl) silane	20.8 (14.3-30.3)	>10	8 hr.	—	—	—	2	1
(2-Carbethoxyethyl) triethoxysilane	22.4 (16.2-30.8)	—	8 hr.	—	—	—	2	1
(2-Carbethoxypropyl) diethoxy(methyl) silane	24.6 (18.8-32.3)	—	8 hr.	—	—	—	3	5
(3-Carbethoxypropyl) diethoxy(methyl) silane	22.4	—	8 hr.	—	—	—	1	5
(2-Cyanoethyl) trichlorosilane	2.00 (1.28-3.13)*	—	8 hr.	—	—	—	6	10
(2-Cyanoethyl) triethoxysilane	5.63*	5.95 (3.57-9.90)	4 hr.	—	—	—	2	1
(3-Cyanopropyl) dichloro(methyl) silane	2.83*	1.49 (0.67-3.29)	8 hr.	—	—	—	6	8
(3-Cyanopropyl) diethoxy(methyl) silane	3.73 (2.68-5.21)*	—	8 hr.	—	—	—	2	1
(3-Cyanopropyl) methylsiloxane, cyclic tetramer	>64	—	8 hr.	—	—	—	1	1
(3-Cyanopropyl) trichlorosilane	2.83*	—	4 hr.	—	—	—	6	9
(3-Cyanopropyl) triethoxysilane	4.92 (3.75-6.46)*	>10	8 hr.	—	—	—	2	1
(3,4-Cyclohexenyl) trichlorosilane	2.83*	0.63 (0.39-1.02)	8 hr.	—	—	—	6	9
Di-(2-ethylhexoxy)-di-(2-ethylbutoxy)silane	56.3 (35.5-89.4)	>20	8 hr.	—	—	—	1	1
Ethyl(vinyl) dichlorosilane	2.83*	0.75 (0.54-1.06)	—	4,000†	4	0/6	6	9
(2-Phenylethyl) trichlorosilane	2.83*	0.74 (0.45-1.24)	8 hr.	—	—	—	6	8
(Phenyl)trifluorosilane	0.31 (0.24-0.40)*	0.64 (0.47-0.86)	15 min.‡	1,000†	4	1/6	5	10
Tetra-(2-ethylhexoxy) silane	>64	>20	8 hr.	—	—	—	2	1

* as gm/kg in a suitable vehicle
** female rats
† inhalation time shown killed all six rats
‡ twice concentration shown killed all six rats
§ caused death when introduced into the rabbit eye

Range-Finding Toxicity Data: List VII

HENRY F. SMYTH, JR., PH.D., CHARLES P. CARPENTER, PH.D., CARROL S. WEIL, URBANO C. POZZANI, JEAN A. STRIEGEL, and JUDITH S. NYSTROM

Carnegie-Mellon University, 4400 Fifth Avenue, Pittsburgh, Pennsylvania 15213

Acute toxicity and irritation data on about 200 compounds are presented, accumulated in a continuing program for screening potential commercial products.

DURING THE SEVEN years since the appearance of our last communication on the range-finding test,[1] many additional data have become available for presentation. Table I lists these data under names conforming to the nomenclature used in *Chemical Abstracts*. Table II lists only the oral LD_{50} values for a number of inorganic compounds. All materials included are either in commercial production or have been evaluated for commercial potential within the past few years.

As has been stated in earlier communications,[1-6] the range-finding test is relied upon only to allow predictions of the comparative hazards of handling new chemicals. Acute toxicity studies, no matter how precisely executed, yield no more than an indication of the degree of care necessary to protect exposed workmen or lead to an opinion that certain technically feasible applications of a chemical may or may not eventually be proved safe.

Our last paper[1] included data under the name of triisooctyl phosphine. The compound should have been entered as triisooctyl phosphite.

Experimental methods have remained unchanged. They are described in our 1962 publication.[1]

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TABLE I

Range-Finding Toxicity Data

Material Studied	Single Oral LD[illegible] for Rats mg/Kg	Single Skin Penetration LD[illegible] for Rabbits mg/Kg	Concentrated Vapor Inhalation for Rats Maximum for 1 hr. 24 hrs.	Inhalation of Metered Vapor Concentration by Rats: Concentration ppm	Time, hr.	Mortality	Irritation on Unprotected Rabbit Eyes	Corneal Injury in Rabbit Eyes
Hydrocarbons								
2-(4-Cyclohexenyl)-bicyclo-(2.2.1)-hept-5-ene	4.29 (3.07 – 5.98)	3.54 (2.13 – 5.72)	8 hr.	–	–	–	3	2
Cyclopentene	2.14 (1.45 – 3.17)	1.59 (0.97 – 2.59)	5 m.	15,000	4	4/6	5	5
5-Ethylidene-2-norbornene	2.83	9.17 (1.83 – 6.63)	–	4,000	4	3/6	5	2
[illegible]	[illegible]	5.66 (2.29 – [illegible])	30 m.	2,000	4	2/6	5	1
[illegible]	[illegible]	>5	5 m.	–	–	–	1	7
[illegible]	19.7 (15.0 – 23.3	14.1 (4.35 – [illegible])	15 m.	8,000	4	2/6	4	1
[illegible]	[illegible] (2.38 – 5.21	14.1 (8.75 – 22.9)	4 hr.	–	–	–	3	2
[illegible]-Tetrahydro-1H-indene	7.53 (6.73 – [illegible]) *	14.1 (8.33 – 23.77)	–	4,000	4	1/6	4	9
Toluene	3.08 (2.49 – 3.31)	20	15 m.	8,000	4	4/6	4	2
4-Vinyl-1-cyclohexene	5.19 (4.22 – 6.41)	15.9 (11.7 – 21.5)	–	4,000	4	4/6	5	2
5-Vinyl-2-norbornene								
Hydroxyl Compounds								
p-tert. Butylphenol	3.25 (2.48 – 4.26)	2.52 (0.93 – 8.58)	8 hr.	–	–	–	6	9
Cyclohexenyl-4-phenol	2.55 *	3.18 (1.94 – 5.18)*	8 hr.	–	–	–	–	9
2-Cyclopenten-1-ol	0.47 (0.34 – 0.66)	0.18	1 hr.	500†	4	0/6	2	5
3,4-Dihydroxy-1,5-hexadiene	1.62 (1.24 – 2.13)	0.40 (0.29 – 0.54)	–	–	–	–	2	6
Eicosanol, mixed isomers	>64	>20	8 hr.	–	–	–	3	1
2-Ethylhexanol	2.46 (1.82 – 3.33)	2.38 (1.70 – 3.34)	8 hr.	–	–	–	3	5
Hydroxydihydrocyclopentadiene	3.25 (2.11 – 4.99)	3.15 (2.33 – 4.26)	8 hr.	–	–	–	5	9
3-Methyl-1-butanol	7.07 (4.32 – 10.4)	3.97 (2.93 – 5.37)	8 hr.	–	–	–	2	8
Methyl-3-cyclohexenyl-methanol	1.41	0.62 (0.42 – 0.92)	8 hr.	–	–	–	3	8
Nonylphenol, mixed isomers	1.62 (1.06 – 2.33)	2.14 (1.52 – 2.99)	4 hr.	–	–	–	6	10
2-Pinanylmethanol	1.62 (1.24 – 2.13) *	0.30	3 hr.	–	–	–	3	9
Tetraethylene glycol, mixed isomers	32.5 (20.1 – 50.3)	7.13 (4.41 – 11.52)	8 hr.	–	–	–	2	1
Ethers								
Diethyl-2,2,1-bicyclo-2,2-heptene-5-methyl ether	[illegible] ([illegible] – [illegible])	[illegible] ([illegible] – [illegible])	[illegible]	–	–	–	[illegible]	1
2-Cyclopentyl ether	11.0	[illegible] ([illegible] – [illegible])	[illegible]	–	–	–	[illegible]	2
Ethyl propenyl ether	19.0 (13.4 – 27.[illegible])	>12	5 m.	[illegible]	4	[illegible]	[illegible]	12
Ethyl vinyl ether	8.16 (7.03 – 9.48)	>20	–	64,000	4	0/6	1	1
Methoxy triethylene glycol vinyl ether	11.3	10.0 (4.56 – 21.9)	8 hr.	–	–	–	2	3
alpha-Methylbenzyl ether	9.80 (6.38 – 15.06)*	–	8 hr.	–	–	–	4	1
2-Methoxypropene	1.87 (1.26 – 2.75)	>20	–	64,000	4	4/6	1	1
1,1,3,3-Tetramethoxyhexane	4.09 (3.32 – 4.76)*	–	8 hr.	–	–	–	2	2
Hydroxy Ethers								
1-Butoxyethoxy-2-propanol	4.00 (2.55 – 6.27)	2.83 (1.75 – 4.57)	8 hr.	–	–	–	3	5
3-Butoxy-1-propanol	5.95 (3.63 – 9.63)	1.59 (1.17 – 2.15)	8 hr.	–	–	–	2	7
Diethoxytetraethylene glycol	4.29 (2.49 – 6.34)	6.35 (3.89 – 10.4)	8 hr.	–	–	–	2	2
3-Ethoxy-1-propanol	13.0 (8.46 – [illegible])	2.83	8 hr.	–	–	–	2	5
2-Ethylbutoxypropanol, mixed isomers	3.56 (2.21 – 5.74)	6.0	8 hr.	–	–	–	2	9
Hexyloxy-2-ethoxyethyl ether	3.73 (2.63 – 5.21)	6.30 (4.66 – 8.52)	8 hr.	–	–	–	2	[illegible]
[illegible]oxypropanol, mixed isomers	7.5	3.56 (1.95 – 6.52)	8 hr.	–	–	–	3	7

TABLE I

Range-Finding Toxicity Data (Continued)

Material Studied	Single Oral LD[illegible] for Rats mg/Kg	Single Skin Penetration LD[illegible] for Rabbits mg/Kg	Concentrated Vapor Inhalation for Rats Maximum for 1 hr. 24 hrs.	Inhalation of Metered Vapor Concentration by Rats: Concentration ppm	Time, hr.	Mortality	Irritation on Unprotected Rabbit Eyes	Corneal Injury in Rabbit Eyes
2-Isopropoxyethanol	5.66 (4.11 – 7.79)	1.60 (1.08 – 2.37)	2 hr.	4,000	4	4/6	3	5
3-Methoxy-1-propanol	13.0 (9.99 – 17.0)	5.66	8 hr.	–	–	–	1	5
2-Propoxyethanol	4.89 (4.16 – 5.73)*	1.95 (0.93 – 6.99)*	–	–	–	–	2	8
Propoxypropanol, mixed isomers	3.23 (2.48 – 4.26)	4.[illegible] (2.7[illegible] – 5.92)	8 hr.	–	–	–	2	7
Aldehydes, Ketones								
Cyclohexanone	1.62 (1.20 – 2.20)	1.00 (0.63 – 1.63)	30 m.	2,000†	4	1/6	1	5
5-Ethyl-3-nonen-2-one	8.12 (7.27 – 9.06)*	8.48 (5.04 – 14.24)	8 hr.	–	–	–	2	1
Hexanal, mixed isomers	9.51 (5.84 – 15.5)	>10	30 m.	–	–	–	2	5
2-Methylcyclohexanone	2.14 (1.48 – 3.10)	1.77	–	2,000	4	3/6	3	5
2-Methyl-1,2,3,6-tetrahydrobenzaldehyde	5.66	3.15 (2.03 – 4.29)	8 hr.	–	–	–	5	5
1-Tetralone	0.81 (0.62 – 1.07)	>20	8 hr.	–	–	–	4	3
[illegible]	[illegible]	[illegible]	15 m.	4,000	4	3/6	2	3
[illegible]								
[illegible]	[illegible]	>20	8 hr.	–	–	–	5	5
[illegible]	[illegible]	>10	–	–	–	–	1	4
Methyltetrahydrophthalic anhydride	2.14 (1.42 – 3.10)	1.41	8 hr.	–	–	–	1	9
Tri-isobutenylsuccinic anhydride	0.64 (0.61 – 1.44)*	0.98 (0.55 – 1.44)*	–	–	–	–	4	[illegible]
Valeric acid, mixed isomers	2.01 (0.90 – 4.44)	0.51 (0.05 – 1.50)	8 hr.	–	–	–	6	6
Esters, Acetals								
Acetic acid, 2-methyl-2,4-pentanediol diester	3.36 (2.05 – 5.48)	16.0 (3.33 – 76.8)	8 hr.	–	–	–	3	2
Acetic acid, 2-methyl-2-propene-1,1-diol diester	0.44 (0.28 – 0.68)	0.441 (0.024 – 0.682)	1 hr.	12.5	4	5/6	5	9
Acetic acid, phenyl ester	1.63 (1.03 – 2.74)	8.6[illegible] (3.65 – 17.5)	8 hr.	–	–	–	2	2
Acetic acid, propyl ester	9.8 (7.5 – 12.9)	>20	30 m.	8,000	4	4/6	1	2
Acetic acid, triethylene glycol diester	22.6	9.[illegible] (1.91 – 33.5)	8 hr.	–	–	–	1	1
Acrylic acid, 2-butoxyethoxy ester	6.50 (4.72 – 8.95)	1.24 (0.89 – 1.04)	8 hr.	–	–	–	5	2
Acrylic acid, decyl ester, mixed isomers	12.3 (9.98 – 15.3)	3.54 (1.17 – 10.7)	8 hr.	–	–	–	5	1
Acrylic acid, hexyl ester	20.0 (12.9 – 30.8)	5.65 (3.51 – 9.14)	4 hr.	–	–	–	5	1
Acrylic acid, 2-methoxyethyl ester	1.23 (0.84 – 1.76)	0.16 (0.11 – 0.25)	8 hr.	–	–	–	5	[illegible]
Acrylic acid, 2-methoxyethoxy ester	1.04 (0.59 – 1.21)	0.25 (0.17 – 0.37)	15 m.	3[illegible]	4	3/6	4	[illegible]
Acrylic acid, 2-morpholinyl ester	5.66	0.28 (0.24 – 1.43)	6 hr.	–	–	–	5	2
Acrylic acid, dihexyl ester	16.1 (10.2 – 25.[illegible])	>20	–	–	–	–	2	1
Acrylic acid, 2-ethylhexanediol diester	20.2 (15.[illegible] – 35.9)	20	8 hr.	–	–	–	2	1
Fumaric acid, ethyl 2,3-epoxypropyl ester	1.62 (1.06 – 2.59)	0.35 (0.22 – 0.57)	–	–	–	–	3	9
Fumaric acid, dimethyl ester	2.24 (1.15 – 4.37)*	1.25 (0.84 – 1.85)*	8 hr.	–	–	–	5	9
Malonic acid, diethyl ester	14.9 (10.7 – 20.6)	>16	8 hr.	–	–	–	5	2
Methacrylic acid, allyl ester	0.43 (0.31 – 0.61)*	0.51 (0.37 – 0.68)	5 m.	250†	4	0/6	5	1
Methacrylic acid, butyl ester	22.6	11.3 (5.19 – 24.7)	8 hr.	–	–	–	3	1
Phthalic acid, butyl decyl ester	20.8 (16.8 – 25.7)	15.9 (11.7 – 21.5)	8 hr.	–	–	–	2	1
Phthalic acid, decyl hexyl ester	49.4 (39.9 – 61.0)	>20	8 hr.	–	–	–	2	1

(Continued on Next Page)

TA[illegible]

Range-Finding To[illegible] (Continued)

Material Studied	Single Oral LD50 for Rats ml/Kg	Skin Absorption Toxicity for Rats (ml/Kg)	Concentrated Vapor Inhalation by Rats Maximum for No Death	Inhalation of Metered Vapor Concentration by Rats: Concentration ppm	Time, hr.	Mortality	Irritation on Uncovered Rabbit Belly	Corneal Injury in Rabbits
Phthalic acid, dioctyl ester	45.2 (23.0 - 73.2)		8 hr.	–	–	–	2	1
Propionaldehyde diacetyl acetal	2.14 (1.34 - 2.99)	[illegible] - 5.72)	30 m.	2,000	4	5/6	4	2
Succinic acid, di-2-[(2-ethylbutoxy)-ethoxy] ester	3.3 *	[illegible] - 7.09)	–	–	–	–	3	2
Epoxy Compounds								
Bicyclopentadiene diepoxide	2.14 (1.34 - 2.99)	[illegible]	8 hr.	–	–	–	3	5
Bis-2,3-epoxybutyl ether	1.07 (0.77 - 1.50)	[illegible] - 0.37)	8 hr.	–	–	–	5	7
3,4-Epoxycyclohexylmethyl [illegible]	4.59 (3.07 - [illegible])	[illegible]	8 hr.	–	–	–	2	1
N,N-Diglycidylaniline	1.62 (1.18 - 2.24)	[illegible] - 7.57)	8 hr.	–	–	–	3	1
2-and 3,4-epoxypropyl furfurate	0.71 (0.48 - 1.04)	[illegible] - 1.70)	8 hr.	–	–	–	5	3
1-[illegible]-2,3-epoxypropyl ether	3.73 (2.48 - 5.21)		8 hr.	–	–	–	5	2
1,2-Epoxycyclohexane	1.09 (0.66 - 1.50)	[illegible] - 1.03)	30 m.	2,000†	4	1/6	3	3
2,3-Epoxypropyl methacrylate	0.77 (0.57 - 1.04)	[illegible] - .93)	2 hr.	–	–	–	5	4
Propylene oxide	1.14 (1.05 - 1.23)	[illegible] - 23.0)	5 m. §	4,000	4	4/6	1	5
Heterocyclic Oxygen								
2-Aminomethyl-2,3-dihydro-[illegible]-pyran	1.00 (0.64 - 1.57)*		4 hr.	–	–	–	6	9
2,5-Dioxotetrahydrofuran	2.46 (1.79 - [illegible])*	[illegible] (0.[illegible] - 3.68)	4 hr.	–	–	–	4	2
3,3-Dihydro-2,4,8,10-tetraoxaspiro-[5,5]-undecane	3.35 (2.48 - 4.53)*	[illegible] (3[illegible] - 12.73)*	8 hr.	–	–	–	2	3
2-Ethoxy-4-methyl-[illegible]	5.16 (4.17 - 6.[illegible])		4 hr.	–	–	–	2	3
Aliphatic and Alicyclic Amino Compounds								
1-Aminoethyl-3-aminomethyl-3,5,5-trimethyl cyclohexanol	1.30 (1.04 - 1.73)	–	8 hr.	–	–	–	7	9
2-Aminomethylcetanaviridopyrrole	0.71 (0.44 - 1.13)	[illegible] (0.[illegible] - 1.14)	4 hr.	–	–	–	6	9
3-Aminomethoxy-2-ethoxy [illegible]	6.50 (4.35 - [illegible])*	[illegible] (4.[illegible] - 8.42)	8 hr.	–	–	–	3	7
Amyl amine, mixed isomers	0.47 (0.33 - 0.65)*	[illegible] (0.[illegible] - 2.05)	30 m. §	2,000	4	4/6	6	9
Bis(2-dimethylaminoethoxy)-methane	2.33	*	–	–	–	–	7	3
Bis(2-dimethylaminoethyl) ether	1.23 (0.94 - 1.62)	[illegible]	8 hr.	–	–	–	6	9
Cyclohexylamine	0.71 (0.51 - 0.97)	[illegible] (0.[illegible] - 0.43)	2 hr.	4,000†	4	0/6	7	10
1,8-Diamino-p-menthane	0.77 (0.62 - 0.95)	[illegible] (0.[illegible] - 0.35)	4 hr.	–	–	–	7	9
2,4-Diamino-1-methylcyclohexane	1.41	[illegible] (0.[illegible] - 0.82)	8 hr.	–	–	–	6	9
Bis(3-aminopropyl) ether of diethylene glycol	4.29 (3.07 - 5.98)	[illegible] (1.14 - 5.48)	4 hr.	–	–	–	6	8
Di(3-aminopropoxy)ethane	2.83 (1.78 - 4.33)	1.25 ([illegible] - 1.95)	8 hr.	–	–	–	6	8
N,N-Di(methyl-N-cyclohexylamine)-methylamine	1.23 (0.94 - 1.62)*	0.21 ([illegible] - 0.30)	8 hr.	–	–	–	7	9
N-Methyl-N-cyclohexylmethylamine	1.41 *	[illegible] (0.[illegible] - 0.85)	4 hr.	–	–	–	6	9
N,N,N',N',N''-Pentamethylenetriamine	1.63 (1.24 - 2.13)	0.28	8 hr.	–	–	–	7	9
N,N,N',N'-Tetramethyl-1,3-[illegible]-propanediamine	0.93 (0.67 - 1.30)	0.10 (0.[illegible] - 0.54)	3 hr.	–	–	–	6	9
N,N,N',N'-Tetramethyl-1,2-[illegible]-ethylenediamine	0.41 (0.31 - [illegible])	0.30 (0.21 - 0.42)	15 m.	1,000	4	2/6	5	9
Tetramethyl-2-tetrazene	1.73 (1.12 - 2.[illegible])	—	4 hr.	–	–	–	2	4
Tripropylamine	0.056 (0.071 - 0.13)*	0.57 (0.[illegible] - 1.23)	8 hr.	250	4	3/6	4	1
Aromatic Nitro and Amino Compounds								
3-Aminopyrene	1.07 (0.77 - 1.50)*	> 1	8 hr.	–	–	–	–	3
N-Methyl-1-naphthylamine	1.41 (1.03 - 1.95)		8 hr.	–	–	–	2	1

TABLE I

Range-Finding Toxicity Data (Continued)

Material Studied	Single Oral LD50 for Rats ml/Kg	Single Skin Penetration LD50 for Rabbits ml/Kg	Concentrated Vapor Inhalation by Rats Maximum for No Death	Inhalation of Metered Vapor Concentration by Rats: Concentration ppm	Time, hr.	Mortality	Irritation to Uncovered Rabbit Belly	Irritation to Covered Rabbit Belly
Amides								
N(2-Acetoxyethyl)-N-ethylacetamide	20.8 (16.8 - 25.7)	>10	8 hr.	–	–	–	2	4
α-Caprolactam	2.14 (1.54 - 2.99)*	1.41	4 hr.	–	–	–	–	[illegible]
N-Ethylacetamide	2.46 (1.83 - 3.25)	1.25 (0.84 - 1.85)	4 hr.	2000	4	3/6	2	[illegible]
Propyl-N-[illegible]	12.93 (8.42 - 19.87)	>20	8 hr.	–	–	–	4	[illegible]
Heterocyclic Compounds								
3,5-Dimethyl-1-(2-chloroethylmercapto)-pyrazole	0.57 *	0.20 (0.15 - 0.27)*	4 hr.	–	–	–	6	10
Isopropyl [illegible]	0.71	0.10 (0.07 - 0.15)	15 m.	500	4	5/6	6	[illegible]
Isopropyl [illegible]	2.83	0.64 (0.47 - 0.86)	8 hr.	–	–	–	6	[illegible]
5-α-Lutidine	0.71 (0.50 - 1.24)	0.14	1 hr.	2500	4	0/6	[illegible]	6
N-Methylmorpholine	1.62 (1.03 - 2.26)	1.41	8 hr.	–	–	–	3	[illegible]
2-Methylpyrazine	7.13 (5.02 - 16.34)	10.8 (6.47 - 16.5)	8 hr.	–	–	–	1	[illegible]
2-Methylpyridine	1.38 (0.71 - 2.57)	0.81 (0.54 - 1.18)	4 hr.	4000	8	4/6	5	[illegible]
1-(Trichloromethylmercapto) pyrazole	0.35 *	0.0630 (0.047 - 0.085)	–	200	1	6/6	7	8
1-(Trichloromethylmercapto)-4-methylpyrazole	1.04 (0.81 - 1.33)	0.0730 (0.059 - 0.107)	2 m. §	–	–	–	7	[illegible]
Cyanide Compounds								
Bis(2-isopropylpentyl) maleate	1.83 (1.21 - 2.79)	>12	8 hr.	–	–	–	3	5
Bis(2-isopropylpentyl) 1-cyclohexene-1,2-dicarboxylate	10.4 (5.55 - 19.4)	> 8	8 hr.	–	–	–	5	[illegible]
Bis(2-methylbutyl) 1-cyclohexene-1,2-dicarboxylate	22.6 (13.4 - 38.2)	16.1 (4.5 - 57.2)	8 hr.	–	–	–	5	[illegible]
3-Butenonitrile	0.1150 (0.075 - 0.1380)*	1.41	5 m.	2500	4	0/6	3	[illegible]
2,3-Dimethylaminopropionitrile	3.25 (2.41 - 4.26)	5.04 (3.73 - 6.82)	8 hr.	–	–	–	2	[illegible]
1,6-Hexamethylene diisocyanate	0.71	0.57 (0.35 - 0.91)	8 hr.	–	–	–	[illegible]	[illegible]
Lactonitrile	0.0570 (0.0410 - 0.1.51)*	0.0200 (0.015 - 0.027)	–	52.50	4	0/6	2	[illegible]
3-Methoxypropionitrile	4.39 (3.07 - 5.96)*	>10	8 hr.	–	–	–	2	[illegible]
Methyl [illegible]	0.0710 (0.027 - 0.18)*	0.22 (0.12 - 0.41)	–	21.25	4	0/6	6	[illegible]
Tridecanol [illegible] isomers	3.73 (2.68 - 5.21)	3.18 (1.43 - 7.06)	8 hr.	–	–	–	4	[illegible]
Halogen Compounds								
1-Bromo[illegible]	4.49 (3.09 - 9.64)	6.00 (4.93 - 13.0)	–	–	–	–	5	[illegible]
2-Chloro[illegible]	20.0 (12.8 - 31.3)	20	5 m. §	[illegible]	4	3/6	1	[illegible]
Chloromethane	0.21 (0.12 - 0.37)	0.28 (0.13 - 0.62)	–	13	4	1/6	6	[illegible]
Dibromo[illegible], mixed isomers	0.21 (0.15 - 0.30)*	0.25 (0.19 - 0.34)	1 hr.	–	–	–	6	[illegible]
Dichlo[illegible], mixed isomers	0.81 (0.50 - 1.31)	0.40 (0.24 - 0.65)	8 hr.	–	–	–	5	[illegible]
1,2-Dichloropropane (propylene dichloride)	1.9 (1.7 - 2.1)	8.75 (6.31 - 9.20)	10 m.	2000	3	3/6	1	[illegible]
trans-2,3-Dichlorobutene	1.41	0.44 (0.27 - 0.71)	4 hr.	–	–	–	5	[illegible]
1,2-Dichloroethylene (acetylene dichloride)	0.77 (0.67 - 0.89)*	3.85 (2.40 - 4.46)	–	–	–	–	[illegible]	[illegible]
Di(2-chloroethyl) [illegible]	0.31 (0.24 - 0.40)*	0.20 (0.14 - 0.30)	1 hr.	–	–	–	3	[illegible]
Fumaroyl [illegible]	0.81 (0.51 - 1.30)	1.41	–	500	4	3/6	6	[illegible]
1,1,1,3,3-Pentachloro-2,2-difluoropropane	0.54 (0.30 - 1.01)	4.53 (2.80 - 7.22)	–	–	–	–	3	[illegible]
m-Nitrobenzotrifluoride	2.46 (1.79 - 3.29)	0.79 (0.49 - 1.30)	8 hr.	–	–	–	5	[illegible]

(Continued on Next Page)

TABLE I
Range-Finding Toxicity Data (Continued)

Material Studied	Single Oral LD50 for Rats ml/Kg	Single Skin Penetration LD50 for Rabbits [illegible]	Concentrated Vapor Inhalation by Rats Maximum for No Death	Inhalation of Metered Vapor Concentration by Rats: Concentration ppm	Time, hr.	Mortality	Irritation on Uncovered Rabbit Belly	Corneal Injury in Rabbits
[illegible]	1.41 (0.83 - 2.40)	3.97 ([illegible] - 3.57)	15 m.	[illegible]	4	3/6	6	10
[illegible]	0.22 (0.19 - 0.27)	0.48 ([illegible] - 0.77)	15 m.	[illegible]	4	1/6	1	8
[illegible]	0.20 (0.16 - 0.27)	3.29 ([illegible] - 5.13)	–	[illegible]	4	3/6	6	–
[illegible]	0.35	0.30 ([illegible] - 0.39)	30 m.	[illegible]	4	5/6	5	9
[illegible]	0.53 (0.47 - 0.71)	3.72 ([illegible] - 4.21)	–	[illegible]	4	4/6	2	2
[illegible]	4.92 (3.73 - 6.46)	>20	–	[illegible]	4	4/6	5	4
[illegible] Compounds								
[illegible]	0.25 (0.19 - 0.35)*	0.141 ([illegible] - 0.378)	8 hr.	–	–	–	2	3
[illegible]	5.19 (2.31 - 9.34)	[illegible] ([illegible] - 5.39)	4 hr.	–	–	–	1	5
[illegible]	2.23 (1.73 - 4.37)	2.83	3 hr.	–	–	–	4	2
[illegible] prime	19.7 (15.0 - 25.8)	>10	8 hr.	–	–	–	2	1
[illegible] phosphate	29.9 (21.4 - 41.8)	15.9 ([illegible] - 21.3)	8 hr.	–	–	–	3	1
[illegible] Compounds								
[illegible]	14.1	11.3	8 hr.	–	–	–	4	2
[illegible]	0.25 (0.19 - 0.32)*	1.00 ([illegible] - 2.31)	8 hr.	–	–	–	7	9
[illegible]	2.14 (1.34 - 2.99)	> 5	1 hr.	–	–	–	3	2
[illegible]	0.37	[illegible] ([illegible] - 1.40)	–	–	–	–	7	5
[illegible]	1.03 (0.64 - 1.37)	1.41 ([illegible] - 2.29)	1 hr.	–	–	–	6	5
[illegible]	13.0 (7.73 - 21.93)	5.04 ([illegible] - 8.23)	8 hr.	–	–	–	2	5
[illegible]	3.25 (2.45 - 4.26)	> 5	–	3,[illegible]	1	4/6	2	2
[illegible] oxide (sulfolane)	1.54 (1.25 - 1.91)	3.13 ([illegible] - 4.3)	8 hr.	–	–	–	2	4
[illegible]	9.25 (7.51 - 12.9)	>15	8 hr.	–	–	–	3	1
[illegible] monobutyrate	2.19 (1.39 - 3.12)*	0.6	–	–	–	–	6	1
[illegible]	1.34 (0.89 - 2.03)*	1.3	–	–	–	–	6	2
[illegible] Compounds								
[illegible]	4.92 (3.73 - 6.46)	10.30 ([illegible] - 21.2)	4 hr.	–	–	–	4	1
[illegible] tetramethoxy silane	12.3 (9.28 - 15.3)	6.30 ([illegible] - 8.2)	8 hr.	–	–	–	3	1
[illegible] tetraethoxy silane	22.6	3.97 ([illegible] - 5.7)	8 hr.	–	–	–	3	2
[illegible]	19.7 (15.9 - 25.8)	>10	8 hr.	–	–	–	3	1
[illegible] silane	2.46 (1.88 - 3.21)	–	2 hr.	–	–	–	3	2
[illegible] silane	2.83 (1.51 - 4.94)	5.66	–	–	–	–	3	2
Methyl [illegible]	12.5 (9.93 - 15.7)	>10	1 hr.	–	–	–	3	2
[illegible]	9.33 (6.09 - 13.9)	6.30 ([illegible] - 8.32)	15 m.	62.5	4	5/6	3	5
[illegible]	11.3	3.54 ([illegible] - 5.6)	2 hr.	1,[illegible]	4	1/6	2	1

* [illegible] vehicle

† [illegible] killed all six rats

‡ [illegible] own killed [illegible] rats

§ [illegible] the rabbit

TABLE II

Acute Oral Toxicity of Some Chemicals for Rats

Material*		Formula	Single Oral LD_{50} for Rats (mg/kg)	Concentration Tabulated (mg/ml)**	
Aluminum isopropylate	T	$Al(OCH(CH_3)CH_3)_3$	11.3 (7.0 - 18.3)	0.200	A
Aluminum nitrate		$Al(NO_3)_3 \cdot 9H_2O$	4.28 (3.66 - 4.76)	0.100	
Ammonium metavanadate	T	NH_4VO_3	0.16 (0.12 - 0.21)	0.0025	B
Ammonium paratungstate	T	$(NH_4)_6W_7O_{24} \cdot 6H_2O$	11.3	0.400	A
Ammonium persulfate		$(NH_4)_2S_2O_8$	0.82 (0.53 - 1.26)	0.200	
Borax		$Na_2B_4O_7 \cdot 10H_2O$	5.66	0.010	
Boric acid		H_3BO_3	5.14 (4.75 - 5.58)	0.250	
Calcium acetate		$Ca(OOCCH_3)_2 \cdot H_2O$	4.28 (3.66 - 4.76)	0.100	
Calcium acrylate	T	$Ca(OOCCH=CH_2)_2 \cdot H_2O$	4.92 (3.75 - 6.46)	0.200	A
Calcium cyanide		$Ca(CN)_2$	0.019(0.0[illegible] - 0.051)	0.001	
Calcium hydroxide		$Ca(OH)_2$	7.34 (4.63 -11.14)	0.100	
Calcium propionate	T	$Ca(OOCCH_2CH_3)_2 \cdot H_2O$	5.16 (4.17 - 6.38)	0.100	
Chromium acetate	T	$Cr(OOCCH_3)_3 \cdot H_2O$	11.35	0.200	
Chromium chloride (ous)	T	$CrCl_2$	1.87 (1.21 - 2.87)	0.050	
Chromium nitrate (ic)		$Cr(NO_3)_3 \cdot 9H_2O$	3.25 (2.13 - 4.94)	0.200	
Cobalt oxide	T	Co_3O	1.70 (1.07 - 2.72)	0.150	A
Copper acetate		$Cu(OOCCH_3)_2 \cdot H_2O$	0.71 (0.60 - 0.83)	0.040	
Copper nitrate		$Cu(NO_3)_2 \cdot 3H_2O$	0.94 (0.61 - 1.43)	0.250	
Copper sulfate		$CuSO_4 \cdot 5H_2O$	0.96 (0.71 - 1.30)	0.050	
Cuprous oxide	T	Cu_2O	0.47 (0.34 - 0.65)	0.100	A
Diammonium decaborane	T	$(NH_4)_2B_{10}H_{10}$	3.56 (1.85 - 6.86)	0.050	
Dicumene chromium	T	$(C_6H_5)_2Cr$	0.61 (0.62 - 1.07)	0.100	M
Ferric nitrate		$Fe(NO_3)_3 \cdot 9H_2O$	3.25 (2.56 - 4.35)	0.250	
Ferrous ammonium sulfate		$(NH_4)_2SO_4 \cdot FeSO_4 \cdot 6H_2O$	3.25 (2.43 - 4.25)	0.500	
Lithium carbonate		Li_2CO_3	0.71	0.200	
Manganese chloride		$MnCl_2 \cdot 4H_2O$	8.1 (7.3 - 9.1)	0.150	
Manganese acetate		$Mn(OOCCH_3)_2 \cdot 4H_2O$	3.73 (2.66 - 5.21)	0.250	
Nickel nitrate		$Ni(NO_3)_2 \cdot 6H_2O$	1.62 (1.15 - 2.50)	0.500	
Potassium acetate		$KOOCCH_3$	3.25 (2.43 - 4.26)	0.100	
Potassium carbonate		K_2CO_3	1.67 (1.51 - 2.60)	0.250	
Potassium hydroxide		KOH	1.23 (0.87 - 1.80)	0.100	
Potassium permanganate		$KMnO_4$	1.09 (0.73 - 1.71)	0.250	
Silver oxide	T	Ag_2O	2.82	0.250	A
Sodium arsenite		$NaAsO_2$	0.041(0.031- 0.053)	0.010	
Sodium cyanide		NaCN	0.045(0.041- 0.051)	0.0[illegible]	
Sodium fluoride		NaF	0.18 (0.12 - 0.28)	0.100	
Sodium nitrite		$NaNO_2$	0.18	0.010	
Sodium phosphate, dibasic		$Na_2HPO_4 \cdot 7H_2O$	12.93 (9.15 -16.97)	0.250	
Sodium phosphate, tribasic		$Na_3PO_4 \cdot 12H_2O$	7.43 (6.81 - 8.03)	0.250	
Sodium tripolyphosphate	T	$Na_5P_3O_{10}$	6.59 (4.62 -10.48)	0.250	
Sulfuric acid		H_2SO_4	2.14 (1.54 - 2.97)	0.250	
Vanadium dichloride	T	VCl_2	0.54 (0.38 - 0.75)	0.010	A
Vanadium oxytrichloride	T	$VOCl_3$	0.14 (0.09 - 0.22)	0.050	M
Vanadium tetrachloride	T	VCl_4	0.16	0.050	M
Vanadium trichloride	T	VCl_3	0.35 (0.22 - 0.57)	0.100	
Zinc acetate		$Zn(OOCCH_3)_2 \cdot 2H_2O$	2.46 (1.60 - 3.78)	0.250	
Zinc naphthenate	T		4.92 (3.75 - 6.46)	0.250	L

* T Preceding formula designates technical grade, or development sample, otherwise reagent grade

** Tabulated concentrations are in water except as noted by following symbols:

M In mineral oil

L In lard

A Suspended in 0.25% agar

B Suspended in 5% bentonite

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Alkali Poisoning.

By DOUW G. STEYN, Section of Pharmacology and Toxicology, Onderstepoort.

I. Weak Alkalis (Sodium Bicarbonate and Sodium Carbonate).

Kobert (1906) states that sodium bicarbonate raises the alkalinity of the tissue fluids and enhances the oxidation processes in the body. Ingestion of excessive quantities of sodium bicarbonate causes dilatation of the stomach, anorexia and anaemia.

Fröhner (1919) reports that a cow which had received 800-900 gm. of sodium carbonate aborted, groaned and bellowed, and had to be slaughtered, and that 10-15 gm. of potassium carbonate is fatal for the dog (heart failure and collapse). Dogs fed daily with 15 gm. of sodium bicarbonate for weeks showed vomiting, diarrhoea and loss in condition (Fröhner, 1919). He also states that two horses which ate sodium carbonate from bags took ill with severe colic, profuse diarrhoea, continuous coma, stomatitis and swelling of the lips.

Reid (1921) describes sodium carbonate poisoning in sheep due to the drinking of effluents from dairy factories. The symptoms were staggering gait and hurried respirations. The animals soon lay down and died. The following were the post mortem appearances: Hyperaemia of the subcutaneous tissues, imperfect clotting of the blood, hyperaemia of the small intestines with subserous haemorrhages, slight congestion of the liver, in very acute cases the abomasum was filled with dark, blood-stained, fluid contents, intense hyperaemia of abomasal mucosa with haemorrhages.

Heller and Larwood (1930) conducted experiments upon rats and other small animals with single salts and also mixtures of calcium chloride, calcium sulphate, sodium bicarbonate, and magnesium sulphate. Drinking water containing 20,000 parts of sodium carbonate per million parts (i.e. a 2 per cent. aqueous solution) was found to be decidedly deleterious and reproduction was interfered with at quite low levels. Sodium bicarbonate proved to be less injurious. In subsequent experiments upon rats Heller (1932 and 1933) established that drinking water containing 1 per cent. (10,000 p.p.m.) of sodium carbonate resulted in unsatisfactory growth of the offspring, while a 1·9 per cent. solution caused a rough coat, red eyes, and diarrhoea in mature rats and a very high

mortality in their young. A 1·5 per cent. solution of sodium bicarbonate caused undersized adults and impeded growth in their offspring, while there were unsatisfactory growth and appearance with 2 per cent. solutions. Experimenting with 0·5 and 1·0 per cent. aqueous solutions of caustic soda (NaOH) he found practically normal growth with the former solution, the latter causing retarded growth, "dirty animals", marked nervousness, sore eyes and diarrhoea.

Linton and Wilson (1933) refer to sodium bicarbonate poisoning in pigs due to the feeding of "Flour sweepings" containing 33·7 per cent. of this alkali. The symptoms seen were excessive thirst, animals appeared to be in pain, stood with their heads lowered and oblivious to their surroundings and staggered on their front legs. Only one pig died after the sweepings were removed and milk given. Autopsy revealed inflammation of the stomach. Linton furthermore describes alkali poisoning in pigs through accidental discharging into their milk of the alkali used for cleansing the milk utensils.

Some time ago the author received for analysis a certain proprietary "scouring powder", which was being sold as a cleansing agent for *kitchen utensils*. Pigs had died after their troughs had been scoured with it. Four grams of this strongly alkaline powder caused death in rabbits within a few hours. This is one of the many cases in which a virulent poison is labelled non-poisonous.

Witter (1936) added sodium bicarbonate to the drinking water of chicks and found that: "(1) Sodium bicarbonate given in the drinking water in the usual dosage ($\frac{1}{4}$ lb. to 5 gallons or 0·6 per cent. solution) caused chicks to drink more water than normal and produce moist droppings. Chicks two weeks old developed pale and swollen kidneys from this dosage, but chicks three weeks old and older were not noticeably injured. (2) A double dose (1·2 per cent. solution) of soda caused chicks to drink more water than those fed the 0·6 per cent. solution and produced watery droppings. Chicks two to eight weeks old were seriously injured by this dosage within one to three days and deaths occurred within this time. (3) Two and four-tenths per cent. solution of soda reduced water consumption below normal for chicks under four weeks of age. The injurious effects of this dosage were noted within a day and deaths occurred within three days. (4) Mature cockerels were injured with a 2·4 per cent. solution of soda, but were not affected by a 1·2 per cent. solution. It was apparent throughout the project that the younger the chicks the more susceptible they were to soda injury. (5) Kidneys from chicks affected by feeding soda became pale, swollen and engorged with urates. The kidney tubules showed degenerative and exudative changes indicating severe injury. (6) Chicks affected by feeding soda showed an increase in kidney weight, and an increase of approximately four times in uric acid per gram of kidney and in uric acid in the blood". He found the injury caused by feeding sodium bicarbonate to chicks similar to the pathology and blood chemistry changes present in visceral gout. Two weeks old chicks consumed from 45 to 113 c.c. of "normal water" daily.

Carbonates, bicarbonates and caustic soda tend to cause alkalinity of the gastrointestinal juices and of the blood and tissue fluids (change in pH), consequently the continued drinking of alkaline waters and the constant taking of alkaline waters and the constant taking of alkaline powders will lead to serious digestive and other disturbances. Carbonates are more harmful than bicarbonates.

Cope (1936) draws attention to the fact that the constant use of alkaline stomach powders by individuals suffering from chronic gastric hyperacidity may have serious consequences. These powders generally contain calcium carbonate, magnesium carbonate, bismuth oxycarbonate, and sodium bicarbonate. He states that the following symptoms may arise in the course of time when alkaline powders are taken over long periods: Loss of appetite; slight vomiting; irritability; thoughtlessness; unreasonableness; depression; vague headaches and muscle pains; red and inflamed conjunctiva (sore eyes); "vision may be slightly blurred, but is as a rule not interfered with"; in some cases the patient himself complains about increasing impairment of mental efficiency; the skeletal muscles are sometimes abnormally excitable to direct percussion; polyuria is usual, but sometimes the urine volume is reduced below normal; the urine is mostly alkaline and always contains albumin; finally there may be coma. "The rapidity with which these successive stages are passed through varies considerably"—from a few days to weeks. Cope states that "very characteristic of all conditions of this type in which the body contains excess alkali is the extremely low concentration of chloride in the urine. This may be very simply demonstrated by the addition of nitric acid and a small quantity of silver nitrate solution." According to Cope the following points are of importance in the diagnosis of alkali poisoning: (1) The symptoms. (2) The presence of albumen in the urine. (3) Low concentration of chloride in the urine. (4) "An alkaline reaction of the urine with effervescence on the addition of acid is of no diagnostic value as it is frequently found in persons taking considerable quantities of alkali in the complete absence of all toxic symptoms." (5) Blood analysis: (*a*) "Estimation of plasma carbonate or alkali reserve is the most certain of interpretation"—a rise above 80 volumes per cent. is practically diagnostic of alkali poisoning. (*b*) "It would seem to be a sate general rule, however, to regard all suspected cases showing a rise in blood urea of about 80 mg. per cent. as cases of alkali poisoning, unless an independent cause for the high value can be definitely found." As treatment he recommends: (1) Removal of the alkaline powders. Alkali sensitive individuals should take di-sodium phosphate or perhaps hydrated magnesium silicate. (2) Administration of sodium chloride *per rectum* facilitates the excretion of bicarbonate. (3) Ammonium chloride can be given as an acidifying agent, but it must be used with care as in all cases of alkali poisoning the kidneys are severely damaged and acidosis may result. It is essential that the urine be examined at frequent intervals.

In experiments upon themselves Joos and Mecke (1934) found that after the administration of sodium bicarbonate there is (1) a marked increase in the sodium content of the blood serum, (2) a

slow fall in the magnesium content, (3) a pronounced and sudden fall in the calcium content, (4) a quick fall in the chloride content, and (5) a slow fall in the phosphorus content of the blood serum.

Little (1937) describes a simple method of estimating the alkaline constituents of washing powders and washing solutions containing mixed alkalies.

The taking of too much alkaline stomach powders has resulted in a serious state of alkalosis (Davies, 1939).

II. Caustic Alkalis (Caustic Soda and Caustic Potash).

A. *Onderstepoort Experiments.*

Some time ago a specimen consisting of a fairly dark-brown, semi-coagulated, jelly-like fluid was submitted by the Government Veterinary Officer, Greytown, Natal, to Onderstepoort for analysis. It was stated that it was a specimen of milk which had turned this colour when placed on the stove to boil. The specimen was submitted in an air-tight fruit jar and was about eight days old when examined. It emitted a very pronounced alkaline odour, was slimy and jellylike, and yielded a marked alkaline reaction with litmus paper. It was impossible to ascertain the degree of alkalinity by means of titration as the filtrate was dark-brown in colour and could not be decolorised with charcoal. It was then decided to determine the sodium content of the specimen. This was found to be far in excess of the sodium content of normal milk.

The following rabbits were drenched by means of a stomach tube with the above specimen:—

Rabbit A (2·0 *Kg.*): 100 *c.c. on* 7/7/38.

Within five minutes after drenching pronounced laboured breathing set in, the animal breathing through its mouth. General weakness (paresis) followed soon and the animal was unable to sit up or hold its head up. Muscular twitchings were seen on different parts of the body. The heart-action was very much accelerated and weak in the beginning and became progressively weaker until it was imperceptible a short while before death. The animal died within ten minutes after the development of symptoms. A few minutes before death occurred the animal became completely paralysed, was comatose and lay prostrate with the head drawn backwards. It died with terrific convulsions (jumping about in the cage) of the whole body. These were probably due to asphyxia.

Post mortem appearances: General cyanosis; pronounced hyperaemia of the lungs, liver and kidneys; pronounced dilatation of both heart ventricles; pronounced hyperaemia and sliminess of the gastric mucosa; contents of small intestine very slimy.

Rabbit B (2·0 *Kg.*): 50 *c.c. on* 7/7/38.

Symptoms very similar to those described above set in within ten minutes after drenching. The only difference in the

sympto logy was that there were no muscular twitchings and that the animal died quietly in a state of paralysis and coma. Death occurred approximately twenty minutes after drenching.

Post mortem appearances: Very similar to those described in rabbit A with haemorrhages in the gastric mucosa.

The following three experiments (specimens 1, 2 and 3) were then conducted:—

Specimen (1):

Four grams of granular caustic soda were added to 200 c.c. of fresh cow's milk. A light yellow colour appeared almost immediately. It soon became more intensely yellow and then developed a brownish colour as the temperature of the milk was raised by means of a gas-flame. As the temperature of the milk rose it became dark yellow-brown and when it was boiling it turned a dark reddish-brown colour. Between 50° and 70° the milk jellified and became fluid again before it boiled. It emitted a pronounced alkaline odour.

Specimen (2):

Twenty grams of granular caustic soda were added to 200 c.c. of fresh cow's milk and boiled for two minutes. The milk showed the same changes as those described under (1); the changes in colour took place more rapidly and the colours were more intense. The cooled specimen was then placed in an air-tight fruit jar and was drenched to two rabbits seven weeks later.

Rabbit A (1·9 *Kg.*): 50 *c.c. on* 14/9/38.

The animal developed dyspnoea and apathy within a few minutes after drenching. After having shown loss of appetite for a day it appeared normal again.

Rabbit B (1·95 *Kg.*): 100 *c.c. on* 14/9/38.

Symptoms more pronounced than those described in Rabbit A set in within five minutes after drenching. The animal appeared normal after twenty-four hours.

Specimen (3):

Forty grams of caustic soda were added to 200 c.c. of fresh cow's milk and boiled for two minutes. The results of the addition of caustic soda and boiling were similar but more pronounced than those described under (2). The appearance, colour and odour of this specimen of milk were identical with those of the specimen submitted by the Government Veterinary Officer, Greytown. Two rabbits were drenched with specimen (3) after it had been kept for seven weeks in an air-tight fruit jar.

Rabbit C (1·25 *Kg.*): 50 *c.c. on* 14/9/38.

The only symptoms the animal showed were transitory laboured respiration and loss of appetite.

Rabbit D (1·65 *Kg.*): 100 *c.c. on* 14/9/38.

Laboured respiration set in within a few minutes after drenching. There were violent intestinal movements within thirty minutes. After having shown pronounced apathy, loss of appetite, accelerated heart-action and after having passed dark brown urine for two days the animal appeared normal again.

The slight toxicity of specimens (2) and (3) was thought to be due to the fact that they had stood too long (seven weeks) before being drenched. A further three specimens (4, 5 and 6) of milk were then treated with caustic soda; boiled for two minutes and left standing for eight days in air-tight fruit jars. The changes in the milk were the same as those noted before. On cooling the specimens jellified.

Specimen (4):

Twenty grams of granular caustic soda were added to 200 c.c. of fresh cow's milk. After standing for eight days the specimen was dark brown in colour and had a jellylike appearance and was slimy. It emitted a pronounced alkaline odour and smelt very strongly of ammonia. It was drenched to two rabbits:—

Rabbit E (0·9 *Kg.*): 50 *gm. in a small quantity of water on* 23/9/38.

The animal retched and vomited within a few minutes after drenching, and died within a further two minutes.

Post mortem appearances: Most pronounced hyperaemia of gastric mucosa with partial dissolution (slimy appearance of the mucosa; similar changes in the small intestine; pronounced congestion of the liver; a fair quantity of vomitus in the lungs.

Rabbit F (1·7 *Kg.*): 100 *c.c. with a small quantity of water on* 23/9/38.

Symptoms identical with those described in Rabbit A, which was drenched with the specimen submitted by the Government Veterinary Officer, Greytown, developed within three minutes after drenching. After a further ten minutes the animal retched, vomited and died soon afterwards.

Post mortem appearances: Gastric wall dark reddish-brown in colour and almost completely dissolved (very slimy); mucosa of small intestine dark reddish-brown and slimy (partial dissolution); pronounced hyperaemia of the liver; no vomitus in lungs.

Specimen (5):

Forty grams of caustic soda were added to 200 c.c. of fresh cow's milk. After eight days appearance and odour of this specimen were the same as those of specimen (4). The colour was dark yellowish-brown. The following rabbits were drenched with it:—

Rabbit G (1·25 *Kg.*): 50 *c.c. with a small quantity of water on* 23/9/38.

The symptoms resembled those described in Rabbit F. The animal vomited and died within ten minutes after dosage.

Post mortem appearances: As in Rabbit F, only more pronounced. No vomitus in lungs.

Rabbit H. (1·65 *Kg.*): 100 *c.c. with a small quantity of water on* 23/9/38.

Symptoms as described in Rabbit F appeared within a few minutes and death occurred within ten minutes after drenching. *Post mortem appearances:* As in Rabbit G. The gastric wall was almost completely dissolved and showed a rupture.

Specimen 6:

Sixty grams of caustic soda were added to 200 c.c. of fresh cow's milk. After eight days the colour, appearance and odour (alkaline and ammonia) were identical with those described under specimen (5).

This specimen was not dosed to rabbits as specimens (4) and (5) had yielded positive results.

In considering the toxicity of milk to which caustic alkalis were added it should be realised that there are two different kinds of poisons present, namely, the caustic alkali and decomposition products caused by the action of the alkali on some of the constituents of the milk, especially the protein. That the alkali causes pronounced destruction of the protein in the milk is evident from the pungent ammonia odour which is emitted after a few days. Toxic amines are probably also formed. It appears that the toxicity of caustic alkali milk decreases in the course of time. The ammonia odour which was not pungent after eight days decreased and eventually disappeared.

On an earlier occasion three groups of four rabbits each were drenched with 1·0 (Group I), 2·0 (Group II) and 5 (Group III) per cent. aqueous solutions of caustic soda respectively. The two rabbits (Group I) which received 40 c.c. of a 1·0 per cent. solution developed no symptoms beyond transient loss of appetite. Those two animals of Group I which were drenched with 80 c.c. died within forty hours. In Group II two rabbits received 40 c.c. and the remaining two 80 c.c. of a 2·0 per cent. solution of caustic soda. They died from within twenty to thirty hours after drenching. The two rabbits in Group III which received 40 c.c. of a 5·0 per cent. solution of caustic soda developed symptoms within a few minutes.

One of these rabbits vomited and died within one-and-a-half hours after drenching, whilst the other one lived for approximately thirty hours. The remaining two rabbits of Group III which received 80 c.c. of a 5·0 per cent. caustic soda solution died within three hours.

The symptoms and post mortem appearances closely resembled those described below under II (B). Even with 80 c.c. of a 1·0 per cent. solution of caustic soda there was pronounced dissolution (corrosion) of the gastric mucosa and wall. In one of these cases the gastric wall was almost perforated in two places. The remaining portion of the internal surface of the stomach wall was very slimy and intensely dark (dirty) reddish-brown (alkali haematin). With higher concentrations of caustic soda the lesions were much more pronounced.

B. *Mode of Action, Toxicity and Symptoms of Caustic Alkalis.*

Caustic alkalis dissolve the epithelium and form soluble alkali albuminates when coming into contact with proteins (skin and mucous membranes). The mucous membrane and skin becomes oedematous, transparent, and soapy, and the corroded area becomes necrotic, soft and slimy. If blood vessels have been corroded by the alkali the affected surface may bleed and alkali haematin is formed. In such cases the colour of the affected area may be reddish-brown, black brown (rare) or often bright red (excessive haemorrhage). In the course of time the colour changes to greyish-white—in dilute solutions within 1-2 hours and in concentrated solutions after longer periods.

Acute alkali poisoning facilitates the deposition of calcium in the bone system, hence the blood and tissues are deficient in this element (Rentz, 1933; Renvis, 1935).

Histologic changes in the parenchymatous organs in cases of alkali poisoning have been described by Jankovich, Incze and Fazekas (Fazekas, 1937), but unfortunately the publication is not obtainable in South Africa. Fazekas (1937) drenched different groups of rabbits with 1, 2, 3, 5 and 10 per cent. solutions of caustic soda, the animals receiving 0·5, 0·75, 1·25, 1·5, 2·25 and 2·5 gm. NaOH after one day starvation. The following changes were noted in the blood chemistry of the animals:—(1) A disturbance of the glucose metabolism in the form of hyperglycaemia. (2) A pronounced increase in the inorganic phosphorus content of the total blood. (3) A decrease in the serum calcium. (4) A moderate decrease in serum chlorine. (5) A slight increase in serum sodium. (6) Definite acidosis in the form of a decrease in serum alkali reserve.

The degree of corrosion caused by caustic alkali depends upon: (1) Their concentration. (2) The quantity swallowed. (3) Length of period of action. (4) Quantity of food present in the stomach and intestine. (5) The resistance of the tissues concerned. Solutions of caustic alkalis under 1·0 per cent. do not as a rule cause fatal corrosion but mostly only inflammation of the mucous membranes. More concentrated solutions and especially solid pieces of alkali

frequently cause severe corrosion and even perforation of mucous membranes and of the stomach wall. When solutions of caustic alkali are thrown in the face it may be aspirated and fatal pneumonia may result.

Balasz (1934) states that 2,134 cases of alkali poisoning were treated in the Budapest hospital from 1924-1933. Most of these cases were suicidal. He gives a detailed description of the symptoms in alkali poisoning.

The following symptoms are seen in cases where solutions of caustic alkalis of 10 per cent. and higher concentrations are swallowed:—The lips are swollen in almost all cases of alkali poisoning. This is differential-diagnostically of great value in comparison with acid poisoning. Very severe pain in all the organs with which the solution comes into contact. Pain is especially experienced in the sternal region and in the back due to oesophagitis and perioesophagitis. On the first day there are stomach pains in the cardiac and epigastric regions. There are hiccoughs and vomiting. The vomitus may be mucoid, slippery and reddish-brown or almost black with alkali haematin or it may be bright red when excessve bleeding occurs. When necrotic material is present it has a very bad odour. There is difficulty in swallowing which may be due to pain, spasms, an acute, transitory, reflex paralysis, or paralysis due to direct alkali action on the soft palate or oesophageal musculature, swelling of the oesophageal mucous membrane, mechanical blocking of the oesophagus by necrotic mucous membranes, food, or due to fibrosis of the muscles of deglutition. The respiration is accelerated and in bad cases the pulse is slow and small (shock) in the beginning, but eventually it becomes accelerated in most cases. Prognostically a fast pulse is unfavourable as it indicates complications which are described below. The blood pressure is in most cases low. The reflexes are generally increased. The pupils are dilated in bad cases. Patients are mostly quite conscious and convulsions occur very rarely. The skin is mostly pale but in laryngeal lesions it is cyanotic. In the course of time the face turns red when fever appears as a result of complications. In very serious cases death occurs in a few hours with a subnormal temperature. Diarrhoea and bloody faeces are rare and only occurs when there is severe corrosion of the duodenum and jejunum. The urine need not necessarily be alkaline and is sometimes acid in serious fatal cases. An alkaline reaction of the urine often sets in a few hours after poisoning but is transitory. There is protein in the urine in serious cases and often also acetone due to inanition or to decomposition of protein.

The direct causes of death in alkali poisoning may be shock, collapse, glottis oedema, pneumonia, exhaustion, perforation, haemorrhage, mediastinitis, pericarditis and (or) peritonitis without perforation. The maximum number of fatalities fall within the first three days and on the eighth to tenth days according to Balász (1934). The shortest period within which death occurred from alkali poisoning was two hours.

The following complications may follow the swallowing of caustic alkalis:—(1) Stenosis of the oesophagus, pylorus and small intestine. (2) Leschke (1932) states that cancer of the stomach may develop in some cases. (3) Inflammatory and purulent phlegmones of the oesophagus and the stomach which may pass over on to the adjoining organs resulting in pleuritis, pericarditis and peritonitis. (4) Extensive internal haemorrhage due to corrosion of large blood vessels. This is rare in comparison with extensive bleeding in cases of parenchymatous haemorrhage. (5) Abortion is rare. (6) Perforation of the oesophagus, stomach wall and (or) intestinal wall resulting in phlegmones, perioesophagitis, pneumonia, pericarditis, pleuritis and peritonitis. (7) Pronounced glottis oedema necessitating tracheotomy. (8) Toxic effects on the heart due to absorption of decomposition products from necrotic tissues. (9) Shock and collapse. (Fröhner, 1919; Länder, 1926; Lewin, 1929; Van Itallie and Bylsma, 1930; Glaister, 1931; Leschke, 1932 and 1934; Rentz, 1933; Fazekas, 1934 and 1937; Balasz, 1934; Schranz, 1934; Smith and Cook, 1934; Renvis, 1935.)

The following table is compiled from information supplied in Balász's (1934) publication and is of great value in differentiating between acid and alkali poisoning:—

Acids.	*Caustic Alkalis.*
1. Coagulate protein of tissues and partly prevent further corrosion.	1. Dissolve tissue protein and enhances further corrosion.
2. Crust is hard.	2. Lesion is soft, transparent and slimy (moist). If corrosion is severe the area may be reddish-brown or bright-red.
3. Very little or no oedema.	3. Pronounced, jellylike oedema.
4. Lips rarely swollen.	4. Lips almost always swollen.
	5. Infections are more frequent in alkali poisoning as the lesions are more favourable for bacterial growth. Hence perioesophagitis and mediastinitis with perforation are more frequent in alkali than in acid poisoning.

The symptoms of alkali poisoning in animals are similar to those described in human beings.

C. *Post Mortem Appearances.*

These depend upon the concentration and quantity of alkali swallowed and are evident from the description of the symptoms.

D. *Detection of Caustic Soda.*

Reaction of specimen is strongly alkaline. Vomit is the best specimen for analysis, and also stomach contents in cases where no chemical antidotes were administered. The specimen, if fluid, should be concentrated by evaporation and then extracted with absolute alcohol and filtered. The filtrate is then evaporated to dryness and tested for the alkali suspected. The dialysis method could also be used. In protracted cases it is difficult and often impossible to detect the alkali which caused the poisoning.

E. *Treatment of Alkali Poisoning.*

Tracheotomy should be performed in cases of glottis oedema. In less severe cases stomach lavage could be executed with small quantities (200 c.c.) of weak acid solutions (diluted vinegar, acetic acid). Milk and eggs should be administered and Belladonna (atropine) to inhibit gastric and intestinal secretion and motility. It is of importance to treat the weak heart. Morphine injections should be given to allay pain, excitement, incessant vomiting and sleeplessness. Infusions are essential in order to replace the loss of fluid. Serious cases of stenosis of the oesophagus, pylorus and small intestine should be operated upon.

III. Summary.

1. Poisoning with weak and caustic alkalis is reviewed and discussed.
2. Analysis of a specimen of milk maliciously poisoned with caustic soda is described.
3. An account is given of (*a*) experiments in which varying quantities of caustic soda were added to fresh cow's milk and also of experiments conducted upon rabbits with this milk. (*b*) The effects of different concentrations of caustic soda on rabbits.
4. The continuous taking of alkaline stomach powders may have serious consequences.

IV. Acknowledgments.

My thanks are due to Dr. J. Louw of the Section of Biochemistry for determining the quantity of sodium in the suspected specimen of milk. To my assistants, Mr. J. G. Malherbe, M.Sc., and Mr. M. G. van Niekerk, I am indebted for assistance rendered in the course of the experiments.

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CHEMICAL FOOD POISONING

AN UNUSUAL SERIES OF EPIDEMICS PRESUMABLY DUE TO SABOTAGE

By

RAY E. TRUSSELL,[1] C. E. STEWART[2] AND ROBERT F. KORNS[1]

(Received for publication September 10th, 1949)

This report describes 3 epidemics of acute gastroenteritis each involving from 400 to 600 inmates of a prison. The clinical and epidemiological patterns in all three were remarkably similar even to the extent of developing at the same time of day on the same day of the week on each occasion. The causative factor and method of transmission, as determined by epidemiological investigation, are sufficiently unusual to justify description.[3]

Clinical manifestations and laboratory tests

The clinical manifestations were the same in all 3 outbreaks. Some men complained of gradually increasing abdominal discomfort, followed by diarrhea; others were taken abruptly ill with severe abdominal cramps and diarrhea. Nausea and vomiting occurred in a small proportion of the patients, but were not a prominent feature of the illness. Temperatures were normal or subnormal. Leucocytes were within normal limits. Blood and mucus were observed in the stools of a few of the severely ill patients. No estimate can be made of the percentage of patients passing blood, since the outbreaks all occurred at night and each inmate had a toilet in his cell. Although the severely ill men were prostrated, the duration of illness was a matter of a few hours and fewer than 5 men were hospitalized among the approximately 1,600 who were sick. However, malaise lasting 1 or 2 days was not uncommon. About 50 per cent of the known illnesses came to the attention of the prison hospital staff for treatment. The other ill men were discovered by interviews during epidemiological survey and in these the attacks had been either so mild as not to require treatment, or they had recovered sufficiently by morning so that the attacks were not reported.

None of the commonly recognized enteric pathogens was isolated from repeated stool cultures submitted from each of the 3 outbreaks. Two stools examined by direct smear contained, in addition to the usual findings, many polymorphonuclear leucocytes per oil immersion field.

Epidemiological investigations

1. *Topography and population groups.* The State Prison and State Hospital are two separately operated institutions adjacent to each other in a village in northern New York State. The prison and hospital are built on a hillside with the business and residential districts of the village in close proximity and in general at a lower level (see figure 1).

The populations under study were estimated as follows:

[1] New York State Department of Health and Albany Medical College, Albany, N. Y.

[2] Dalhousie University, Halifax, Canada.

[3] The assistance of the many members of the New York State Department of Health and the staff of the prison concerned herein without whose cooperation this investigation could have been impossible, is gratefully acknowledged.

Village	2,000
State Hospital inmates	1,100
Prison employees	330
Prison inmates (1946)	1,565
(1947)	1,719

2. *Onset of illness.* The 3 epidemics each occurred on Sunday evenings, the first two on successive week ends, the third approximately 10 months later. The approximate hour of onset as given by each ill convict interviewed is listed in table 1. The median time of onset did not differ appreciably in the 3 outbreaks and ranged from 12 to 14 hours after the preceding noon meal.

Table 2 illustrates the similarity of time distribution of onsets in each cell block in the first two epidemics. No striking differences are apparent.

3. *Attack rates.* The numbers of inmates reporting as ill to the prison hospital in the 3 outbreaks, respectively, were 356, 310 and 204. However, systematic surveys of approximately 100 men or more in each cell block revealed many additional cases.[1] These interviews were usually made after the evening meal to avoid conflict with prison routine during the day. Men in consecutive cells along each corridor were interviewed. As the evening wore on many were found asleep and some were passed by until it was realized that this was a source of error, because men who had lost sleep due to illness were more likely to be asleep. Hence it was necessary to interview all in a given group.

The numbers and percentages of men reporting ill from each cell block are given in table 3. On the basis of these proportions it was possible to estimate the total numbers of men ill in the prison population in the 3 epidemics, respectively, as 560, 595, and 430.

Other population groups investigated included the prison guards and other paid employees, the inmates and employed personnel in the adjacent state

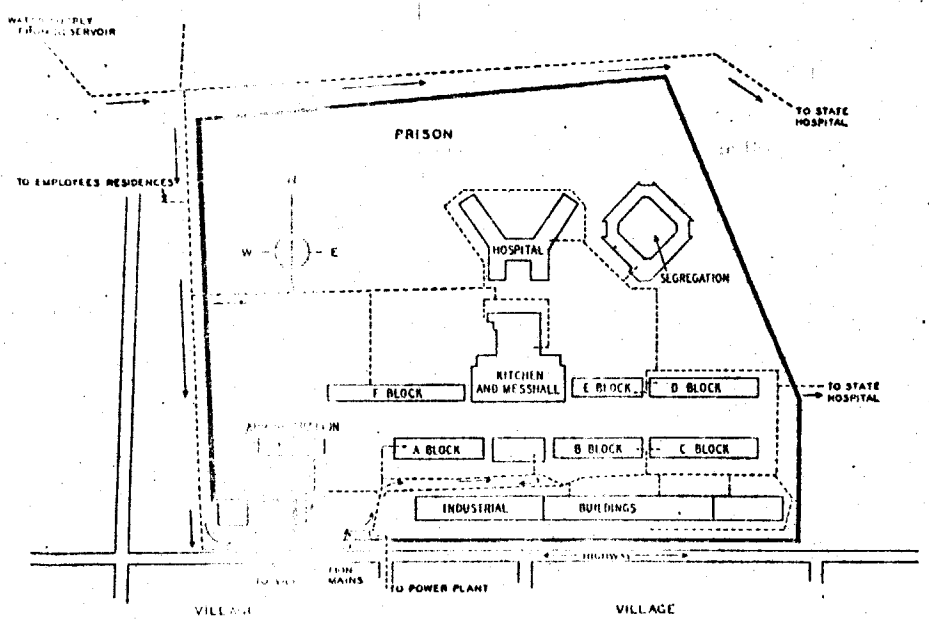

FIGURE 1. Arrangement of State Prison buildings. Dotted lines indicate the location of the main water distribution system within the prison.

TABLE 1

Estimated times of onset of illness obtained during cell-to-cell interviews from men who had been ill in each of the 3 epidemics

Estimated time of onset (to the nearest hour)	Number of men becoming ill on Sunday and Monday		
	July 28–29, 1946	August 4–5, 1946	[illegible] 26, [illegible]
Sunday:			
1:00 P.M.	0	0	1
2:00	0	2	1
3:00	1	3	0
4:00	3	2	2
5:00	2	5	6
6:00	3	8	2
7:00	5	11	1
8:00	6	9	12
9:00	4	15	13
10:00	15	32	17
11:00	17	16	22
12:00	21	23	27
Monday:			
1:00 A.M.	15	20	26
2:00	41	27	25
3:00	38	18	27
4:00	16	22	14
5:00	15	13	12
6:00	26	9	5
7:00	11	11	4
8:00	11	10	3
9:00	5	6	0
10:00	1	0	1
11:00	3	1	0
12:00	3	1	0
1:00 P.M.	2	0	0
2:00	2	0	0
3:00	2	3	0
Totals	271	267	224
Approximate median time of onset	2:00 A.M.	1:30 A.M.	12:30 A.M.

hospital, and the residents of the village logically divided into 4 groups on the basis of water supply, as discussed below. In summary of detailed data to be presented later, it can be said that there was no evidence that any one of these groups was involved in the 3 outbreaks. Moreover, reference to table 3 shows that the convicts living in the lumber camp outside the prison were not ill. Thus, the outbreaks were limited to individuals within the prison. The large numbers of men ill and the explosive and constant character of each outbreak indicate a common-source etiologic agent, and the selection for convicts to the exclusion of guards and other employees totaling approximately 330, points to a vehicle restricted to the use of the inmates. In retrospect, it is easy to point out that this could only have been food, but the epidemiological investigation of the first outbreak was badly confused by a coincidental massive disturbance in the prison water supply, which preceded the Sunday noon meal by about 2 hours. It was necessary, therefore, to spend some time studying the water supplies to the institutions and the village.

4. *Water supplies.* a. Prison. The dotted lines in figure 1 indicate the location of the major water distribution system within the prison. This water is pumped from a lake several miles from the prison and is chlorinated before reaching the distributing mains. The system not only supplies the prison, but also supplies the State Hospital and is connected with the water main system supplying a major portion of the village for emergency aid in case of drought or fire. It is believed that the bulk of the water supplied to the prison enters through the front (south) wall main, and that a neutral point of pressure between this and the water entering through the west wall main is established somewhere along the east side of the circuit, although constantly in fluctuation due to variation in demand throughout the day.

[illegible] inadequate water in the village

TABLE 2

Occurrence of illness in terms of time and place as determined by interview following the first two epidemics

Estimated time of onset	Number of men becoming ill on Sunday and Monday													
	July 28–29, 1946							August 4–5, 1946						
	Cell block							Cell block						
	A	B	C	D	E	F	Hospital	A	B	C	D	E	F	Hospital
Sunday:														
2:00 P.M.	—	—	—	—	—	—	—	1	—	1	—	—	—	—
3:00	—	1	—	—	—	—	—	—	1	1	—	1	—	—
4:00	3	—	—	—	—	—	—	1	—	—	—	—	—	1
5:00	—	—	—	—	1	1	—	—	—	—	—	2	1	2
6:00	2	—	—	—	—	1	—	2	—	1	—	3	2	—
7:00	2	—	—	1	—	1	1	2	1	2	—	3	1	2
8:00	2	1	2	—	—	—	1	—	2	1	1	3	—	2
9:00	1	—	—	1	1	—	1	1	—	1	—	9	2	2
10:00	—	3	1	1	8	2	—	1	2	5	2	15	—	7
11:00	3	1	3	3	3	4	—	1	1	4	1	3	3	3
12:00	3	3	3	1	8	6	—	6	7	3	1	1	2	3
Monday:														
1:00 A.M.	3	3	1	3	2	2	1	6	2	3	3	3	2	1
2:00	9	7	7	4	8	5	1	2	3	6	3	4	3	6
3:00	9	9	6	4	6	4	—	3	3	1	7	—	1	3
4:00	5	1	3	2	4	1	—	1	3	1	6	1	1	6
5:00	1	1	3	4	2	4	—	2	4	2	2	—	1	2
6:00	1	3	1	11	5	5	—	—	2	4	1	—	2	—
7:00	1	1	1	5	2	1	—	—	3	5	1	1	1	—
8:00	1	2	1	—	5	2	—	—	2	1	—	3	2	2
9:00	—	1	1	—	3	—	—	—	3	—	—	1	1	1
10:00	—	—	1	—	—	—	—	—	—	—	—	—	—	—
11:00	1	—	1	—	—	—	1	—	—	—	—	—	1	—
12:00	—	—	1	—	1	1	—	—	—	—	—	—	1	—
1:00 P.M.	1	—	1	—	—	—	—	—	—	—	—	—	—	—
2:00	—	—	1	1	—	—	—	—	—	—	—	—	—	—
3:00	—	1	—	—	1	—	—	—	1	—	1	—	1	—
Totals	48	38	38	41	60	40	6	29	40	42	29	53	31	43
Per cent sick (approx.)	39	41	38	42	74	27	8	30	40	42	30	67	31	52
Approximate median time of onset	1:30 A.M.	2:00 A.M.	2:00 A.M.	4:00 A.M.	2:00 A.M.	2:00 A.M.	11:00 P.M.	12:00 P.M.	2:00 A.M.	1:00 A.M.	2:30 A.M.	9:30 P.M.	2:00 A.M.	12:00 P.M.

TABLE 3

Incidence of illness as determined by cell-to-cell interviews after each epidemic

Location	July 28-29, 1946				August 4-5, 1946				May 25-26, 1947			
	Population	No. interviewed	No. interviewed who were ill	Per cent	Population	No. interviewed	No. interviewed who were ill	Per cent	Population	No. interviewed	No. interviewed who were ill	Per cent
A block	185	123	43	35	[illegible]	100	30	30	180	100	20	20
B block	182	100	41	41	[illegible]	100	40	40	183	100	25	25
C block	215	100	38	38	215	100	42	42	239	100	12	12
D block	235	100	42	42	[illegible]	100	30	30	226	100	10	10
E block	115	84	62	74	115	84	56	67	143	100	36	36
F block	445	152	42	27	445	100	31	31	454	100	39	39
Segregation block	33		Known* 16	[illegible]	35		Known* 23	70	27		Known* 17	63
Hospital	135	73	6	[illegible]	[illegible]	81	44	52	152	152	32	21
Lumber camp	18	9	0	0	16	111	0	0	16	16	0	0
Dormitory	Not in use				Not in use				99		Known* 19	19

* Reported by guards and hospital personnel.

reservoir as a result of dry weather. The village reservoir supply main was shut off on Sunday, July 28th, about [illegible] A.M. and the prison main connecting with the village system was opened wide at a rapid rate. This created temporary major disturbances in water pressures in both the prison and village mains which were manifested by the appearance of very dirty water in the lower (southern) groups of cell blocks, in the entire prison hot water supply (which initially was cold water drawn from the area of maximum disturbance), and in the houses in the village drawing water from the same supply.

b. Village. House-to-house surveys in the village were made in areas supplied by spring water, by the village reservoir (and, thus, temporarily by the prison supply) and by the prison mains directly (employees' homes). Table 4 indicates the incidence of gastroenteric complaints obtained on July 28th and 29th, 1946, from 428 village residents. There was no evidence of any increase in gastroenteritis above that usually observed in such surveys. In addition to this lack of statistical evidence for involvement of the village residents in the outbreak is the fact that the prison physician, who was also the only practicing physician in the area, was not called to treat any cases of diarrhea in the village.

In view of the inability to incriminate

TABLE 4

Incidence of illness among village residents according to source of drinking water as determined by house-to-house survey

Water supply	Number interviewed	Gastroenteritis, July 28-29
Spring supplied water mains	92	1
Town reservoir (prison supply temporarily)		
a. Using spring water or boiled water	172	4
b. Using water from mains	118	6
Employee residences supplied from prison mains before system enters prison	46	0
Totals	428	11

other common-source vehicles in the first outbreak as discussed below, an engineering opinion was offered that the excessive mineral and organic content stirred up in the prison mains might have acted as a cathartic. It was considered possible from an engineering point of view, because of the dual water supply entering the prison and the fact that the small prison hospital,[4] where the inmates essentially were spared in the outbreak, was at a higher elevation than the cell blocks, that the "polluted" water might not have reached the hospital. It was also suggested that the hydraulic disturbance could have been counterbalanced in time to limit the major portion of the "pollution" to the prison system, thus sparing the townspeople. Against this hypothesis were the following:

a. Inmates in E block, where the highest attack rate occurred, did not notice discoloration of the drinking water.

[4] This is the hospital within the prison walls. The large State Hospital for Insane Criminals adjacent to the prison was not involved in any of the epidemics.

b. The attack rate was 27 per cent in F block, which was more remotely removed from the area of water disturbance than the hospital in which the attack rate was 8 per cent (6 patients with very mild symptoms).

c. The approximately 60 prison guards were spared in the outbreak. Most of the other employed personnel were off duty on Sunday and presumably not exposed. The guards did not eat food prepared in the prison, but did drink water. However, it was pointed out that it was the custom of many guards to prepare buckets and pitchers of iced water early in the morning for use through the day, and that they might have been spared because of this habit.

Laboratory studies of the many water specimens collected within the prison and throughout the village were essentially negative with two exceptions. The mess hall water supply was found to be contaminated, the poorest specimen showing a total bacterial count of 44,000 per ml and a most probable number of coliform organisms as 8.8. A spring-fed drinking faucet in the prison yard was delivering water with a total count of 35,000 and a most probable number of 38 coliform organisms. Both of these observations were regarded as incidental, however, because E block inmates did not have access to either point of distribution since, for disciplinary reasons, they were in continuous confinement in the cell block. The water specimens collected in E block the day after the outbreak were bacteriologically satisfactory and chemical analyses revealed nothing unusual.

Technically, by opening 3 valves at the right moment, back-siphonage of dyes from the dye vats of the industrial plant could have occurred. In view of the fact that the plant was closed on

Saturdays and Sundays and chemical analyses of the water were not suggestive, this possibility seemed remote.

There were no disturbances in water supply during the second and third outbreaks, and laboratory studies of specimens collected at these times did not incriminate water as a causative factor. Both village and state hospital inmates were spared again. The first group was still consuming prison water during the second epidemic. The state hospital derived its water from the same supply during the entire series.

5. *Milk.* The prison maintained a dairy herd large enough to supply the needs of the mess hall and prison hospital and operated a pasteurizing plant within the main kitchen. The pasteurizing plant also froze ice cream mix prepared in large volume in the main kitchen.

Gross bacterial contamination or introduction of chemical into the milk supply would ultimately reach a large number of convicts; (*a*) on cereals served at breakfast, (*b*) in coffee, (*c*) through extra rations issued to hospital patients and the painting crew and (*d*) in ice cream.

The dairy herd was examined by the state veterinarian, who found among 69 animals, 7 with questionable evidence of mastitis. The milk samples showed no unusual total counts or microscopic findings. The pasteurizing plant procedures and records were thoroughly reviewed, and with the exception of two minor deficiencies in cooling methods, were considered satisfactory.

The distribution of milk followed a constant pattern and was such that the milk consumed in the prison hospital at breakfast each morning by all hospital patients was a day older than that served to the rest of the prison inmates. Tuberculous patients and about one-fourth of the other patients received extra milk rations in the morning and afternoon, and this milk was from the same lot as that served to the rest of the prison population for breakfast.

In view of this pattern of milk distribution, it is unlikely that liquid milk was the common source vehicle because: (*a*) among the 72 tuberculous patients, there was a 42 per cent attack rate during the second outbreak which paralleled the incidence for the rest of the population, in spite of the increased consumption of milk. In the first epidemic the incidence was extremely low in all hospital patients including those with tuberculosis. (*b*) There were 16 hospital patients ill in the second outbreak who did not receive the extra milk ration. Although the milk which they drank at breakfast was a day older than that served to the other inmates, the onsets of illness in these 16 men occurred during the same time period as those in the rest of the prison population. (*c*) The eleven painters who received an extra quart of milk a day were not ill during either of the first two outbreaks.

6. *Other foods.* In favor of a vehicle consisting of one or more foods prepared in the main mess hall are the following:

a. The prison guards who brought their lunch to work were not ill.

b. The lumber camp personnel who drew supplies from the prison stores but ate a partially different menu than the men within the prison were not ill.

c. The highest attack rates occurred in E block men continuously confined to their cells and eating essentially only what was brought to them from the mess hall. Kitchen personnel also had high attack rates. Other inmates with "yard" privileges commonly prepared meals in the yard from foods purchased or sent as gifts from outside the prison. This would tend to reduce the amount of food which was consumed in the mess halls and the lower attack rates did occur in these groups.

TABLE 5

Extract of food-eating histories from first outbreak

Food	Eating history	Totals	Ill	Not ill	Attack rate
Hamburger (Saturday noon)	Eaten	276	155	121	56.2
	Not eaten	23	6	17	26.1
Milk (Sunday morning)	Eaten	278	156	122	56.1
	Not eaten	20	4	16	20.0
Pork (Sunday noon)	Eaten	277	155	122	55.9
	Not eaten	22	6	16	27.3
Ice cream (Sunday noon)	Eaten	275	152	123	55.3
	Not eaten	24	9	15	37.5

The significant food-eating histories obtained in the 3 outbreaks are extracted below, together with a commentary on each.

a. *First epidemic.* Review of the food-eating histories in the first outbreak, as shown in table 5, indicated that only 4 foods were eaten in the preceding 24 hours by 95 per cent or more of the men who were ill. No other foods were consumed by more than 70 per cent of those ill. The hamburger and ice cream were consumed by men working in the lumber camps. These men were not ill. Milk has been discussed above.

Bacteriological studies of the hamburger, bread, cake, potato salad, pork, ice cream, butterscotch pudding powder and condensed milk used in the 24 hours preceding the outbreak were noncon-

TABLE 6

Extract of food-eating histories from second epidemic

Food	Eating history	Totals	Ill	Not ill	Attack rate
Milk (Sunday morning)	Eaten	159	94	65	59.1
	Not eaten	8	6	2	75.0
Coffee (Sunday morning)	Eaten	150	92	58	61.3
	Not eaten	17	8	9	47.1
Beef (Sunday noon)	Eaten	153	87	56	63.4
	Not eaten	15	4	11	26.7
Bread (Sunday noon)	Eaten	165	100	65	60.6
	Not eaten	3	1	2	33.3
Ice cream (Sunday noon)	Eaten	161	97	64	60.2
	Not eaten	7	4	3	57.2

tributory. Other foods were not available for study.

b. *Second epidemic.* The food eating histories obtained after the second outbreak revealed 5 foods eaten by 90 per cent or more of the men who were ill, as shown in table 6.

Milk was ruled out on an epidemiologic basis for the reasons given above. Unused portions of ice cream and roast beef which had been prepared for the Sunday noon meal were available under refrigeration. Inmates and one investigator voluntarily offered to eat these foods 2 days after the epidemic. Fifty-eight were given ice cream, while 33 ate the beef. None of the 91 became ill. Included in this group were both those who had and who had not been ill during the epidemic.

TABLE 7

Extract of eating histories from third epidemic

Food	Eating history	Totals	Ill	Not ill	Attack rate
Sunday noon					
Beef	Eaten	357	217	140	60.8
	Not eaten	30	8	22	26.7
Potatoes	Eaten	321	192	129	59.8
	Not eaten	66	33	33	50.0
Turnip	Eaten	266	156	110	58.7
	Not eaten	121	69	52	57.0
Gravy	Eaten	303	182	121	60.1
	Not eaten	84	43	41	52.4
Ice cream	Eaten	323	177	146	54.8
	Not eaten	64	48	16	75.0
Bread	Eaten	352	204	148	57.9
	Not eaten	35	21	14	60.0
Coffee	Eaten	326	191	135	58.6
	Not eaten	61	34	27	55.7
Sunday evening					
Soup	Eaten	186	125	61	67.2
	Not eaten	201	100	101	49.8
Cake	Eaten	261	153	108	58.6
	Not eaten	126	72	54	57.2
Bread	Eaten	250	147	103	58.8
	Not eaten	137	78	59	56.9
Tea	Eaten	166	92	74	55.4
	Not eaten	221	133	88	60.2

In view of these negative human volunteer experiments and the subsequent failure of the bacteriological laboratory to incriminate sausage, beans, gravy, potatoes, onions, beef, bread, cake, ice cream, coffee and soup served in the 24 hours preceding the outbreak, consideration was given to the possibility that a toxic chemical, such as roach powder, had been introduced into the bread mix in the prison bakery which was so situated as to be well isolated from continuous observation. However, analyses of the bread failed to demonstrate significant amounts of fluorides which had been used as a roach powder in the prison and the investigation again came to a standstill.

c. *Third epidemic.* This episode occurred 10 months later and appeared identical with the first two in every respect. The food eating histories collected by Dr. John Myers from 337 men for the Sunday noon and evening meals are shown in table 7.

The discrepancies in attack rates which attract attention are those occurring in men who ate beef, potatoes and gravy at the noon meal, and soup at the evening meal. Rearrangement of these data into observed and theoretical numbers of men ill, using the group attack rate of 58.2 per cent, gives the results shown in table 8. The differences between the observed and theoretical numbers ill are too small to be statistically significant. However, if the data are tabulated in terms of those men who did or did not eat any of the 4 foods (beef, potatoes, gravy and soup) the interesting significant difference in table 9 is observed. Men who consumed none of these items suffered no illness. Those who ate one or more of the four foods had a 60 per cent attack rate. (The X^2 value for this 4-fold table is approximately 20.1. Thus the probability that

TABLE 8

Observed and expected illnesses in terms of foods consumed

Food item	Total who ate	Total ill		Difference O-T
		Observed O	Theoretical T	
Roast beef	357	217	208	+9
Potatoes	321	192	187	+5
Turnip	266	156	155	+1
Gravy	303	182	176	+6
Ice cream	323	177	188	−11
Bread	352	204	205	−1
Coffee	326	191	190	+1
Soup	186	125	108	+17
Cake	261	153	152	+1
Bread	250	147	145	+2
Tea	166	92	97	−5

a difference as great or greater might be observed by chance is less than 1 in 100,000.)

This peculiar association between the consumption of 4 different foods served at two meals, and the excessive incidence of illness became less confusing when it was realized that there was a factor common to the four: namely, meat stock. When roasts are cooked in the prison, the juices which run out of the meat are collected into one large steam-heated kettle in the kitchen and are usually left there overnight. This meat stock ob-

TABLE 9

Observed illnesses in terms of individuals who did or did not consume one of 4 food items

History of eating one or more	History of illness			
Beef, potatoes, gravy, soup	Ill	Not ill	Totals	Per cent ill
Eaten	225	148	373	60.0
Not eaten	0	14	14	0
Totals	225	162	387	58.2

tained from roasts is used only for week end meals because it is more tasty than that prepared from other sources. Preceding the third epidemic, the beef stock obtained from the roasts cooked on Saturday was left overnight in a kitchen steam kettle and then was distributed as follows:

a. over the sliced roast beef warmed just before serving;
b. in the gravy which was poured over the potatoes;
c. in the soup served at the evening meal.

Bacteriological studies of roast beef, ice cream, bread and coffee, turnips, potatoes, soup and gravy failed to reveal unusual numbers of bacteria commonly sought in food poisoning studies. However, two of several mice-feeding experiments gave positive results:

a. Six mice were fed gravy and 5 died.
b. Seven mice were fed soup and 4 died.

Mice which were fed beef, ice cream, bread, turnip and potato remained well.

Soup and gravy were then subjected to chemical examination with the idea in mind that cleaning powder or some washing compound had been deliberately introduced into the kettle containing the meat stock, since this kettle was located in the main kitchen next to the dishwashing machine. The findings of significance were:

a. *Both soup and gravy had pH values greater than 13.*
b. No fluoride was demonstrable.
c. No silicate was demonstrable.
d. Phosphates were present in small quantities.

In the search for a chemical within the prison which would produce such excessive alkalinity, the detergents used in the dishwashing machine were ruled out on the basis of their high silicate and phosphate content, and the laundry soda occasionally used to clean kitchen drains was eliminated because it had a maximum demonstrable pH in solution of 10.1.

These findings pointed by exclusion to lye as the only chemical which might be in use in the prison which would produce such extreme alkalinity. It was found that pure sodium hydroxide stored in the pharmacy safe had occasionally been issued to a guard for cleaning a plugged kitchen drain, but this source of lye had not been used for some time prior to the first outbreak. However, several hundred pounds of commercial caustic soda are used as one component of a bleach each year in the industrial dye plant located in the prison. This is issued to the plumbers for use in cleaning badly plugged drains, among which are those in the kitchen floor below the outlets of the steam-heated kettles used for meat stock, soups, etc. The plumbers who do this work are convicts who, because of the number of calls for assistance, are permitted to work without continuous direct supervision in some instances.

In addition to this logical use of lye by the plumbers, the prison authorities occasionally detect small amounts of the compound in the possession of other convicts. It is believed that this is stolen by some of the 47 convicts working in the industrial plant, where there are only two civilian supervisors. It is used for a variety of purposes by the convicts, not the least interesting of which is the preparation of hair "straightener" by the colored inmates through mixing the chemical with soap, oil and other ingredients.

Since lye was never used in cleaning the steam-heated kettles in the kitchen, the introduction of the compound into the meat stock must have been a malicious act. Since caustic soda was brought in by plumbers, or could have been smuggled in by any of the 114 convicts assigned to the kitchen and mess hall, it is obvious that opportunities for sabotage existed.

Discussion

This series of outbreaks presented a number of confusing factors of interest:

A. *Etiologic agent suggested by the clinical picture.* The clinical features of cramps, diarrhea, blood, mucus and pus in the stools and the estimated median incubation period of about 12 hours suggested an infectious etiologic agent, possibly an alpha streptococcus. The rapid clinical recovery, the normal or subnormal temperatures, the leucocyte counts within normal limits, and the negative stool cultures did not support such a hypothesis. The relative lack of nausea and vomiting, and the estimated length of the incubation periods did not suggest such toxic agents as staphylococcus toxin, fluorides and cadmium.

B. *Reliability of interviews.* Large numbers of men had decided that certain food items or personnel were to blame and lost no opportunity to attempt to bias the interviewers by strong statements supporting their belief. Often, if an inmate was associated with the preparation of certain kinds of food or had friends who were, he would attempt to attract interest away from that food item. A few reported that they had not been ill and their food histories were included in the appropriate group until a check of the hospital records revealed a visit to the dispensary for treatment during the epidemic.

C. *Water supply.* The coincidental massive disturbance in water supply occurring during the day of the first epidemic could not have been better timed to confuse the epidemiological picture. Fortunately, there were no changes in water supply during the second and third outbreaks.

D. *Human volunteer experiments.* During the second outbreak, there was good statistical reason to incriminate roast beef as the vehicle. Large proportions of men who were ill had also consumed milk, ice cream and bread. However, milk was ruled out for reasons discussed above, and meat consumed by 33 men, and ice cream consumed by 58 men failed to produce illness. Bread was left under suspicion by exclusion, but laboratory studies failed to incriminate this item.

It is now realized that the meat eaten by the human volunteers was meat which was taken directly from uncut roasts in the icebox, and which had not been covered with meat stock and heated. Since there is reason to believe that meat stock was "contaminated" with caustic soda, it is obvious that the meat consumed by the volunteers should not have caused illness.

E. *The occurrence of the first two outbreaks on two successive week ends.* After it was realized that meat stock was the vehicle in the third outbreak in 1947, notes taken during the investigation of the first two outbreaks in 1946 were reviewed. It was found that during confidential interviews with the kitchen and mess hall helpers, 3 had volunteered the information that the pork stock, cooked out of the roasts just before the first epidemic, had been used both week ends with proper refrigeration during the intervening time. Thus, if lye was put into the kettle of pork stock, illness could have occurred due to this one item on both week ends. This

possibility was denied by the civilian cook who regarded it, and practically every question, as direct challenge to his ability as a cook. Hence his statements were subject to some bias.

F. *Failure of meat and potatoes collected from foods served preceding the third outbreak to kill mice in feeding experiments.* The meat and potatoes collected for laboratory investigation of the third outbreak, although statistically incriminated, failed to kill mice when fed. This was explained when it was realized that the meat examined was from an uncut roast which had not been covered with the alkali-bearing meat stock before heating. Likewise, the potatoes were not covered with the alkaline gravy.

It should be emphasized that from an epidemiological point of view the third epidemic is reasonably well-understood. The identical pattern of the first and second epidemics tempts the conclusion that they were due to the same chemical agent but there is no supporting laboratory proof. Moreover, while it is clear that the 4 significant foods involved in the third epidemic transported a highly alkaline chemical it has not been shown that the illness was due to the alkalinity. Commercial caustic soda is not pure sodium hydroxide and until more definite laboratory studies are made no conclusion is supportable favoring the sodium hydroxide over its accompanying impurities as the gastrointestinal irritant. The effect of the chemical on the foods should also be considered from the standpoint of reactions leading to irritating substances.

Summary

Three epidemics of gastroenteritis causing approximately 1,600 illnesses occurred in a prison. The clinical and epidemiologic pattern of each epidemic was identical even to the extent of occurring at the same time on the same day of each week of occurrence.

Epidemiologic investigations of the first two episodes were unsuccessful in describing the source, but the third epidemic was shown to be limited to men who consumed one or more of 4 different foods served at two different meals. Meat stock prepared in one large kettle and poured over meat slices being heated, made into gravy for potatoes and used in soup was common to the 4 foods. The soup and gravy were toxic for mice and had pH values above 13. Commercial caustic soda issued to plumbers from one of the prison industries for cleaning plugged floor drains could have been introduced into the meat stock as a malicious act. It is not known whether the irritant effect on the gastrointestinal mucosa was due to the alkalinity, to impurities in the commercial caustic soda or to compounds formed by the alkali reacting with the foods.

U.S. Bureau of the Census, Department of Commerce, 1965-1972

U.S. Imports for Consumption and General Imports

U.S. Department of Commerce, Washington, D.C.

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THE EFFECT OF KRAFT PULP MILL WASTES ON SOME AQUATIC ORGANISMS

WILLIS M. VAN HORN, J. B. ANDERSON, AND MAX KATZ[1]
The Institute of Paper Chemistry, Appleton, Wisconsin

ABSTRACT

A study has been made of the toxic substances which may be found in kraft pulp-mill waste-waters. It has been determined that the sulphides, mercaptans, resin acid soaps, and sodium hydroxide constitute the greatest hazard. The minimum lethal concentration of these and other materials to fresh-water minnows, *Daphnia*, and aquatic insect larvae has been established. Methods have been devised for evaluating these materials in kraft-waste waters, and data from the examination of the wastes of a typical northern kraft mill are presented.

INTRODUCTION

The pulp and paper industry has for years been interested in problems relating to stream improvement since large amounts of water are required in the making of its products. This water is not a part of the final product but is used, among other ways, as a medium for pulp refining and sheet formation. During its use, solids are added to it, either in solution or suspension, and when, after its use, the water is returned to the stream, these solids can have an adverse effect on the stream.

There are at least three ways by which any industrial or domestic waste can affect the aquatic environment. First, the waste may contain toxic substances which will destroy the fauna and/or flora; second, it may contain materials in solution, the stabilization of which uses so much oxygen that the aquatic forms are adversely affected; and third, there may be a combination of these two types of action.

In the current study, attention has been directed to the first of these three possibilities in relation to kraft mill operation. Until recently this problem has received comparatively little attention. Cole (1935) investigated the effects of kraft black liquor on perch, bluegills, largemouth black bass, and rock bass, and found that the material was harmless in concentrations up to 5,000 p.p.m. In Scandinavia, Bergström and Vallin (1937) and Bergstrom (1939) studied the effect on fish of various components of kraft mill effluents, including digester condensates, dilute wash waters, and other wastes. He found that, of all the sources of waste arising from the kraft process, those containing the sulphate soap carried the greatest potential danger. This same fact had been reported earlier by Ebeling (1931). Hagman (1936) found that resin

[1]Environmental Health Center, U. S. Public Health Service, Cincinnati, Ohio.

acids in sufficient concentrations would harm fish, and stated that the safe concentration of these acids was 2 p.p.m. or less. Extrom and Farner (1943) concluded that bass *(Huro salmoides)*, bluegills *(Lepomis macrochirus)*, and sunfish *(Lepomis gibbosus)* were unaffected by "river concentrations" of kraft mill wastes although the fish could not live in the wastes themselves.

The inconclusive nature of the work cited above, together with the need for more specific information, impelled segments of the kraft pulping industry to conduct and support an investigation designed to clarify the entire relationship between the kraft mill and the stream. Accordingly, the work started by Cole and continued by Extrom and Farner was brought to The Institute of Paper Chemistry in 1943. At the Institute, the study was first supported by the Committee on Kraft Waste Disposal, a group representing various kraft mills in Wisconsin. For the past 5 years, however, the investigation has been supported and extended by the National Council for Stream Improvement, Inc. (an organization of pulp and paper companies) whose purpose is to study this and other pollution problems in the pulp and paper industry. The objectives of the present investigation may be listed as follows: (1) to determine if and to what extent the waste liquors from a kraft mill are toxic to aquatic organisms; (2) to devise methods for the detection and measurement of these toxic components; and (3) to discover measures which will prevent these wastes from reaching the streams.

There are several ways by which chemical wood pulp can be manufactured, among which are the sulphite process, the kraft or sulphate process and, more recently, the semichemical process. Of these the kraft method is the most widely used, with the sulphite process second in importance. In the sulphite process the liquor used for digesting the wood is an aqueous solution of sulphurous acid in which lime or some other base has been dissolved. The result is, therefore, a solution of a bisulphite of the base containing an excess of sulphurous acid. When calcium[1] is used as the base, the nature of this liquor, as well as the mechanics of the sulphite process, makes it economically unfeasible to recover the spent liquor. It is, therefore, wasted and is commonly known as sulphite waste liquor.

In the kraft process, on the other hand, the wood is cooked or digested with an alkaline liquor in which the principal components are sodium hydroxide and sodium sulphide, the latter comprising up to 45 percent of the total alkali. From the point of view of the problem at hand, the important aspect of the kraft process is that instead of being wasted the spent liquor is evaporated and burned, and the chemicals in the ash are re-used and the heat is recovered.

[1] In recent years the Weyerhaeuser Timber Company has developed an acid cooking process in which magnesium instead of calcium is used. In theory, this process permits the recovery of the chemicals and burning of the residue for heat recovery, thus materially reducing, if not eliminating the problem of stream pollution. However, this development is still in the experimental stage.

In order to delineate more clearly the problem of stream pollution which may result from a kraft mill, it is desirable to consider briefly the general aspects of the process. The wood is cut into small chips and introduced into digesters, the cooking liquor is added, and the digester closed. Heat is then applied gradually, by direct or indirect steaming, and the cooking process is started.

The cooking cycle of any type of chemical pulp production consists of three phases: (1) a period when the liquor is permeating the chips, (2) a period of full pressure when the cellulose fibers are being freed from their bonding material, and (3) a gassing-down period after the cook when the digester's internal pressure is gradually relieved to the point where the pulp can be blown out. At the conclusion of the first phase, the accumulated air and other gases in the digester are drawn off and passed through a condenser for heat recovery. Turpentine is recovered from the condensate, and the residue becomes waste. Similarly, at the conclusion of the second phase, the relief gases are condensed and may become waste.

After the pulp is blown from the digester, the spent cooking liquor (black liquor) is separated from it and sent to the recovery plant. The pulp is washed with water that is re-used until the black liquor concentration is high enough for wash waters to be sent to the recovery plant. In the final washing process, however, the waters may be so dilute that their chemicals cannot be recovered economically and, therefore, they may be sent to the sewer.

The spent cooking liquor and the concentrated wash waters are evaporated under vacuum to a consistency of approximately 50 percent solids. In most mills this vacuum is produced by a jet condenser and its effluent may contain in solution some of the non-condensable gases from the process of black liquor evaporation. This effluent may be sent to the sewer or, in some mills, it is used for pulp washing and eventually finds its way back to the recovery plant. Use of surface condensers instead of the jet type will reduce, if not eliminate, the possibility of pollution from this source. The concentrated black liquor, containing most of the non-fibrous portion of the original wood as well as the spent chemicals, is then burned in furnaces designed to recover the chemicals and the heat of combustion.

When the black liquor from the blowpits (into which it is blown from the digester) is sent to the recovery plant, it is usually stored in large receptacles for varying periods. As the material stands, a thick foamy material, called sulphate soap, rises to the surface. Actually, it is composed of the sodium salts of resin and fatty acids which were present in the wood. Currently, this soap is being recovered and sold, but small amounts of it may be found in the dilute wash water.

In summary, it can be stated that the major potential stream polluting products from the normal operation of a kraft mill are: (1) the initial

[illegible] condensate, (2) the final blowdown condensate, (3) the evaporator condensate, and (4) the soaps and other material in the dilute [illegible] waters.

Method of Testing Toxicity of Wastes

According to Sutermeister (1941), kraft waste liquors may contain the following compounds: sodium carbonate, sodium hydroxide, sodium sulphide, sodium sulphate, sodium sulphite, sodium chloride, hydrogen sulphide, methyl mercaptan, and sodium combined with organic acids, such as the sodium salts of resin and fatty acids (soaps). It is apparent that [illegible] of these compounds might prove toxic if they are present in sufficient amounts. The first problem, therefore, was to determine the minimum lethal concentration of these materials to various aquatic species.

In [illegible] test animals, the more susceptible species of each group were selected. A secondary consideration was the environmental relationship of the groups, i.e., fish and fish-food organisms. The fish used were [illegible] of minnows taken from various sources in the vicinity of Appleton, Wisconsin. Lake Emerald *(Notropis a. atherinoides)* and spotfin *(Notropis sp. lepterus)* shiners were most commonly used but, at times, the bluntnose minnow *(Hyborhyncus notatus)* and the rosyface shiner *(Notropis rubellus)* were also used. Two types of fish-food organisms were used as test animals. The first of these was *Daphnia sp.* grown in the laboratory for the purpose, and the second was aquatic insect larvae of various species taken from Lake Winnebago and adjacent waters.

The method using minnows as test animals was a modification of that employed by Powers (1917) and has been previously described by the senior author (Van Horn, 1943). Two-liter portions of the materials to be tested were placed in open battery jars immersed in a constant temperature ([illegible] C.) water bath. From one to five fish, depending on the [illegible] of the test solution, were then placed in each jar, and [illegible] were made hourly up to 5 days. The survival time of the [illegible] recorded if death resulted. Checks were made on the [illegible] content of the test solution at the conclusion of the [illegible] those cases where this level fell below 4 p.p.m., the data were [illegible] checks were also made on pH and alkalinity to make sure [illegible] were within limits favorable to fish life. If these [illegible] outside of these limits, which was very seldom, the [illegible] discarded.

[illegible] *Daphnia* was used as the test animal, two specimens were [illegible] containing 25 milliliters of the test solution. The vials [illegible] each of which carried 26 test vials and 6 controls. [illegible] vations were made for a period of 48 hours, and the [illegible] elapsing between the time of placing the specimens in the

solution and immobilization was recorded. Checks on dissolved oxygen, pH, and alkalinity were made before and after each test.

When aquatic insect larvae were employed as test animals, the method used for *Daphnia* was modified to accommodate the physiological requirements of the insect larvae. The larvae were placed in test solutions contained in glass vessels of suitable size and were permitted to remain there for a period of 48 hours. In the meantime, a close watch was kept on them. Extraneous variables, such as pH, alkalinity, and dissolved oxygen, were checked as described above. The species used in these studies were taken from Lake Winnebago and adjacent waters and included mayflies of the genus *Blasturus* and *Leptophlebia*, the damsel fly *Agrion*, and the dipteran *Chironomus*. All tests were made using stabilized Fox River water. This water is relatively hard, having a pH of 7.6 to 7.8 and a total alkalinity of from 140 to 160 p.p.m.

Toxicity of Kraft Mill Wastes

In order to determine which of the components of a typical kraft mill waste would be significant in this study, a number of screening tests were made on 15 compounds, using the Lake Emerald and spotfin shiners as test animals (Table 1). It is apparent that the compounds of greatest

Table 1.—*Minimum lethal concentrations*[1] *of kraft pulp-mill waste components killing Lake Emerald and spotfin shiners in 120 hours.*

Compound	Concentration, p.p.m.
Sodium hydroxide	100.0
Sodium sulphide	3.0
Methyl mercaptan	0.5
Hydrogen sulphide	1.0
Sodium thiosulphate	5.0
Sodium chloride	2,500.0
Sodium carbonate	250.0
Sodium sulphate	100.0
Formaldehyde	50.0
Crude sulphate soap	5.0
Sodium salt of fatty acid fraction of crude soap	5.0
Sodium salt of resin acid fraction of crude soap	1.0
Sodium oleate (stock room)	5.0
Phytosterol (pure)	3.0
Sodium abietate (pure)	3.0

[1]In this work the "minimum lethal concentration" was defined as the lowest concentration of a toxic material which would kill any of the test animals within a period of 120 hours, when held in a test solution at 18° C., and factors of pH, total alkalinity, and dissolved oxygen being held to conditions approximating those of control tests. 100 percent survival of controls was required.

interest in this study are sodium hydroxide, the sulphides, methyl mercaptan, and the crude sulphate soap and its components. Furthermore, under normal operating conditions, kraft waste waters are relatively constant in their composition and in the proportion of one component to another. For these reasons, particular attention was directed to the four compounds indicated, and an extensive series of tests, in addition

described for minnows, was made on *Daphnia* and two atic insect larvae.

ontained in Table 2 indicate that the minimum lethal con- as roughly the same for the species of minnows tested, the mayfly larvae. While mayfly larvae were less sensitive p.p.m.), they were more sensitive than either of the other lium sulphide. The midge larvae (*Chironomus* sp.) are sitive to all the toxic compounds tested.

minimum lethal concentrations of kraft pulp waste components minnows,[1] Daphnia, and two species of aquatic insect larvae.[2]

nd	Minimum Lethal Concentration, p.p.m.			
	Minnows	*Daphnia*	Mayfly Larvae[2]	*Chironomus* Larvae
.....	100.0	100.0	100.0	700.0
.....	3.0	10.0	1.0	1,000.0
.....	1.0	1.0	1.0	750.0
.....	0.5	1.0	1.0	50.0
.....	5.0	5.0	10.0	50.0
p	1.0	3.0	....	50.0
n acid fraction of soap	5.0	1.0	....	....
y acid fraction of soap				

r (*Notropis spilopterus*) and the Lake Emerald shiner (*Notropis a. atherinoides*). d were of the genera *Blastomus* and *Leptophlebia*. No attempt was made to

ANTITY OF TOXIC MATERIALS IN WASTE WATER

tablished the maximum safe concentration of the potentially ials found in kraft pulping wastes, the next step was to amount passing to the streams. The methods of analysis being reported in detail elsewhere but they may be briefly ere.

pparent that the various sulphides need not be determined and, for the sake of convenience, it would be better if the ould be collectively evaluated by a single analysis. Tamele l (1936), Tamele, *et al.* (1941), Lykken and Truemnler, d Borlew and Pascoe (1946) have described such a method olves the use of a potentiometric-argentimetric titration in a aline solution. The applicability of this method was thoroughly and it was determined that it would be effective in the current Furthermore, if chlorides are not present in any great amount, le to use the same titration for mercaptan evaluation. For the s, an adaptation of the Liebermann reaction was used. The oxide analysis consisted of a simple potentiometric titration acid to a pH of 8.4.

the period from May to September, 1947, the waste waters of l northern kraft mills were subjected to frequent analyses for mercaptans, resin soaps, odium hydroxide, and pH. Care

was taken to sample under all types of operating conditions. All samples taken were grab samples. It should be noted that 24-hour composite samples of these wastes will not show extremes in variation of their chemical content. It has been demonstrated, for example, that, if a sample having a high pH is permitted to stand for an hour or so, its pH will gradually change in the direction of neutrality. For that reason, grab samples were taken to determine the degree of variation in the nature of the waste waters. At the time the samples were taken, data on the volume of sewer discharge and the volume of river discharge were secured. A typical analysis for one mill is shown in Table 3.

It will be noted from Table 3 that, in 13 of the 35 analyses of raw waste, the concentration of sulphides in the waste water exceeded the minimum lethal concentration (1 p.p.m.); in all cases, the concentration of mercaptans exceeds the minimum lethal concentration (0.5 p.p.m.); in all cases, content of the resin acid soaps exceeded the minimum lethal concentration (1 p.p.m.); and, in 4 of the 35 analyses, the concentration

TABLE 3.—*The results of waste water analyses of a typical northern kraft mill, June 24 to September 12, 1947.*

Sample No.	River dilution ratio[1]	Parts per Million				
		Sulphides	Mercaptans	Resin soaps	Sodium hydroxide	pH
1	170	0.7	1.5	5.0	6.6	8.6
2	166	0.6	0.7	5.0	8.0	8.8
3	178	1.5	4.1	18.0	61.4	9.9
4	218	1.6	1.8	8.0	24.2	9.4
5	185	1.0	1.2	5.0	12.0	9.0
6	193	0.2	1.9	5.0	0.0	7.8
7	275	18.4	12.0	18.0	114.0	10.6
8	232	0.5	2.5	3.0	6.6	8.7
9	224	0.2	0.7	3.0	0.0	8.2
10	138	0.4	0.9	2.0	0.0	8.2
11	142	0.9	1.6	3.0	0.0	8.2
12	126	0.0	1.0	3.0	0.0	8.1
13	123	0.8	1.6	3.0	14.7	8.7
14	193	0.0	2.7	5.0	10.6	8.7
15	173	0.2	1.4	5.0	0.0	8.3
16	191	0.2	0.9	5.0	14.7	8.8
17	173	0.2	1.2	2.0	0.0	7.6
18	176	0.0	1.1	5.0	0.0	7.9
19	166	0.0	1.2	3.0	0.0	7.9
20	177	0.2	1.1	3.0	0.0	7.7
21	153	0.2	1.1	3.0	0.0	8.4
22	152	0.0	0.8	3.0	0.0	8.1
23	157	0.0	0.6	2.0	0.0	8.3
24	144	0.3	1.0	2.0	2.6	8.5
25	156	2.1	1.9	3.0	13.3	8.9
26	161	88.4	15.8	18.0	391.0	10.5
27	113	0.4	1.6	3.0	4.0	8.5
28	103	0.3	1.7	3.0	17.3	9.0
29	96	0.0	3.1	5.1	9.3	8.7
30	94	2.8	3.3	8.0	21.4	8.8
31	94	25.8	8.1	18.0	115.0	9.4
32	134	0.4	1.0	3.0	144.0	10.7
33	136	3.6	5.1	5.0	13.7	8.9
34	127	2.8	7.2	3.0	9.4	8.8
35	109	1.3	0.9	5.0	0.2	8.5

[1]The river dilution ratio is calculated by dividing the volume of river discharge by the volume of sewer discharge.

of sodium hydroxide exceeded the minimum lethal concentration (100 p.p.m.). As soon as the wastes are discharged, dilution with river water takes place at varying rates depending on the volume of river flow. The lowest dilution ratio observed at this particular mill was 1 to 94 (Table 3). If total dilution is assumed, the concentrations of the toxic materials would be brought to a point below their minimum lethal concentration as soon as the necessary dilution had occurred.

In the entire study, including phases not presented here, the lowest ratio of dilution found in the two receiving streams was 1 to 73. Similarly, the highest concentrations of the compounds under study were sulphides 88.4 p.p.m., mercaptans 45 p.p.m., resin acid soaps 28 p.p.m., and sodium hydroxide 3,763 p.p.m. Although these conditions did not occur simultaneously, they do form a theoretical picture of what could happen under the worst conditions in the mills under study. In each case, however, the concentrations of the materials after dilution are brought to a point where their danger is reduced to a minimum, if not eliminated.

Discussion

The primary purpose of this investigation was to determine whether or not a problem relating to aquatic organism toxicity is associated with the normal operation of a kraft pulp mill and, if there is a problem, to determine its seriousness. From the data presented it can be stated that, under normal operating conditions in the locality where the study was made, there is little danger to the aquatic environment from the toxic properties of kraft pulp-mill wastes. While the majority of analyses of waste waters indicated that they contained toxic materials in excess of the minimum lethal concentration, their concentration was reduced to a level safe for fish and other forms after dilution in the stream. It should be pointed out also that, when fish or other aquatic organisms come into contact with these materials in lower concentrations, death does not occur instantly. For example, in a concentration of methyl mercaptan of 1 p.p.m. (twice the minimum lethal concentration), fish survive for as long as 2 days before exhibiting any of the reactions of poisoning.

If a kraft pulp mill is operating normally, there seems little danger that it may be responsible for destroying an aquatic environment by the operation of its wastes, providing enough receiving water is available for efficient dilution. Abnormal or accidental incidents may sometimes occur in the operation of any industrial installation. For that reason, this investigation is being continued to determine at which step in the process the potentially toxic materials occur, and to find ways and means of preventing their passage to the stream in the event of accidents or other abnormal conditions.

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Effect of Calcium on the Toxicity of Gentamicin

GERALD H. WAGMAN, EDWIN M. ODEN, MARVIN J. WEINSTEIN, and SAMUEL IRWIN[1]
Department of Microbiology, Schering Corporation, Bloomfield, New Jersey

A gentamicin hydrochloride-calcium complex has been prepared which displays a marked reduction in acute toxicity in mice by intravenous and subcutaneous routes. Toxicity of the calcium complex was reduced threefold compared with gentamicin sulfate, and it was five times less toxic than the parent hydrochloride on an equal microbiological activity basis. Derivatives of gentamicin hydrochloride prepared in the same manner with sodium, potassium, and magnesium ions showed no reduction in toxicity compared with gentamicin hydrochloride. Simultaneous subcutaneous injections in mice of calcium chloride and gentamicin sulfate also resulted in a reduction of toxicity. Chronic studies of the gentamicin hydrochloride-calcium complex in cats showed no significant difference in toxicity from gentamicin sulfate.

A number of investigators have demonstrated that the in vivo acute toxicity of certain oligosaccharide antibiotics is influenced by calcium ions. Calcium chloride has been shown to reverse the acute toxicity of streptomycin in mice (2, 6, 11), and in rats, guinea pigs, and rabbits (7). Reversal of streptomycin chronic toxicity in mice has also been demonstrated (11). The latter investigators have shown that calcium gluconate and heptagluconate are also effective, and it has been demonstrated that the pantothenate salt also resulted in decreased toxicity (2).

Corrado and Ramoz reported that the curarizing and ganglioplegic actions of neomycin on dogs and cats were antagonized by calcium ions (3). These authors noted the same antagonism against kanamycin (4), and they also found that the neuromyic blocking effect of kanamycin in pigeons was similarly reversed. Di Marco and Bertazzoli (5) have postulated that the acute toxic effect caused by aminosidin [determined to be identical to paromomycin (8)] may be due to displacement of calcium from the nervous and contractile structures. Sobek et al. (9) observed that, by lowering the calcium level by administration of ammonium oxalate to guinea pigs and mice, neomycin toxicity was significantly increased. On the basis of their experiments, they assumed that ionized calcium is bound rapidly in the organism by neomycin, with which it forms a complex compound. An extension of this work (10) indicated that acute toxicity of neomycin administered by the intracerebral route was reduced by simultaneous injection of calcium chloride [illegible] route.

Gentamicin, the most [illegible] to this family of [illegible] antibiotics, was studied for [illegible] to this calcium ion effect, not only in acute toxicity experiments in mice but also for the influence of calcium ions [illegible] administration.

[1]Present address: University of Oregon Medical School, Portland.

Materials and Methods

Acute toxicity studies were done in 20-g CF-1 (Carworth Farms, New City, N.Y.) male mice. Mice were injected with calcium chloride by the subcutaneous route simultaneously with injection at an opposite site of a toxic amount of gentamicin sulfate. A quantity of calcium chloride equal to 8 mg per mouse was used as a control, as was a toxic dose of gentamicin sulfate (12.5 mg per mouse).

Another experiment was designed to determine more accurately the ratio of calcium chloride to gentamicin to be used, and also to determine whether both compounds could be administered together. Solutions of gentamicin sulfate were made up containing 25 mg/ml (as the base), and calcium chloride was added to these solutions to give concentrations of 1, 2, 4, 8, 16, and 32 mg/ml (as the salt). Precipitates of calcium sulfate formed in the latter two solutions upon standing, and were removed by centrifugation before injection (1 ml per mouse) of the mixtures.

Because of the precipitation problem encountered in mixing the antibiotic sulfate with calcium chloride, a gentamicin hydrochloride-calcium complex was prepared by the following procedure. Gentamicin hydrochloride (632 μg mg) was dissolved in water to give a concentration of 100 mg/ml. This solution was brought to a final pH of 8.0 by the slow addition of solid $Ca(OH)_2$ and vigorous stirring. A slight turbidity resulted, and the insoluble material was filtered off. The solution was concentrated to an oil, dissolved in methanol, and precipitated with 10 volumes of acetone. The precipitate was filtered off, washed twice with acetone, and vacuum-dried. The calcium chloride complex thus formed gave positive tests for both chloride ($AgNO_3$) and calcium (oxalate). The material, which assayed 578 μg/ml, was tested in mice for efficacy against a virulent strain of *Staphylococcus aureus* in comparison with gentamicin sulfate and hydrochloride.

A chronic toxicity study was done in cats to compare neurological effects of gentamicin sulfate and gentamicin hydrochloride-calcium complex. Twenty cats of varying weight and age were used, balanced into five treatment groups of two males and two females each. One group served as a saline control. The remaining groups received two dose levels (60 and 120 mg/kg) of the free base equivalent of either gentamicin sulfate or gentamicin hydrochloride-calcium complex. Doses were administered subcutaneously once daily with the solution concentrations adjusted so that the milliliter volume injected daily was one-half the kilogram body weight of the animals. The doses chosen were based on previously [illegible] which indicated these levels [illegible]

Ataxia was scored observationally on the basis of a 0 to 8 rating scale of severity of gait: "2" represented slight impairment; "4," moderate; and "8," extreme impairment. In evaluating impairment of the righting reflex, the rate of recovery from a supine position was first observed and recorded as absent, sluggish, or normal. If recovery was sluggish, the animals were further tested by being inverted and dropped to the ground from heights of 60, 45, or 30 cm for four successive trials, depending upon the number of failures from each height. The number of failures to land correctly out of the four trials was used as an index of the degree of impairment present. The higher the score, the greater the impairment was, with a score of 8 being maximal. The actual system of scoring employed, with a rating scale of 0 to 8, is shown in Table 1. The body weight of the animals was determined both before and twice weekly during the course of drug administration. The subcutaneous injection site was rotated daily in clockwise fashion about the back surface of the animal during the course of each week's treatment. Previous experience with gentamicin had suggested that neurological impairment would not develop until after approximately 10 days of drug administration. As a consequence, after predrug evaluation, scoring for impairment was not undertaken until the 5th day of drug administration. Measurement of the righting reflex was not initiated until the 8th day of drug administration. Therefore, observations were made daily.

Table 1. *Rating scale for impaired righting reflex in cats*

Supine position	Drop distance failures[a]			Score
	60 cm	45 cm	30 cm	
Absent				8
Sluggish	4			7
Normal	2-3			6
	1	4		5
	0	2-3		4
		1	4	3
		0	2-3	2
			1	1
			0	0

[a]Number of failures out of four trials.

Results

Acute toxicity studies. Simultaneous injections of calcium chloride and gentamicin sulfate into mice clearly produced a drop in acute toxicity at levels of antibiotic which would normally be toxic to

the animals. The calcium chloride control was not toxic at the level used, whereas the gentamicin control was toxic. The data are summarized in Table 2.

The effects of injecting mixtures of gentamicin sulfate and calcium chloride are presented in Table 3. It is shown that in the mice receiving the 8- and 16-mg quantities of calcium chloride optimal reduction in toxicity was observed by both the intravenous and subcutaneous routes. The dosage of 32 mg of calcium chloride per mouse was toxic by both routes, undoubtedly owing to the large excess of calcium ions present.

The calcium complex, which was tested in mice by the subcutaneous route, reduced toxicity to less than one-fifth that of the starting material (gentamicin HCl) and caused toxicity to decrease to less than one-third that of gentamicin sulfate (Table 4).

In preparation of the complex, the *p*H at which the titration was stopped was significant. If the *p*H was below 7.7, the acute toxicity of the compound increased toward that of the starting material as the *p*H decreased; material with a *p*H of over 8.0 and up to 10.0 had no lower toxicity than did material titrated to *p*H 8.0. The PD_{50} of the complex against *Staphylococcus aureus* infections in mice was the same as that for the equivalent base quantity of the hydrochloride or sulfate of gentamicin.

To determine whether the decrease in toxicity was due to the complexing effect of calcium as opposed to some other cation, hydroxides of sodium, potassium, and magnesium were used to prepare their respective derivatives of gentamicin hydrochloride in exactly the same manner as with calcium. These were injected into mice by the subcutaneous route to determine whether any of the cations had an effect similar to that of calcium.

None of the other gentamicin hydrochloride derivatives prepared with sodium, potassium, or magnesium ions was less toxic than the parent antibiotic.

Table 2. *Effect of calcium chloride on acute toxicity of gentamicin in mice*

Compound injected[a]		
Gentamicin sulfate (mg/mouse, as base)	Calcium chloride (mg/mouse)	Survivors/total
10	8	10/10
15	8	8/10
20	8	2/10
12.5	0	2/10
0	8	10/10

[a]Animals treated with both compounds were injected subcutaneously on opposite sides.

Table 3. *Effect of varying concentrations of calcium chloride on acute toxicity of gentamicin in mice*

Calcium chloride (mg/mouse)	Survivors/total	
	Subcutaneous route, 12.5 mg/mouse[a]	Intravenous route, 2.5 mg/mouse
0	0/5	1/10
1	0/5	
2	0/5	
4	3/5	
8	5/5	6/10
16	5/5	8/10
32	0/5	1/5

[a]Gentamicin sulfate (as base).

Table 4. *Acute toxicity of gentamicin salts in mice*[a]

Gentamicin (mg/mouse, as base)	Survivors/total		
	Hydrochloride	Sulfate	Calcium complex
60	—	—	2/15
45	—	—	9/15
30	—	—	9/15
25	—	—	10/15
22	—	—	15/15
20	—	—	15/15
11	—	0/15	—
10	—	8/15	—
9	0/15	—	—
8	1/15	9/15	—
7	5/15	15/15	—
6	10/15	15/15	—
5	15/15	15/15	—
4	15/15	—	—

[a]Subcutaneous route.

Because the only molecular change was the addition of the respective cations, the reduction in acute toxicity must, therefore, be attributed specifically to the presence of calcium in the preparation.

Chronic studies. The neurotoxic data are detailed in Tables 5 and 6 and the means are plotted in Fig. 1 and 2. In Fig. 1 and 2, numbers in parentheses indicate the number of animals that had become moribund or had died. In Table 5 and Fig. 1, it is demonstrated that there is little difference between equivalent high levels of the calcium complex and the sulfate or between the lower levels tested. The observations reveal the toxicity, in terms of ataxia, of the calcium complex of gentamicin hydrochloride to be equal to, or possibly slightly greater than, that of gentamicin sulfate, particularly at the higher dose.

The neurotoxic data based on impairment of righting reflex are summarized by the graphs shown in Fig. 2 and detailed in Table 6. Significantly impaired equilibrium was noted with the 60 mg/kg doses after approximately 12 days, with the 120 mg/kg doses, impaired equilibrium occurred after 5 days with the calcium complex and after 10 days with the gentamicin sulfate. In this study, it was noted that the male animals seemed significantly more sensitive to the neurotoxic effects of the drugs than did the females.

Table 5. *Effect of gentamicin-calcium complex and gentamicin sulfate on ataxia in cats*

Animal no.	Sex	Treatment	Dose (mg/kg)	Day of treatment										
				5	8	9	10	11	12	13	14	15	16	17
13	M	Saline	—	0	0	0	0	0	0	0	0	0	0	0
M8	M			0	0	0	0	0	0	0	0	0	0	0
35	F			1	0	0	0	0	0	0	0	0	0	0
6	F			0	0	0	0	0	0	0	0	0	0	0
Mean				0.2	0	0	0	0	0	0	0	0	0	0
44	M	Gentamicin-calcium complex	60	1	1	1	0	0	0	2	4	8	—[a]	
L92	M			0	0	0	0	0	1	4	6	7	8	8
40	F			0	0	0	0	0	0	0	2	6	6	6
45	F			0	0	0	0	0	0	0	2	2	4	4
Mean				0.2	0.2	0.2	0	0	0.2	1.5	3.5	5.8	6.5	7.0
37	M	Gentamicin sulfate	60	0	1	1	0	0	0	1	2	7[b]		
J41	M			0	0	0	0	0	3	4	6	6[b]		
52	F			0	0	0	0	0	0	1	1	1	1	1
L79	F			0	0	0	0	0	1	0	2	5	7	7
Mean				0.2	0.2	0.2	0	0	1.0	1.5	2.8	4.8	5.2	5.2
3	M	Gentamicin-calcium complex	120	2	6[a]									
49	M			2	5	6	8	8	8	—[a]				
47	F			0	0	2	3	3	6	6	6	—[b]		
2	F			0	3	2	7	8	8					
Mean				1.0	3.5	5.5	6.5	6.7	7.5	7.5	7.5			
14	M	Gentamicin sulfate	120	0	0	0	1	6	6	6	6	7[b]		
48	M			0	0	0	1	6	6	7	—[a]			
57	F			0	0	0	2	3	6	6	7	7[b]		
5	F			0	0	0	1	7	7	8				
Mean				0	0	0	1.5	6.3	6.2	6.8	7.5	7.5		

[a]Died (score of "8" used subsequently for averaging).

[b]Sacrificed (score presacrifice used for subsequent averaging).

Table 6. *Effect of gentamicin-calcium complex and gentamicin sulfate on impairment of righting reflex in cats (0-8 scale)*

Animal no.	Sex	Treatment	Dose (mg/kg)	Day of treatment										
				5	8	9	10	11	12	13	14	15	16	17
13	M	Saline	—	0	0	0	0	0	0	0	0	0	0	0
M8	M			0	6	4	0	0	0	0	0	0	0	0
35	F			1	1	0	0	4	2	0	0	0	0	0
6	F			0	0	0	0	0	0	0	0	0	0	0
Mean				0.2	1.7	1.0	0	1.0	0.5	0	0	0	0	0
44	M	Gentamicin-calcium complex	60	0	0	0	4	4	4	5	6	8	—[a]	
L92	M			0	2	3	5	5	5	7	7	8	8	8
40	F			0	0	0	0	0	0	2	3	7	7	7
45	F			0	3	3	0	3	0	1	3	3	6	7
Mean				0	1.2	1.5	2.3	4.0	2.3	3.8	4.8	6.5	7.2	7.5
37	M	Gentamicin sulfate	60	0	1	1	0	0	0	3	3	8[b]		
J41	M			0	0	0	0	0	5	7	7	8[b]		
52	F			0	6	5	6	3	4	3	3	3	0	0
L79	F			0	4	4	5	0	2	2	3	7	6	7
Mean				0	2.7	2.5	2.8	0.7	2.7	3.8	4.0	6.3	5.2	5.5
3	M	Gentamicin-calcium complex	120	2	6[a]									
49	M			2	6	6	6	8	8	—[a]				
47	F			0	4	5	7	6	7	7	7	—[b]		
2	F			0	4	6	8	8	8	—[a]				
Mean				1.0	5.0	6.3	7.2	7.5	7.7	7.8	7.8	7.8		
14	M	Gentamicin sulfate	120	0	2	2	2	7	7	8	8	8[b]		
48	M			1	1	2	0	7	7	8	—[a]			
87	F			0	0	0	3	7	7	7	8	8[b]		
5	F			0	2	2	4	7	7	8[a]				
Mean				0.2	1.2	1.5	2.3	7.0	7.0	7.8	8.0	8.0	8.0	8.0

[a]Died (score of "8" used subsequently for averaging).
[b]Sacrificed (score presacrifice used for subsequent averaging).

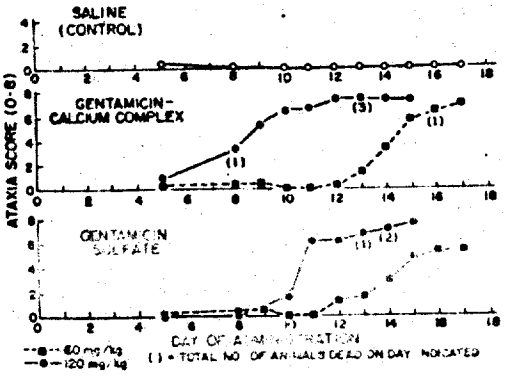

Fig. 1. *Effect of gentamicin-calcium complex and gentamicin sulfate on ataxia in cats.*

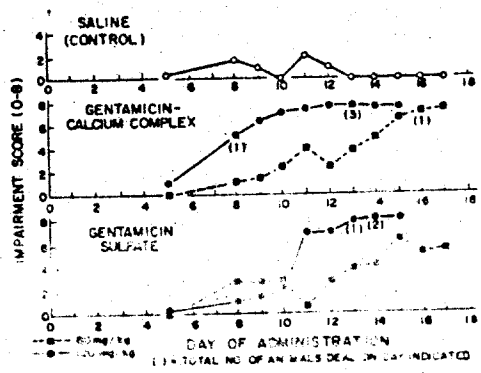

Fig. 2. [illegible] *gentamicin sulfate on impairment of righting reflex in cats.*

Diminished intake of food or water, or both, often was noted just prior to, or commensurate with, the first indications of neurological impairment. One animal receiving 120 mg/kg of the calcium complex exhibited bloody urine on the 5th day of drug administration. Treatment with the 120 mg/kg dose of gentamicin hydrochloride-calcium complex was terminated on the 15th day; the balance of the study was terminated on the 17th day. Surviving animals were sacrificed at that time.

The data on body weights of the cats during the course of drug administration are summarized in Table 7. As shown in the table, almost all drug-treated animals exhibited a loss of body weight, particularly with the higher doses of drug.

Discussion

Acute toxicity studies in mice have shown the calcium complex of gentamicin hydrochloride to be significantly less toxic than gentamicin hydrochloride on an equal antibiotic activity basis. A separate study was undertaken in cats, in which it was determined that a similar diminution in neurotoxicity did not occur under conditions of subchronic daily subcutaneous administration.

A number of theories have been proposed by various investigators on the effect of calcium on the reduction of acute toxicity of other basic antibiotics. It is thought that the acute toxicity is due to a rapid decrease in serum calcium ions which are bound or complexed by

Table 7. *Comparative changes in body weights of cats*

Cat no.	Sex	Compound administered	Daily dose (mg/kg, as base)	Initial wt (kg)	Final wt (kg)[a]		Wt change (kg) Individual	Wt change (kg) Total
13	M	Saline	—	2.10	2.15	(17)	+.05	
M8	M			4.60	4.30	(17)	-.30	
35	F			2.30	2.40	(17)	+.10	
6	F			3.00	3.15	(17)	+.15	0.00
Avg				3.00	3.00			
44	M	Gentamicin-calcium complex	60	3.35	3.10	(15)	-.25	
L92	M			3.50	3.05	(15)	-.45	
40	F			1.80	1.75	(15)	-.05	
45	F			3.10	2.75	(15)	-.35	-1.10
Avg				2.94	2.66			
37	M	Gentamicin sulfate	60	3.20	3.00	(15)	-.20	
J41	M			3.60	3.20	(15)	-.40	
52	F			2.15	2.20	(17)	+.05	
L79	F			3.10	2.75	(17)	-.35	- .90
Avg				3.01	2.79			
3	M	Gentamicin-calcium complex	120	2.50	2.20	(11)	-.30	
49	M			4.40	3.90	(14)	-.50	
47	F			2.40	2.40	(14)	-.00	
2	F			2.90	2.60	(14)	-.30	-1.10
Avg				3.05	2.78			
11	M	Gentamicin sulfate	120	2.10	[illegible]	(15)	[illegible]	
48	M			3.80	[illegible]	(14)	[illegible]	
87	F			[illegible]	2.20	(14)	-.30	
5	F			[illegible]	2.70	(14)	-.60	-1.50
Avg				2.92	2.55			

[a]Day weight was measured is given in parentheses. Weights were measured on the 17th day or on the day the animal died.

the antibiotic. Sobek and co-workers (9) demonstrated a decrease in calcium ions and a significant rise of the phosphorus level within 3 hr after administration of neomycin to guinea pigs; they also reported that a significant decrease in calcium leads to lengthening of magnesium narcosis. In that study, the addition of calcium to replace those ions bound by neomycin reversed the toxic effects. A similar effect has been demonstrated in the present study with gentamicin. Preliminary experiments in our laboratory have shown a decrease in serum calcium in mice after administration of gentamicin. These studies are still in progress and will be reported in detail at a later time.

The data presented on the effect of calcium on the acute toxicity of gentamicin clearly demonstrate that the reduction in toxicity is similar to that described for streptomycin and neomycin as well as for other antibiotics in this group, and the mechanism is probably the same.

In the chronic cat studies, death is due to renal damage, and the gentamicin-calcium complex apparently has no effect on accumulation of the antibiotic nor will it eliminate this pathological phenomenon. Furthermore, this toxicity may not be attributed to a decrease in serum calcium per se because much lower serum levels of gentamicin are attained during chronic administration and the antibiotic is already complexed with calcium prior to treatment.

The experimental evidence presented demonstrated that the calcium complex of gentamicin hydrochloride offers no significant advantages in terms of neurotoxicity over gentamicin sulfate under the described conditions of chronic administration in cats as opposed to the significant decrease in toxicity found in the acute studies in mice.

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POISONING BY CAUSTIC SODA

BY

STANLEY G. WILLIMOTT, PH.D.CANTAB. AND LIVERP., B.Sc.

GOVERNMENT ANALYST, CYPRUS

AND

MINNIE GOSDEN, M.B., B.S.LOND., D.T.M. AND H.

GOVERNMENT BACTERIOLOGIST, CYPRUS

(With Special Plate)

Fortunately poisoning by the fixed caustic alkalis is comparatively rare in England, although in Europe fatal cases appear to be not uncommon. Balázs,[3] for example, records in three years 754 alkali poisonings, of which 163, or 21.6 per cent., were fatal; and further statistics are given in the works of Popper[1] and Leschke.[2] In England ammonia is more frequently employed as a fatal poison, and, according to the Registrar-General's returns[4] for the three years 1930 to 1932, it accounted for ninety-two deaths, of which seventy-eight were suicidal and the remainder accidental.

It is therefore somewhat remarkable that in a period covering little more than one year no fewer than eight cases of poisoning by caustic soda should have occurred in Cyprus, an island with a population of only 348,000. In England poisoning by the fixed alkalis has nearly always been the result of accident or negligence, and exceptionally of suicide, but in the cases in Cyprus, of which the available data are summarized in the table, half were accidental and half suicidal, the former occurring in children and the latter in adults. A number of typical cases of poisoning by caustic soda or caustic potash and their effects are discussed in the well-known works of Taylor,[5] Glaister,[6] and Wynter Blyth,[7] so that little need be said here; while details of certain cases occurring in Central Europe are given in the texts of Popper and Leschke, already referred to. The majority of such cases appear to fall into two distinct groups: (*a*) those ending fatally within a few days, and (*b*) those ending fatally some months or years afterwards.[8]

Source of Caustic Soda

The use of caustic soda locally has greatly increased during the last few years, and it would appear that its misuse has increased correspondingly. Caustic soda is used in small quantities by the Cypriot community for domestic washing purposes, and is cheap and freely available. It has always been the practice in washing clothes to add some form of alkali in order to soften the generally prevailing hard waters of the plains of Cyprus. Formerly the alkali used consisted exclusively of wood ashes supplied from domestic sources. It is perhaps noteworthy that "plant ashes in order to Mennu" were at one time produced in certain villages of Cyprus for export for industrial purposes. Within recent years, however, this has given place to washing soda ($Na_2CO_3 10H_2O$), and during the last two or three years, in its turn, to caustic soda. The reasons given for this change are the cheapness and availability of caustic soda, and, it is claimed, the better results in the wash. In all these cases in Cyprus the alkali was obtained in this way, and its ready availability under the conditions prevailing constituted a serious problem. Practically the whole amount of the caustic soda imported from Europe is absorbed in the local soap industry, but, in view of recent experience, it was considered desirable to restrict its general domestic use. Accordingly it was advised that caustic soda and caustic potash should be placed on the Poisons Schedule, and this recommendation was adopted by Government in February, 1934. A similar situation appears to have existed in Hungary,[1] where poisoning by caustic alkalis was common. There the difficulty was overcome in 1926 by subjecting the sale of caustic alkalis to restrictions.

The caustic soda as sold was in the form of broken fused lumps, and analysis showed an average content of 77 per cent. NaOH with 10 per cent. sodium carbonate. This latter impurity usually accompanies commercial grades. For washing purposes it is usual to dissolve about 50 grams in a little water, and to add this to about 120 litres of water.

General Features of Poisoning

In the table are summarized the available data of known cases of poisoning by caustic soda occurring since December, 1932, although there are possibly others unknown to the authorities. In spite of the difficulties of obtaining reliable data, especially in regard to the amount of alkali taken, it has been possible to study four of this series (Nos. 1, 4, 7, and 8), all affecting adults, in some detail. Starkenstein[10] regards 10 to 20 grams of solid caustic alkali as the fatal dose, but it is clear from our own experience (see table) that a quantity less than 10 grams can be lethal. The fatal dose is unknown, but it is noteworthy that 2.6 grams (40 grains) of caustic potash have proved fatal (Taylor[5]).

Cases of Poisoning by Caustic Soda

Case	Patient	Age	Cause	Dose (grams)	Symptoms	Result
1	Turkish woman	33	Suicide	60	Burning pains in mouth, throat, and stomach; vomiting and exhaustion	Died 29½ hours after ingestion
2	Turkish child	1½	Accident	?	Lips, tongue, and palate burnt	Died 25 hours after ingestion
3	Greek child	3	Accident	Sucked a lump of NaOH	Mouth, lips, and tongue burnt	Recovery in 3 days
4	Turkish woman	20	Suicide	8.5	Burning of mouth and palate; vomiting blood; shock	Died in 11 days
5	Turkish child	3	Accident	?	Oesophageal stricture; unable to swallow any liquid	Gastrostomy; recovering
6	Greek child	5	Accident	?	Emaciation; oesophageal stricture	Gastrostomy; died
7	Turkish girl	16	Suicide	5	Stricture of oesophagus and pylorus	Palliative jejunostomy. Died after 74 days
8	Turkish girl	15	Suicide	?	Burning of lips, tongue, pharynx; vomiting; shock	Died 26 hours after

The immediate effect of swallowing caustic alkali is to produce excoriation and destruction of the mucous membrane, which gives rise to intense pain, often extending from the mouth to the abdomen. The severity of the lesions depends upon the concentration of the alkali, the stomach contents, and the interval elapsing before treatment. Alkali corrosions are usually oedematous and mucous as compared with those from acids, which are dry and hard. The alkali extracts water from the tissues with which it comes into contact, and attacks the protein, forming alkali-albuminate, which becomes gelatinous, swells, and in the presence of much water commences to dissolve—a process termed "colliquation." Because of this, alkali burns penetrate deeply into the tissues, although cases of perforation are few compared with those which develop stricture later on.

Treatment follows the accepted lines of avoidance of stomach lavage, and the administration of organic acids and liquids in order to reduce the concentration of the alkali. The use of the bougie at the proper time is regarded as being very important, especially in Continental practice, in order to avoid later complications of oesophageal stricture.

Details of some Medical Cases

The first case of this series, which occurred in December, 1932, was one of determined suicide.

First Example.—A Turkish woman of Nicosia, a mother of five children, swallowed apparently no less than 60 grams (925 grains) of caustic soda dissolved in about 120 c.cm. of water. Symptoms of distress came on almost immediately, with vomiting and burning pains in the mouth, throat, and stomach, and with great exhaustion and collapse. It was observed that the buccal cavity, tonsils, and pharynx were soft and oedematous, and of the deep chocolate colour suggestive of recent burning by alkali, while the lips and tongue were red and swollen. The mouth gave a strong reaction to litmus paper, and the vomited matter consisted of a brownish liquid, due to the presence of blood, and contained portions of detached mucous membrane. This liquid gave a strongly alkaline reaction to litmus, which on analysis proved to be due to the presence of 1.6 per cent. of free caustic soda. The patient was allowed lemon juice *ad lib.*, but, although she appeared to have recovered from shock, her condition had deteriorated by next morning, and death took place twenty-nine and a half hours after ingestion of the poison. Unfortunately it was not possible to carry out a post-mortem examination, but it was considered that death had resulted probably from perforation of the stomach. A more detailed account of this case has been published elsewhere.[6]

Second Example.—The second case of interest (No. 4) again concerned a Turkish woman, a domestic servant, who, at 9 p.m. on August 31st, 1933, swallowed about 8.5 grams (123.4 grains) of caustic soda dissolved in a little water, to which had been added some rosewater in order to mask the burning taste. From the woman's statement the alkali had not completely dissolved, and apparently some was swallowed solid, a fact which may account for the oesophageal perforations found after death. The amount of caustic soda taken was more than three times the known fatal dose of caustic potash (40 grains).

The same symptoms as previously observed—that is, burning pains extending from the mouth to the stomach—came on immediately, with, later, vomiting of blood and mucus. On arrival at hospital, one and a half hours later, the patient was conscious, but suffering from shock, with the extremities very cold. Burning of the surface of the buccal cavity, however, was not so severe as that observed in the first case. Vomiting of blood persisted, and the vomitus gave a marked reaction to red litmus. The patient complained of severe pain in the throat and stomach. The temperature was 97° F., and the pulse rate 90, and during the eleven days of observation the temperature varied from 97° to 101°, the pulse rate between 84 and 130, and the respiration rate between 28 and 36. The patient recovered from shock, but swelling of the tissues which had been in contact with the alkali—especially the lips and tongue—was marked. By the fifth day the tongue was healing, though red, but no liquids could be swallowed, and feeding was carried out with liquid glucose per rectum. After the first week it was clear that the patient was not progressing, although the burned parts appeared to have healed, and to be covered with shiny mucous membrane. Later, signs of bronchopneumonia were observed, and the patient died on September 11th—that is, eleven days after swallowing the poison. In view of the amount of destruction of tissue in the oesophagus and stomach the length of survival appears remarkable. The coroner's finding at inquest was that death was due to exhaustion and bronchopneumonia resulting from suicide by swallowing corrosive poison.

Some interesting conditions were seen at necropsy, which was carried out five hours after death. Externally there were no unusual appearances. The lips were covered with [illegible] and somewhat shining mucous membrane, and the skin and mucous membranes were generally pale. Rigor mortis was present in all parts, and post-mortem staining in the dependent parts. The anterior half of the tongue was covered with smooth, shining mucous membrane, and at the back of the tongue the papillae were well marked. The mucous membrane in the upper part of the oesophagus was again smooth and shining, while the tip of the epiglottis was absent. At a point 9 cm. below the latter the walls of the oesophagus were very soft and adherent to the vertebral bodies, and at this level an oval perforation, with smooth edges, 1.5 cm. long, was found between the oesophagus and the trachea. One centimetre below this there was a second circular perforation 1.1 cm. in diameter, also leading into the trachea (Fig. 1 on Special Plate). From the site of these perforations to the pylorus the mucous membrane of the oesophagus and stomach was very soft and pulpy, but the muscular wall was firm and intact. From the level of the lower perforation extending to the pylorus, a dark red, lightly adherent slough was found forming a complete cast of the oesophagus and stomach (Fig. 1 on Plate). The stomach was filled with a dark brown fluid, which gave an alkaline reaction. The mucous membrane of the duodenum was deeply injected, but below the pylorus there was no erosion. The trachea was filled with purulent material and the left lung exhibited patches of [illegible] bronchopneumonia. The liver showed cloudy swelling, and the kidneys were injected with pinpoint haemorrhages. Sections from the liver, kidney, and stomach near the cardiac end were prepared and examined. It was found that the stomach wall near the cardiac end exhibited remains of highly oedematous and necrotic mucous membrane, in which remnants of glands were recognizable. The muscular wall was little affected (Fig. 2 on Plate). Degeneration and desquamation of the tubular epithelium were seen in the kidney, with area of complete necrosis containing blood pigment (Fig. 3 on Plate). In the liver there were again areas of slight focal necrosis (Fig. 4 on Plate). The presence of such areas in the liver and kidney does not appear to have been observed in the recorded cases of poisoning by the fixed alkalis.

Third Example.—Case 8 was again one of suicide, in which a Turkish girl, aged 15, bought caustic soda, and made a concentrated solution with water. She swallowed a mouthful at 5.30 p.m. on April 2nd, 1934, and came to hospital an hour later vomiting and in a collapsed condition. She was treated by gastric lavage and lemon juice *ad lib.*, but swallowing was difficult and painful. During the next twenty-four hours there was a purulent exudate from the nose and mouth, increasing pulse rate, pain, and collapse, and death occurred twenty-six hours after taking the poison. A post-mortem examination, performed fifteen hours after death, showed burning of the tip and back of the tongue, the inner surface of the lips, and all over the tonsils and pharynx. The burnt areas were covered with a soft, brown slough. The oesophagus was deeply injected, but the mucous membrane was not eroded. In the stomach a burnt area, 15 cm. by 10 cm., and deep brown in colour, was found extending over the centre of the greater curvature. The mucous membrane was soft, but the wall of the stomach under the burnt area was firmer than the surrounding wall. The stomach contents consisted of a yellow fluid of slightly acid reaction. There was oedema of the lungs, but no consolidation. The liver exhibited cloudy swelling and the kidney congestion. The thymus was large and glandular, and all the lymphatic glands, especially in the neck, were large, soft, and dark red in colour.

Surgical Cases

The remainder consisted of a group of surgical cases under the care of Dr. C. H. Cuff, to whom we are indebted for certain clinical data. Stricture having developed, it was necessary to resort to gastrostomy in two instances and jejunostomy in another. It is interesting to note that stricture of the oesophagus was successfully treated by operation (gastrotomy), for the first time in 1885, by Loreta,[11] in the case of a patient of 24, who had swallowed caustic alkali. Since then surgical treatment had been resorted to with considerable frequency. In cases of non-malignant stricture of this [illegible] Gray Turner has recently [illegible] [illegible] operation [illegible] gastrostomy.

In the first of these cases (No. 6) gastrostomy, on Frank's method, was successfully performed, but the patient, aged 3, died the next day from peritonitis.

The second of this group (No. 5) was that of a Turkish girl, aged 3, who accidentally swallowed a little caustic soda solution on July 3rd, 1933, the exact amount being unknown,

The usual symptoms came on, and some burning of the face, lips, and tongue with oedema were observed on arrival at hospital shortly after. This appeared to be a mild case, and after two days the parents removed the child against medical advice. The subsequent history illustrates the necessity for continued observation and treatment in subjects which appear at first sight to be mild cases of alkali poisoning. On December 18th, 1933, the patient re-entered hospital unable to swallow any liquid, and it was impossible to pass any kind of sound through the stricture of the oesophagus which had developed. Radioscopic examination revealed oesophageal stenosis opposite the third costal cartilage, and gastrostomy was performed on December 21st. During three months the patient has made uninterrupted progress on a diet consisting of soup, eggs and milk, dried milk preparations, orange juice, etc., given at four-hourly intervals.

The last case of this group (No. 7) concerned a Turkish girl, aged 16, who attempted suicide on January 19th, 1934, by taking about 5 grams (77.2 grains) of strong caustic soda solution. On arrival at hospital within the hour the patient was suffering from shock, and complained of burning pain in the mouth and stomach. There was also purging and much vomiting of blood. The body temperature was subnormal, varying between 97° and 98° F. It was possible to pass a small sound, and lemon juice was given freely. The patient recovered from shock and appeared to be definitely improving when the relatives removed her after she had been one week in hospital. On February 12th she returned to hospital—this time unable to swallow even liquids—and was, in consequence, much emaciated. Radioscopy revealed a stricture of the oesophagus opposite the fifth costal cartilage. The patient continued to lose strength, and it was suspected that the pylorus also was stenosed. On operation this was found to be the case, and palliative jejunostomy was carried out (March 2nd). The patient was afterwards maintained on a diet of citrated milk, eggs, soup, meat extracts, olive oil, liquid glucose, etc., but the emaciation was progressive, and death took place on April 2nd.

Conclusions

Poisoning by caustic alkali in Cyprus has increased remarkably during the last year, and has justified Government action in restricting its free use for domestic purposes. In all the cases studied the poison was obtained in this way. Of these the suicides were among the adults[4] and the accidental cases among the children. The fatal dose of caustic soda is less than 10 grams, and a quantity of 5 grams, in solution, has produced the most serious complications of stricture. There was perforation of the oesophagus in one fatal case, and possibly perforation of the stomach in another, while in another three cases stricture developed. Among the pathological conditions found post mortem, areas of necrosis in the liver and kidney have been observed (Case 4). It is evident from a study of these cases that the prognosis in alkali poisoning is doubtful, and that even in what appear to be mild cases the need for long-continued observation and treatment is essential.

We are indebted to Dr. C. H. Cuff, acting director of health, for permission to publish the results of this investigation.

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TOXIC EFFECT OF SODIUM HYDROXIDE ON TUBERCLE BACILLI*

DIRAN YEGHAN, D.V.M., AND VERA BUDD

From the Laboratory of the Ray Brook State Tuberculosis Hospital, Ray Brook, New York

Culture for tubercle bacilli before, during and after chemotherapy is a necessary procedure in the evaluation of chemotherapeutic agents. The limitations of the present methods for concentration and culture must be recognized because although in many instances the sputum is rendered microscopically and culturally negative for tubercle bacilli it is quite possible that specimens of pathologic material may contain a few viable bacilli and yield no growth.

In order to obtain tubercle bacilli in pure culture the pathologic material must be digested and decontaminated. The digestant most commonly used is sodium hydroxide but unfortunately this agent, as well as others employed for the same purpose, will kill an undetermined number of tubercle bacilli. Therefore it seemed important to study quantitatively the toxic effect of sodium hydroxide, particularly on small numbers of tubercle bacilli. This report includes data obtained from such a study of several strains of tubercle bacilli suspended in albumin-water and also of bacilli present in pathologic material.

MATERIALS AND METHODS

The tubercle bacilli studied in this experiment included various stock strains (Table 1) as well as those found in pathologic material. Sodium hydroxide in a final concentration of 2 per cent was the digestant.

Stock cultures of tubercle bacilli in liquid Tween-albumin medium were diluted sufficiently to contain small numbers of bacilli, too few to be seen microscopically. The final dilution was made up in equal parts of suspension in diluent (0.1 per cent of bovine albumin in distilled water) and 4 per cent NaOH. The control suspension contained the same concentration of bacilli in a corresponding total volume of diluent.

Immediately after the addition of NaOH, 5 ml. of the suspension was removed and neutralized with dilute hydrochloric acid. A 5-ml. portion of the control suspension was likewise taken and to this was added a volume of diluent equal to the amount of hydrochloric acid required to neutralize the treated sample. One-milliliter quantities of each of these were then placed in sterile petri dishes, and approximately 20 ml. of melted and cooled oleic-acid medium[1] was poured into the plates, which were rotated to insure mixing of the inoculum. Five such plates were made for each suspension. The remainder of each of the original suspensions was kept at 37 C. as in the routine procedure for digesting sputum. At 30 and again at 60 minutes after the addition of NaOH, 5-ml. samples of each were removed, neutralized and plated as described. Following inoculation the plates were incubated at 37 C. and, after evaporation of any excess moisture, were sealed with wide rubber bands. Colony counts were made after 42 days of incubation. The results are shown in Table 1.

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To determine any possible protective effect of sputum on tubercle bacilli a similar experiment was carried out, the only difference being the substitution

TABLE 1

THE EFFECT OF SODIUM HYDROXIDE-EXPOSURE ON TUBERCLE BACILLI SUSPENDED IN 0.1 PER CENT ALBUMIN IN DISTILLED WATER

STRAIN OF TUBERCLE BACILLI		AVERAGE NUMBER OF COLONIES PER PLATE IN DESIGNATED TIME OF EXPOSURE			
		0 Min.	5 Min.	30 Min.	60 Min.
Stock strain H37Rv	Control*	242		241	250
	2% NaOH		57	13	15
SM-sensitive from sputum	Control*	117		120	109
	2% NaOH		88	50	40
PAS-resistant variant of H37Rv	Control*	413		421	388
	2% NaOH		292	157	23
SM-PAS-resistant from sputum	Control*	96		71	73
	2% NaOH		46	17	11
Resting cells, stock H37Rv	Control*	405			
	2% NaOH		47	4	2
*1 colony from H37Rv after 60 minutes' exposure to NaOH	Control*	85			
	2% NaOH		18	8	<1
*2 colony from H37Rv after 60 minutes' exposure to NaOH	Control*	121			
	2% NaOH		2	<1	<1

* Control suspensions were not exposed to NaOH but were diluted correspondingly, incubated at 37 C., and plated at the same time-intervals.

TABLE 2

THE EFFECT OF SODIUM HYDROXIDE ON STRAIN H37Rv SUSPENDED IN HOMOGENIZED SPUTUM

	AVERAGE NUMBER OF COLONIES PER PLATE IN DESIGNATED TIME OF EXPOSURE			
	0 Min.	5 Min.	30 Min.	60 Min.
Experiment 1:				
Control	125		101	98
NaOH		44	12	4
Experiment 2:				
Control	53		38	29
NaOH		34	8	2

of the NaOH-digested, neutralized, negative sputum for the diluent. These findings are shown in Table 2.

The method for determining the effect of NaOH on tubercle bacilli in sputum

was, of necessity, somewhat different, since it was not possible to determine the actual number of viable bacilli present before homogenization. Equal portions of 4 per cent NaOH and purulent sputum, known to contain tubercle bacilli, were shaken by machine and incubated at 37 C. When the mixture became homogeneous, usually after 30 to 50 minutes, a 10-ml. portion was removed and neutralized. A sample (1 ml.) of this was diluted serially in albumin water and 5 other acid pour plates were made. The remainder of the neutralized sample was reincubated to serve as a control for subsequent platings.

The sputum NaOH mixture was likewise incubated and at intervals of 1 and 2 hours after the first sample was neutralized, additional 10-ml. portions were removed. After neutralization these were diluted to correspond with the first sample and plated. Fresh dilutions of the control neutralized sample were also prepared and plates made at the same time-intervals.

RESULTS

Analysis of the data presented in Table 1 reveals that the number of colonies in the control series was surprisingly constant. Incubation at 37 C. for 1 hour had no appreciable effect on the viability of the mycobacteria. The bacilli were well distributed as evidenced by close agreement of individual plate counts. In view of these findings the 30- and 60-minute control plates were not made with the last 3 strains listed in the table.

The most significant finding in the NaOH-treated group is the marked reduction in the number of viable bacilli in the first time-interval (5 minutes). This period represents the time, immediately after adding NaOH, required to remove a tubed sample and titrate to neutrality with dilute hydrochloric acid, using phenolphthalein as an indicator. The survival rate in the first 4 strains tested averaged 56 per cent in this short period. At the end of 30 minutes the average survival rate was 28 per cent and after 60 minutes it had dropped to 11 per cent.

The resting-cell experiment was included because physiologically young cells are generally more sensitive to adverse conditions, and it was thought that these nonmultiplying cells, being older, might show increased resistance to the action of NaOH. This did not prove to be so, as shown by figures in the table.

The last two strains tested, #2 and #4, were cultures from individual colonies chosen at random from the plating of stock H37Rv after 60 minutes' exposure to NaOH. The findings in this case proved that these were chance survivors and not more resistant to NaOH than the cells in the original culture.

When strain H37Rv was tested, using homogenized sputum for the diluent (Table 2), there was an average survival rate of 44 per cent for the 5-minute interval of exposure to NaOH, while the 30- and 60-minute platings showed average rates of 16 and 5 per cent, respectively.

Clinical specimens of sputum, known to contain tubercle bacilli, were also treated; but the findings are not tabulated since the necessary preliminary treatment with NaOH made it impossible to determine either the number of bacilli present before exposure to the decontaminant or the mortality rate during the first time-interval. It is of interest, however, that a very purulent sputum requiring

30 minutes' pre-digestion showed only one or two less colonies per plate after additional 1- and 2-hour periods of exposure to NaOH, while a thin specimen that digested well in 30 minutes showed a further reduction of viable bacilli to 26 and 23 per cent, respectively, following additional 1- and 2-hour periods of exposure to NaOH.

DISCUSSION

It is known that an indefinite number of tubercle bacilli, present in pathologic material as well as in aqueous suspensions, are killed by sodium hydroxide and other digestants. This series of experiments was carried out primarily to study the toxic effect of this agent on material containing small numbers of bacilli. Quantitative data on this subject were obtained by means of bacteriologic methods that have only recently been applied in the study of tubercle bacilli.[5]

The most unexpected and significant finding was the high mortality rate of the bacilli during the 5-minute period of exposure to NaOH and neutralization with acid. Almost 50 per cent of the bacilli were killed by this process. This finding was observed when bacilli from cultures were suspended in albumin-water or in digested and neutralized negative sputum. Whether similar results would be obtained with previously untreated positive sputum is impossible to determine for technical reasons. However, there is no reason to believe the contrary. After the initial period of exposure the death rate is significant although not proportionally as great.

The mode of action of sodium hydroxide on tubercle bacilli seems to be similar to that of other injurious chemical and physical agents on bacteria in general; the first unit of time shows the largest number of deaths, while the last few survivors often require considerable exposure before they are killed, and yet as a rule they do not yield cultures of higher resistance than the original strain.[3] Our findings confirm this observation.

Accurate determination of the mortality rate of tubercle bacilli in sputum was impossible for the reasons given. The first time-unit exposure to sodium hydroxide was of necessity prolonged to 30 or more minutes, and prior to this critical time-interval the death rate is obviously not known.

The results obtained when tubercle bacilli were suspended in predigested, neutralized sputum were comparable to those obtained with albumin-water suspensions and therefore our findings are in agreement with those of Spendlove and associates[4] that sputum exerts no demonstrable protective effect against sodium hydroxide. The fact that nonmultiplying cells were in no way more resistant to the action of sodium hydroxide would seem to indicate that the physiologic status of the tubercle bacilli present in clinical material is not an important factor.

On the basis of this study it is unlikely, in instances where the tubercle bacilli are too few to be found in stained preparations, that more than a very few will survive the action of sodium hydroxide during the concentration process. The "negative" culture should therefore be interpreted with an appreciation of the limitations of present laboratory methods for the culture of tubercle bacilli.

SUMMARY

Quantitative data on the toxicity of sodium hydroxide for small numbers of tubercle bacilli have been presented. The mortality rate was highest (about 50 per cent) during the first 5-minute period of exposure to sodium hydroxide and neutralization. The action of this agent on tubercle bacilli is comparable to that of other injurious agents on bacteria in general. The laboratory significance of these findings is discussed.

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