VOLUME 5

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SODIUM CHLORIDE, POTASSIUM CHLORIDE

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prepared for

THE FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION AND WELFARE

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prepared by

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FOOD AND DRUG Research LABORATORIES, INC.

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FINAL REPORT

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date January 7, 1974

Laboratory No. 1767 e
Contract No. FDA 71-260

Sample: Fine white crystalline material

Marking: FDA 71-70 (Sodium chloride)

Examination Requested: Teratologic evaluation of FDA 71-70 in rabbits

Procedure: (See Appendix I)

Results: See Tables 1 through 4 and Appendix II

Conclusion:

On the basis of the data presented herein, the following conclusion appears to be warranted:

"The administration of up to 363 mg/kg (body weight) of the test material to pregnant rabbits for 13 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

FOOD and DRUG RESEARCH LABORATORIES, INC.

Kenneth Morgareidge, Ph.D.
Director

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FOOD AND DRUG RESEA LABORATORIES, INC.

Groups: 311 & 312; 317 through 320

Material : FDA 71-70

Date: December 7, 1973

Laboratory No. : 1767 e

Table 1

Fate Summary
(Rabbits)

Group	Material	Dose ** mg/kg	Total		Surviving at Term	
			Mated	Pregnant	Total	Pregnant[1]
311	Sham	0.0	20	10	20	10
312	6-AN*	2.5	27	9	27	9
317	FDA 71-70	3.63	28	13	26	12
318	FDA 71-70	16.85	25	15	24	14
319	FDA 71-70	78.2	34	14	29	11
320	FDA 71-70	363.0	27	12	24	10

* Positive Control : 2.5 mg/kg of 6-aminonicotinamide dosed on Day 9
** Administered as a water solution (See Appendix I)
1) Includes all dams examined at term

FOOD AND DRUG RESEAR LABORATORIES, INC.

Gro 311 & 312; 317 through 320 | Ta. 2 | Date: December 7, 1973

Material: FDA 71-70 | Reproduction Data (Rabbits) | Laboratory No.: 1767 e

Group:	311	312	317	318	319	320
Dose (mg/kg):	Sham	6-AN**	3.63	16.85	78.2	363.0
Pregnancies						
Total No.	10	9	13	15	14	12
Died or Aborted (before Day 29)	0	0	1	1	3	2
To term (on Day 29)	10	9	12	14	11	10
Corpora Lutea						
Total No.	103	107	168	112	169	160
Average/dam mated	8.58	5.94	10.5	7.47	8.05	9.41
Live Litters						
Total No.*	10	7	12	12	10	10
Implant Sites						
Total No.	46	43	70	64	42	45
Average/dam*	4.60	4.78	5.83	4.57	3.82	4.50
Resorptions						
Total No.*	2	11	2	10	2	3
Dams with 1 or more sites resorbed	2	6	2	5	2	3
Dams with all sites resorbed	--	2	--	2	1	--
Per cent partial resorptions	20.0	66.7	16.7	35.7	18.2	30.0
Per cent complete resorptions	--	22.2	--	14.3	9.09	--
Live Fetuses						
Total No.	42	32	64	50	40	42
Average/dam*	4.20	3.56	5.33	3.57	3.64	4.20
Sex ratio (M/F)	1.21	1.64	1.17	1.40	1.22	1.63
Dead Fetuses						
Total No.*	2	--	4	4	--	--
Dams with 1 or more dead	1	--	2	1	--	--
Dams with all dead	--	--	--	--	--	--
Per cent partial dead	10.0	--	16.7	7.14	--	--
Per cent all dead	--	--	--	--	--	--
Average Fetus Weight, g	37.9	31.0	38.1	38.5	41.3	40.6

* Includes only those dams examined at term.

** Positive Control: 2.5 mg/kg of 6-aminonicotinamide dosed on Day 9

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 311 & 312; 317 through 320 Laboratory No. 1767 e

Table 3

Material FDA 71-70 Date December 7, 1973

Summary of Skeletal Findings*

(Rabbits)

Findings / Group No.:	311	312	317	318	319	320
Dose (mg/kg):	Sham	6-AN**	3.63	16.85	78.2	363.0
Live Fetuses Examined (at term)	42/10	29/6[a]	64/12	50/12	40/10	42/10
Sternebrae						
Incomplete oss.	2/1			1/1		
Scrambled						
Bipartite		2/2		3/3		
Fused		2/2			2/1	
Extra	1/1		1/1	5/5	1/1	5/2
Missing		1/1				
Other						
Ribs						
Incomplete oss.		1/1				
Fused/split		8/4				1/1
Wavy				1/1		
Less than 12						
More than 13						
Other						
Vertebrae						
Incomplete oss.						
Scrambled		11/4				
Fused		2/1				
Extra ctrs. oss.		1/1				
Scoliosis		4/1				
Tail defects	1/1	23/5			2/1	1/1
Other						
Skull						
Incomplete closure						
Missing						
Craniostosis						
Other ; harelip		4/1				
Extremities						
Incomplete oss.						
Missing						
Extra ; club feet		6/2				
Miscellaneous						

* Numerator=Number of fetuses affected; Denominator=Number of litters affected.

** Positive control: 2.5 mg/kg of 6-aminonicotinamide dosed on Day 9

[a] 3 pups lost in processing

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 311 & 312; 317 through 320 Date December 7, 1973

Material FDA 71-70 Laboratory No. 1767 e

Table 3-a
Summary of Soft Tissue Abnormalities
(Rabbits)

Group	Material	Dose Level mg/kg	Dam	Number of Pups	Description
312	6-AN*	2.5	Z 7572	7	Anopia
			Z 7596	4	Anopia; medial rotation of hind limbs; short tail
			Z 7598	4	Anopia
			Z 7599	4	Medial rotation of hind limbs; short tail; anopia
				1	Short tail; anopia

*Positive Control: 2.5 mg/kg of 6-aminonicotinamide dosed on Day 9

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 311 & 312; 317 through 320

Date December 7, 1973

Species Rabbits

Laboratory No. 1767 e

Table 4

Average Body Weights*

Group	Material	Dose Level	Day 0	6	12	18	29**
		mg/kg	g				
311	Sham	0.0	1.96	2.03	2.04	2.13	2.27 (10)
312	6-AN***	2.5	2.07	2.11	2.16	2.34	2.31 (9)
317	FDA 71-70	3.63	2.12	2.25	2.26	2.34	2.50 (12)
318	FDA 71-70	16.85	2.09	2.22	2.33	2.26	2.45 (14)
319	FDA 71-70	78.2	1.92	2.04	2.02	2.12	2.25 (11)
320	FDA 71-70	363.0	2.14	2.20	2.24	2.26	2.35 (10)

* Of pregnant dams
** Number of surviving dams in parentheses (c.f. Table 1)
*** Positive control: 2.5 mg/kg of 6-aminonicotinamide dosed on Day 9

Appendix I

Teratology Study in Rabbits

Virgin, adult, Dutch-belted female rabbits were individually housed in mesh bottom cages in temperature and humidity-controlled quarters with free access to food and fresh tap water. On Day 0, each doe was given an injection of 0.4 ml of human chorionic gonadotropin (400 IU) via the marginal ear vein. Three hours later, each doe was inseminated artificially with 0.3 ml of diluted semen from a proven donor buck using approximately 20×10^6 motile sperm according to the procedure described by Vogin et al (Pharmacologist 11, 282 (1969)). Beginning on Day 6 and continuing daily through Day 18 the females were dosed with the indicated dosages by oral intubation. The controls were sham treated with the vehicle at a level equivalent to the group receiving the highest test dose. The test material was prepared and doses calculated according to the following table:

Dosage (mg/kg)	Dose (ml/kg)	Concentration (mg/ml)
≦ 250	1	≦ 250
251 - 500	2	125 - 250
501 - 750	3	133 - 250
751 - 1000	4	187 - 250
1001 - 1250	5	200 - 250
1251 - 1500	6	208 - 250
1501 - 1600	6.4	235 - 250

Body weights were recorded on Days 0, 6, 12, 18, and 29 of gestation. All animals were observed daily for appearance and behavior, with particular attention to food consumption and body weight in order to rule out any abnormalities which may have occurred as a result of anorexic effects in the pregnant female animal.

On Day 29 all does were subjected to Caesarean section under surgical anesthesia, and the numbers of corpora lutea, implantation sites, resorption sites and live and dead fetuses were recorded. Body weights of the live pups were also recorded. The urogenital tract of each animal was examined in detail for normality. In addition all fetuses underwent a detailed gross examination for the presence of external congenital abnormalities. The live fetuses of each litter were then placed in an incubator for 24 hours for the evaluation of neonatal survival. All surviving pups were sacrificed, and all pups examined for visceral abnormalities (by dissection). All fetuses were then cleared in potassium hydroxide (KOH), stained with alizarin red S dye and examined for skeletal defects.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 311

Material Sham

Dose 0.0 mg/kg

Appendix II

Reproduction Data in Rabbits (Individual)

Date December 7, 1973

Laboratory No. 1767

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
S 7556	P	9	8	7		3	4	1	26.1	
S 7557	NP	8	0						----	
S 7558	P	10	3	2		1	1	1	47.0	
S 7559	P	9	6	6		4	2		41.0	
S 7560	NP	0	0						----	
S 7561	P	9	1	1		1	0		50.1	
S 7562	P	10	5	5		3	2		38.4	
S 7563	NP	--**	0						----	
S 7564	NP	--**	0						----	
S 7565	NP	--**	0						----	
S 7566	P	10	7	7		3	4		26.9	One Neonatal Death
S 7567	NP	--**	0						----	
S 7568	P	8	2	2		2	0		46.2	
S 7569	P	12	3	1	2	0	1		20.3	
S 7570	NP	--**	0						----	
S 7571	NP	--**	0						----	
S 7572	P	9	5	5		3	2		43.3	
S 7573	NP	--**	0						----	
S 7574	P	9	6	6		3	3		39.5	
S 7575	NP	--**	0						----	

* P = Pregnant; NP = Not Pregnant

** Not Recorded

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 312

Material 6-AN

Dose 2.5 mg/kg

Appendix II

Reproduction Data in Rabbits (Individual)

Date December 7, 1973

Laboratory No. 1767

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
Z 7556	NP	--**	0						----	
Z 7557	NP	--**	0						----	
Z 7558	NP	7	0						----	
Z 7559	NP	7	0						----	
Z 7560	NP	0	0						----	
Z 7561	NP	0	0						----	
Z 7562	NP	9	0						----	
Z 7563	NP	3	0						----	
Z 7564	NP	3	0						----	
Z 7565	NP	5	0						----	
Z 7566	P	6	4					4	----	
Z 7567	NP	--**	0						----	
Z 7568	NP	--**	0						----	
Z 7569	P	7	3	3		--**	--**		----**	
Z 7570	NP	--**	0						----	
Z 7571	P	8	5	4		2	2	1	27.0	Four Neonatal Deat
Z 7572	P	8	8	7		4	3	1	29.3	Four Neonatal Deat
Z 7573	NP	6	0						----	
Z 7574	NP	5	0						----	
Z 7575	NP	--**	0						----	
Z 7596	P	10	7	4		2	2	3	26.8	Four Neonatal Deat
Z 7597	NP	--**	0						----	
Z 7598	P	7	4	4		3	1		43.0	One Neonatal Death
Z 7599	P	9	6	6		4	2		20.5	Five Neonatal Deat
Z 7600	NP	--**	0						----	
Z 7594	P	7	5	4		3	1	1	39.1	
Z 7595	P	--**	1					1	----	

* P = Pregnant; NP = Not Pregnant

** Not Recorded

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 317

Material FDA 71-70

Dose 3.63 mg/kg

Appendix II

Reproduction Data in Rabbits (Individual)

Date December 7, 1973

Laboratory No. 1767 e

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
E 7001	NP	--**	0						----	
E 7002	NP	--**	0						----	
E 7003	NP	--**	0						----	
E 7004	P	23	6	5		2	3	1	37.5	
E 7005	NP	--**	0						----	
E 7006	P	10	6	6		4	2		41.4	
E 7007	P	13	7	7		5	2		41.6	
E 7008	P	14	7	7		5	2		44.2	
E 7009	NP	--**	0						----	
E 7010	P	8	7	7		3	4		32.4	One Neonatal Death
E 7011	NP	--**	0						----	Died Day 10
E 7012	NP	--**	0						----	
E 7013	P	9	10	--		--	--		----	Died Day 17
E 7014	NP	--**	0						----	
E 7015	NP	--**	0						----	
E 7016	P	12	8	8		4	4		36.2	
E 7017	NP	--**	0						----	
E 7018	NP	--**	0						----	
E 7019	NP	6	0						----	
E 7020	NP	--**	0						----	
E 7091	P	8	6	6		3	3		37.5	
E 7092	NP	5	0						----	
E 7093	P	10	9	6	3	2	4		33.8	One Neonatal Death
E 7100	P	8	2	1		1	0	1	50.3	
E 7101	P	16	7	7		4	3		30.9	One Neonatal Death
E 7102	NP	5	0						----	
E 7113	P	14	2	1	1	--**	--**		30.1	
E 7114	P	7	3	3		1	2		41.6	

* P = Pregnant; NP = Not Pregnant

** Not Recorded

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 318

Material FDA 71-70

Dose 16.85 mg/kg

Appendix II

Reproduction Data in Rabbits (Individual)

Date December 7, 1973

Laboratory No. 1767 e

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
E 7021	NP	--**	0						----	
E 7022	NP	--**	0						----	
E 7023	P	10	4	4		2	2		36.5	
E 7024	NP	--**	0						----	
E 7025	NP	--**	0						----	
E 7026	NP	--**	0						----	
E 7027	NP	--**	0						----	
E 7028	P	5	1	1		1	0		49.4	
E 7029	P	7	5	--		--	--		----	Died Day 28
E 7030	NP	--**	0						----	
E 7031	P	15	9	9		5	4		29.9	
E 7032	P	4	3					3	----	
E 7033	P	10	7	7		4	3		30.4	
E 7034	P	--**	3	3		2	1		42.1	
E 7035	P	10	7	6		3	3	1	36.3	
E 7036	P	--**	6	5		2	3	1	36.8	One Neonatal Death
E 7037	P	8	3					3	----	
E 7038	P	5	1	1		1	0		52.2	
E 7039	P	7	4	2		2	0	2	40.3	
E 7040	NP	4	0						----	
E 7081	P	8	5	5		2	3		32.2	
E 7082	NP	--**	0						----	
E 7083	P	7	4	4		2***	1***		43.8	
E 7089	NP	0	0						----	
E 7090	P	12	7	3	4	2***	0***		31.8	

* P = Pregnant; NP = Not Pregnant

** Not Recorded

*** Not all Recorded

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FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 319

Material FDA 71-70

Dose 78.2 mg/kg

Appendix II

Reproduction Data in Rabbits (Individual)

Date December 7, 1973

Laboratory No. 1767 e

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
E 7041	NP	6	0						----	
E 7042	P	10	3	3		2	1		40.9	
E 7043	NP	--**	0						----	
E 7044	NP	8	0						----	
E 7045	NP	--**	0						----	
E 7046	NP	--**	0						----	
E 7047	NP	19	0						----	Died Day 16
E 7048	P	7	4	--		--	--		----	Died Day 13
E 7049	NP	--**	0						----	
E 7050	NP	--**	0						----	
E 7051	NP	--**	0						----	Died Day 8
E 7052	P	5	1	1		1	0		----**	
E 7053	NP	3	0						----	
E 7054	NP	--**	0						----	
E 7055	NP	5	0						----	
E 7056	P	9	3	3		2	1		43.0	
E 7057	P	6	5	5		3	2		40.9	
E 7058	NP	--**	0						----	
E 7059	P	10	3	--		--	--		----	Died Day 15
E 7060	NP	--**	0						----	
E 7084	NP	--**	0						----	
E 7085	NP	--**	0						----	
E 7086	NP	--**	0						----	
E 7087	NP	--**	0						----	
E 7088	P	3	1	1		1	0		45.2	
E 7096	P	6	1					1	----	

Continued on next page.

* P = Pregnant; NP = Not Pregnant

** Not Recorded

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 319

Material FDA 71-70

Dose 78.2 mg/kg

Appendix II

Reproduction Data in Rabbits (Individual)

Date December 7, 1973

Laboratory No. 1767 e

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
Continued.										
E 7097	NP	1	0						----	
E 7098	P	7	5	--		--	--		----	Died Day 14
E 7103	P	14	5	5		2	3		43.1	
E 7104	P	14	9	9		4	5		30.0	Two Neonatal Death
E 7106	P	12	4	4		2	2		46.3	
E 7115	P	11	7	7		4	3		36.7	
E 7116	NP	3	0						----	
E 7117	P	10	3	2		1	1	1	45.3	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 320

Material FDA 71-70

Dose 363.0 mg/kg

Appendix II

Reproduction Data in Rabbits (Individual)

Date December 7, 1973

Laboratory No. 1767 e

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
E 7061	NP	15	0						----	Died Day 8
E 7062	P	8	2	2		0	2		41.9	
E 7063	P	8	6	6		3	3		35.6	
E 7064	NP	--**	0						----	
E 7065	P	14	7	7		5	2		41.5	
E 7066	NP	10	0						----	
E 7067	P	7	6	6		3	3		35.8	
E 7068	NP	--**	0						----	
E 7069	NP	--**	0						----	
E 7070	NP	--**	0						----	
E 7071	P	--	--	--		--	--		----	Aborted Day 17
E 7072	P	15	3	3		3	0		41.1	
E 7073	NP	--**	0						----	
E 7074	NP	--**	0						----	
E 7075	NP	13	0						----	
E 7076	P	8	3	3		2	1		44.4	
E 7077	NP	--**	0						----	
E 7078	NP	--**	0						----	
E 7079	NP	--**	0						----	
E 7080	P	9	4	3		2	1	1	35.7	
E 7099	P	5	3	2		1	1	1	41.5	
E 7107	P	6	3	--		--	--		----	Died Day 27
E 7108	P	11	5	4		3	1	1	39.4	One Neonatal Death
E 7109	P	21	6	6		4	2		48.8	
E 7110	NP	3	0						----	
E 7111	NP	5	0						----	
E 7112	NP	2	0						----	

* P = Pregnant; NP = Not Pregnant

** Not Recorded

FOOD AND DRUG Research LABORATORIES, INC.

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FINAL REPORT

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date October 25, 1973

Laboratory No. 1765 e
Contract No. FDA 71-260

Sample: Fine white crystalline material

Marking: FDA 71-70 (Sodium chloride)

Examination Requested: Teratologic evaluation of FDA 71-70 in mice.

Procedure: See Appendix I

Results: See Tables 1 through 4 and Appendix II

Conclusion:

On the basis of the data presented herein, the following conclusion appears to be warranted:

"The administration of up to 480 mg/kg (body weight) of the test material to pregnant mice for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

FOOD and DRUG RESEARCH LABORATORIES, INC.

Kenneth Morgareidge

Kenneth Morgareidge, Ph.D.
Vice President

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FOOD AND DRUG RESEA LABORATORIES, INC.

Groups. 311 & 312; 317 through 320

Date: September 25, 1973

Table 1

Material: FDA 71-70

Laboratory No.: 1765 e

Fate Summary
(Mice)

Group	Material	Dose** mg/kg	Total		Surviving at Term	
			Mated	Pregnant	Total	Pregnant[1]
311	Sham	0.0	25	22	25	22
312	Aspirin*	150.0	25	20	25	20
317	FDA 71-70	4.80	25	22	25	22
318	FDA 71-70	22.3	30	22	30	22
319	FDA 71-70	100.4	25	23	25	23
320	FDA 71-70	480.0	25	21	25	21

* Positive Control: 150.0 mg/kg

** Administered as a water solution (10 ml per kg of body weight); (See Appendix I)

1) Includes all dams examined at term

FOOD AND DRUG RESEAR LABORATORIES, INC.

Gr 311 & 312; 317 through 320

Date: September 25, 1973

Material: FDA 71-70

Laboratory No. 1765 e

Table 2
Reproduction Data
(Mice)

Group:	311	312	317	318	319	320
Dose (mg/kg):	Sham	Aspirin**	4.80	22.3	100.4	480.0
Pregnancies						
Total No.	22	20	22	22	23	21
Died or Aborted (before Day 17)	0	0	0	0	0	0
To term (on Day 17)	22	20	22	22	23	21
Live Litters						
Total No.*	22	20	22	21	23	20
Implant Sites						
Total No.	249	210	256	261	269	236
Average/dam*	11.3	10.5	11.6	11.9	11.7	11.2
Resorptions						
Total No.*	11	10	17	28	18	17
Dams with 1 or more sites resorbed	7	8	11	12	8	8
Dams with all sites resorbed	--	--	--	1	--	1
Per cent partial resorptions	31.8	40.0	50.0	54.6	34.8	38.1
Per cent complete resorptions	--	--	--	4.55	--	4.76
Live Fetuses						
Total No.	232	196	236	232	251	216
Average/dam*	10.6	9.80	10.7	10.6	10.9	10.3
Sex ratio (M/F)	0.92	0.94	0.95	0.83	0.79	0.89
Dead Fetuses						
Total*	6	4	3	1	--	3
Dams with 1 or more dead	5	3	3	1	--	2
Dams with all dead	--	--	--	--	--	--
Per cent partial dead	22.7	15.0	13.6	4.55	--	9.52
Per cent all dead	--	--	--	--	--	--
Average Fetus Weight, g	0.89	0.87	0.94	0.86	0.88	0.89

* Includes only those dams examined at term.
** Positive Control: 150.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 311 & 312; 317 through 320 Laboratory No. 1765 e

Table 3

Material FDA 71-70 Date September 25, 1973

Summary of Skeletal Findings*
(Mice)

Findings / Group No.:	311	312	317	318	319	320
Dose (mg/kg):	Sham	Aspirin**	4.80	22.3	100.4	480.0
Live Fetuses Examined (at term)	162/22	137/20	163/22	163/21	175/23	151/20
Sternebrae						
Incomplete oss.	16/7	13/11	4/3	37/14	15/9	13/8
Scrambled						
Bipartite	2/2	13/10	3/3		9/8	3/2
Fused						
Extra						
Missing	18/7	17/9	11/6	17/9	20/9	23/11
Other						
Ribs						
Incomplete oss.				2/1		
Fused/split						
Wavy				2/2		
Less than 12				2/1		
More than 13	20/13	45/13	34/16	35/14	27/12	30/13
Other						
Vertebrae						
Incomplete oss.			1/1	7/5	7/5	3/3
Scrambled						
Fused						
Extra ctrs. oss.						
Scoliosis						
Tail defects						
Other						2/1
Skull						
Incomplete closure				3/2	1/1	
Missing						
Craniostosis						
Other						
Extremities						
Incomplete oss.		1/1	1/1	6/4	6/3	1/1
Missing						
Extra						
Miscellaneous						
Hyoid; missing	21/9	30/12	23/9	42/17	39/15	41/13
Hyoid; reduced	8/4	10/6	8/7	17/9	18/10	8/4

* Numerator=Number of fetuses affected; Denominator=Number of litters affected.

** Positive control: 150.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 311 & 312; 317 through 320 Date September 25, 1973

Material FDA 71-70 Laboratory No. 1765 e

Table 3-a
Summary of Soft Tissue Abnormalities
(Mice)

Group	Material	Dose Level mg/kg	Dam	Number of Pups	Description
311	Sham	0.0	S 5999	1	Cleft palate
			S 6005	1	Meningoencephalocele
312	Aspirin*	150.0	A 6020	1	Gastroschisis
317	FDA 71-70	4.80	E 5007	1	Umbilical hernia
318	FDA 71-70	22.3	E 5033	1	Cleft palate
319	FDA 71-70	100.4	E 5082	1	Cleft palate

*Positive Control: 150.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 311 & 312; 317 through 320

Date September 25, 1973

Table 4

Species Mice

Laboratory No. 1765 e

Average Body Weights*

Group	Material	Dose Level	Day 0	Day 6	Day 11	Day 15	Day 17**
		mg/kg	g				
311	Sham	0.0	26.8	30.4	35.3	43.6	48.8 (22)
312	Aspirin***	150.0	26.4	29.6	32.2	38.4	43.8 (20)
317	FDA 71-70	4.80	26.6	31.1	33.1	43.0	48.6 (22)
318	FDA 71-70	22.3	28.3	32.1	36.5	43.7	49.6 (22)
319	FDA 71-70	100.4	26.7	31.0	34.0	42.3	47.8 (23)
320	FDA 71-70	480.0	27.7	31.8	35.1	42.9	48.0 (21)

* Of pregnant dams
** Number of surviving dams in parentheses (c.f. Table 1)
*** Positive control: 150.0 mg/kg

Appendix I

Teratology Study in Mice

Virgin adult female albino CD-1 outbred mice were gang-housed in disposable plastic cages in temperature and humidity-controlled quarters with free access to food and fresh tap water. They were mated with young adult males, and observation of the vaginal sperm plug was considered Day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, the females were dosed with the indicated dosages by oral intubation; the controls were sham treated.

Body weights were recorded on Days 0, 6, 11, 15, and 17 of gestation. All animals were observed daily for appearance and behavior with particular attention to food consumption and weight, in order to rule out any abnormalities which may have occurred as a result of anorexic effects in the pregnant female animal.

On Day 17 all dams were subjected to Caesarean section under surgical anesthesia, and the numbers of implantation sites, resorption sites, and live and dead fetuses were recorded. The body weights of the live pups were also recorded. The urogenital tract of each dam was examined in detail for anatomical normality.

All fetuses were examined grossly for the presence of external congenital abnormalities. One-third of the fetuses of each litter underwent detailed visceral examinations employing the Wilson technique. The remaining two-thirds were cleared in potassium hydroxide (KOH), stained with alizarin red S dye and examined for skeletal defects.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 311

Material Sham

Dose 0.0 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date September 25, 1973

Laboratory No. 1765

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
S 5996	P	12	11	1	5	6		0.93	
S 5997	P	13	13		6	7		0.90	
S 5998	P	9	7		4	3	2	0.92	
S 5999	P	8	8		5	3		0.96	
S 6000	P	12	12		8	4		0.89	
S 6001	P	11	11		5	6		1.11	
S 6002	NP	0						----	
S 6003	P	11	9		4	5	2	0.79	
S 6004	NP	0						----	
S 6005	P	12	11	1	7	4		0.81	
S 6006	P	10	10		5	5		0.82	
S 6007	P	10	10		5	5		0.75	
S 6008	P	12	12		5	7		0.75	
S 6009	NP	0						----	
S 6010	P	9	9		6	3		0.81	
S 6011	P	13	13		7	6		0.88	
S 6012	P	14	12	2	3	9		0.80	
S 6013	P	7	6		4	2	1	0.89	
S 6014	P	13	13		7	6		0.93	
S 6015	P	15	12	1	5	7	2	0.74	
S 6016	P	15	13		5	8	2	1.07	
S 6017	P	11	10		5	5	1	1.15	
S 6018	P	15	14	1	3	11		0.83	
S 6019	P	9	8		3	5	1	0.84	
S 6020	P	8	8		4	4		0.97	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 312
Material Aspirin
Dose 150.0 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date September 25, 1973
Laboratory No. 1765

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
A 5996	P	9	9		4	5		0.81	
A 5997	P	11	10		5	5	1	0.84	
A 5998	P	6	6		3	3		0.96	
A 5999	P	6	6		3	3		1.31	
A 6000	NP	0						----	
A 6001	P	12	10		4	6	2	0.85	
A 6002	NP	0						----	
A 6003	P	9	9		3**	4**		1.10	
A 6004	P	10	9	1	4	5		0.83	
A 6005	P	9	8		3	5	1	0.73	
A 6006	P	14	14		9	5		0.84	
A 6007	P	13	11		3	8	2	0.83	
A 6008	P	14	11	2	5	6	1	0.75	
A 6009	NP	0						----	
A 6010	P	12	11		5	6	1	0.85	
A 6011	P	9	9		6	3		0.89	
A 6012	P	12	11	1	5	6		0.78	
A 6013	P	9	8		4	4	1	0.87	
A 6014	NP	0						----	
A 6015	P	11	11		6	5		0.80	
A 6016	NP	0						----	
A 6017	P	12	12		6	6		0.79	
A 6018	P	12	12		5	7		0.84	
A 6019	P	5	4		3	1	1	0.97	
A 6020	P	15	15		8	7		0.82	

* P = Pregnant; NP = Not Pregnant
** Not all recorded

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 317

Material FDA 71-70

Dose 4.80 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date September 25, 1973

Laboratory No. 1765 e

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
E 5001	P	13	10		5	5	3	0.88	
E 5002	NP	0						----	
E 5003	P	12	11		6	5	1	1.04	
E 5004	P	11	10		3	7	1	0.80	
E 5005	P	10	9		3	6	1	0.89	
E 5006	P	11	10		5	5	1	1.08	
E 5007	P	11	8		4	4	3	0.92	
E 5008	P	13	12		6	6	1	0.96	
E 5009	P	14	13		7	6	1	0.83	
E 5010	P	14	14		5	9		0.87	
E 5011	P	12	12		8	4		0.96	
E 5012	P	9	7	1	4	3	1	0.85	
E 5013	NP	0						----	
E 5014	P	9	9		3	6		1.21	
E 5015	P	11	11		5	6		0.85	
E 5016	P	13	12	1	8	4		1.16	
E 5017	P	12	11		5	6	1	0.88	
E 5018	P	11	10	1	4	6		0.99	
E 5019	NP	0						----	
E 5020	P	10	10		6	4		0.96	
E 5021	P	12	9		4	5	3	1.02	
E 5022	P	10	10		3	7		0.78	
E 5023	P	13	13		8	5		0.84	
E 5024	P	12	12		7	5		1.09	
E 5025	P	13	13		6	7		0.88	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 318

Material FDA 71-70

Dose 22.3 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date September 25, 1973

Laboratory No. 1765 e

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
E 5031	NP	0						----	
E 5032	P	11	7		5	2	4	1.17	
E 5033	P	15	13		5	8	2	0.87	
E 5034	P	11	10		4	6	1	0.89	
E 5035	NP	0						----	
E 5036	NP	0						----	
E 5037	P	12	10		3	7	2	0.88	
E 5038	NP	0						----	
E 5039	NP	0						----	
E 5040	P	11	11		3	8		0.90	
E 5041	P	13	13		6	7		0.91	
E 5042	P	12	12		8	4		0.91	
E 5043	P	9					9	----	
E 5044	P	9	8	1	5	3		1.01	
E 5045	P	16	14		6	8	2	0.84	
E 5046	P	9	8		2	6	1	0.77	
E 5047	P	9	9		5	4		0.84	
E 5048	P	11	11		4	7		1.18	
E 5049	P	14	14		6	8		0.79	
E 5050	P	8	6		2	4	2	0.65	
E 5051	P	13	13		5	8		0.76	
E 5052	P	14	13		8	5	1	0.76	
E 5053	NP	0						----	
E 5054	P	11	10		4	6	1	0.80	
E 5055	P	15	13		8	5	2	0.83	
E 5056	NP	0						----	
E 5057	NP	0						----	
E 5058	P	14	14		6	8		0.77	
E 5059	P	11	10		5	5	1	0.83	
E 5060	P	13	13		5	8		0.78	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 319

Material FDA 71-70

Dose 100.4 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date September 25, 1973

Laboratory No. 1765 e

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
E 5061	P	12	11		6	5	1	0.93	
E 5062	P	11	11		6	5		1.04	
E 5063	P	13	8		4	4	5	0.86	
E 5064	P	13	13		4	9		1.06	
E 5065	NP	0						----	
E 5066	P	11	8		4	4	3	0.93	
E 5067	P	10	10		5	5		0.80	
E 5068	P	14	14		6	8		0.90	
E 5069	P	12	12		6	6		0.97	
E 5070	NP	0						----	
E 5071	P	10	9		6	3	1	0.89	
E 5072	P	15	14		9	5	1	1.21	
E 5073	P	14	14		6	8		0.75	
E 5074	P	14	14		7	7		0.94	
E 5075	P	10	9		2	7	1	0.83	
E 5076	P	10	10		2	8		0.73	
E 5077	P	12	12		2	10		0.70	
E 5078	P	6	6		2	4		0.74	
E 5079	P	8	8		2	6		0.93	
E 5080	P	14	14		5	9		0.75	
E 5081	P	11	10		7	3	1	0.78	
E 5082	P	11	6		4	2	5	1.01	
E 5083	P	12	12		5	7		0.84	
E 5084	P	13	13		5	8		0.79	
E 5085	P	13	13		6	7		0.82	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 320

Material FDA 71-70

Dose 480.0 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date September 25, 1973

Laboratory No. 1765 e

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
E 5091	P	10	9		4	5	1	0.90	
E 5092	P	13	12		5	7	1	0.87	
E 5093	P	13	11		4	7	2	0.89	
E 5094	P	12	11	1	5	6		0.98	
E 5095	P	11	11		6	5		0.96	
E 5096	P	12	12		8	4		0.86	
E 5097	NP	0						----	
E 5098	P	12	11		7	4	1	1.00	
E 5099	P	10	10		4	6		0.87	
E 5100	NP	0						----	
E 5101	NP	0						----	
E 5102	P	7	6		2	4	1	0.77	
E 5103	P	15	14		6	8	1	1.06	
E 5104	P	12	12		5	7		0.75	
E 5105	P	7	7		3	4		1.17	
E 5106	P	11	11		4	7		0.78	
E 5107	P	12	12		5	7		0.85	
E 5108	P	14	14		8	6		0.82	
E 5109	P	14	11	2	5	6	1	0.74	
E 5110	P	13	13		8	5		0.81	
E 5111	P	2	2		1	1		1.22	
E 5112	P	15	15		6	9		0.77	
E 5113	NP	0						----	
E 5114	P	12	12		6	6		0.80	
E 5115	P	9					9	----	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG Research LABORATORIES, INC.

WAVERLY DIVISION
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(607) 565-2931

FINAL REPORT

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date October 25, 1973

Laboratory No. 1766 e
Contract No. FDA 71-260

Sample: Fine white crystalline material

Marking: FDA 71-70 (Sodium chloride)

Examination Requested: Teratologic evaluation of FDA 71-70 in rats

Procedure: See Appendix I

Results: See Tables 1 through 4 and Appendix II

Conclusion:

On the basis of the data presented herein, the following conclusion appears to be warranted:

"The administration of up to 368 mg/kg (body weight) of the test material to pregnant rats for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

FOOD and DRUG RESEARCH LABORATORIES, INC.

Kenneth Morgareidge

Kenneth Morgareidge, Ph.D.
Vice President

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FOOD AND DRUG RESEA LABORATORIES, INC.

Group 311 & 312; 317 through 320

Date: September 25,

Material: FDA 71-70

Laboratory No.: 1766 e

Table 1

Fate Summary
(Rats)

Group	Material	Dose ** mg/kg	Total		Surviving at Term	
			Mated	Pregnant	Total	Pregnant[1]
311	Sham	0.0	25	21	24	21
312	Aspirin*	250.0	25	21	25	21
317	FDA 71-70	3.68	25	21	24	21
318	FDA 71-70	17.1	25	22	25	22
319	FDA 71-70	79.4	25	23	25	23
320	FDA 71-70	368.0	25	19	25	19

* Positive Control: 250.0 mg/kg
** Administered as a water solution (See Appendix I)
1) Includes all dams examined at term

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FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group: 311 & 312; 317 through 320

Date: September 25, 1973

Material: FDA 71-70

Laboratory No. 1766 e

Table 2
Reproduction Data
(Rats)

Group:	311	312	317	318	319	320
Dose (mg/kg):	Sham	Aspirin**	3.68	17.1	79.4	368.0
Pregnancies						
Total No.	21	21	21	22	23	19
Died or Aborted (before Day 20)	0	0	0	0	0	0
To term (on Day 20)	21	21	21	22	23	19
Live Litters						
Total No.*	21	21	21	21	23	19
Implant Sites						
Total No.	231	240	237	232	260	224
Average/dam*	11.0	11.4	11.3	10.6	11.3	11.8
Resorptions						
Total No.*	5	55	3	2	4	3
Dams with 1 or more sites resorbed	2	10	2	2	3	3
Dams with all sites resorbed	--	--	--	1	--	--
Per cent partial resorptions	9.52	47.6	9.52	9.09	13.0	15.8
Per cent complete resorptions	--	--	--	4.55	--	--
Live Fetuses						
Total No.	226	185	234	230	256	221
Average/dam*	10.8	8.81	11.1	10.5	11.1	11.6
Sex ratio (M/F)	1.11	0.73	1.19	0.80	1.33	1.10
Dead Fetuses						
Total*	--	--	--	--	--	--
Dams with 1 or more dead	--	--	--	--	--	--
Dams with all dead	--	--	--	--	--	--
Per cent partial dead	--	--	--	--	--	--
Per cent all dead	--	--	--	--	--	--
Average Fetus Weight, g	3.69	2.30	3.73	3.70	3.74	3.74

* Includes only those dams examined at term.
** Positive Control: 250.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 311 & 312; 317 through 320 Laboratory No. 1766 e

Table 3

Material FDA 71-70 Date September 25, 1973

Summary of Skeletal Findings*

(Rats)

Findings / Group No.:	311	312	317	318	319	320
Dose (mg/kg):	Sham	Aspirin**	3.68	17.1	79.4	368.0
Live Fetuses Examined (at term)	157/21	127/20[a]	162/21	161/21	178/23	154/19
Sternebrae						
Incomplete oss.	51/16	50/19	38/14	49/15	28/15	37/13
Scrambled						
Bipartite	1/1	18/12				
Fused						
Extra						
Missing	12/8	125/20	9/6	8/6	11/5	10/7
Other						
Ribs						
Incomplete oss.		32/11			3/3	
Fused/split		3/3				
Wavy	21/9	70/17	17/10	22/11	19/10	19/10
Less than 12	1/1					
More than 13	4/3	109/20	4/2			1/1
Other						
Vertebrae						
Incomplete oss.	22/9	94/19	9/6	28/13	10/5	16/7
Scrambled						
Fused						
Extra ctrs. oss.						
Scoliosis						
Tail defects						
Other						
Skull						
Incomplete closure	23/10	77/17	26/13	28/13	31/13	30/12
Missing		5/4				
Craniostosis						
Other						
Extremities						
Incomplete oss.		11/5				
Missing						
Extra						
Miscellaneous						
Hyoid; missing	13/5	65/16	16/9	15/7	14/6	5/4
Hyoid; reduced	32/15	13/7	23/12	28/11	27/12	13/9

* Numerator=Number of fetuses affected; Denominator=Number of litters affected.

** Positive control: 250.0 mg/kg

[a] One litter (2 pups) lost in processing

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 311 & 312; 317 through 320 Date September 25, 1973

Material FDA 71-70 Laboratory No. 1766 e

Table 3-a
Summary of Soft Tissue Abnormalities
(Rats)

Group	Material	Dose Level mg/kg	Dam	Number of Pups	Description
311	Sham	0.0	S 6005	1	Cleft palate
312	Aspirin*	250.0	A 6996	2	Encephalomyelocele
			A 6998	1	Acystia; agonadal
			A 6999	1	Hydrocephalus
			A 7008	2	Encephalomeylocele; gastroschisis
				1	Meningoencephalocele
				1	Acystia
			A 7011	1	Hydrocephalus
			A 7017	1	Encephalomyelocele; exophthalmos
				1	Spina bifida
320	FDA 71-70	368.0	E 7107	1	Umbilical hernia

*Positive Control: 250.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 311 & 312; 317 through 320

Date September 25, 1973

Table 4

Species Rats

Laboratory No. 1766 e

Average Body Weights*

Group	Material	Dose Level	Day 0	6	11	15	20**
		mg/kg	g				
311	Sham	0.0	225	241	255	278	339 (21)
312	Aspirin***	250.0	225	245	256	274	321 (21)
317	FDA 71-70	3.68	213	231	236	269	338 (21)
318	FDA 71-70	17.1	227	247	266	284	349 (22)
319	FDA 71-70	79.4	214	233	249	267	335 (23)
320	FDA 71-70	368.0	222	241	256	279	351 (19)

* Of pregnant dams
** Number of surviving dams in parentheses (c.f. Table 1)
*** Positive control: 250.0 mg/kg

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FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 311

Material Sham

Dose 0.0 mg/kg

Appendix II

Reproduction Data in Rats (Individual)

Date September 25, 1973

Laboratory No. 1766

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
S 6996	NP	0						----	
S 6997	P	12	12		7	5		3.84	
S 6998	P	10	10		5	5		3.76	
S 6999	--	--	--		--	--		----	Sacrificed Day 6
S 7000	P	8	8		4	4		4.02	
S 7001	P	14	14		9	5		3.95	
S 7002	P	11	11		7	4		3.89	
S 7003	P	11	11		6	5		3.62	
S 7004	P	12	12		4	8		3.59	
S 7005	P	10	10		5	5		3.25	
S 7006	P	10	10		4	6		3.53	
S 7007	P	12	12		6	6		3.43	
S 7008	P	12	12		6	6		3.69	
S 7009	P	13	13		6	7		3.69	
S 7010	P	12	12		6	6		3.90	
S 7011	P	12	11		9	2	1	3.99	
S 7012	P	13	13		5	8		3.53	
S 7013	P	11	11		6	5		3.93	
S 7014	P	10	10		5	5		3.47	
S 7015	NP	0						----	
S 7016	P	13	9		5	4	4	3.61	
S 7017	P	9	9		5	4		3.68	
S 7018	P	8	8		3	5		3.30	
S 7019	P	8	8		6	2		3.79	
S 7020	NP	0						----	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Appendix II

Reproduction Data in Rats (Individual)

Group 312

Material Aspirin

Dose 250.0 mg/kg

Date September 25, 1973

Laboratory No. 1766

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
A 6996	P	13	13		7	6		2.43	
A 6997	P	10	10		4	6		2.66	
A 6998	P	14	4		2	2	10	2.06	
A 6999	P	11	8		3	5	3	2.24	
A 7000	P	12	12		6	6		2.41	
A 7001	NP	0						----	
A 7002	NP	0						----	
A 7003	P	12	1		0	1	11	1.78	
A 7004	P	13	13		6	7		2.69	
A 7005	P	10	7		2	5	3	1.99	
A 7006	P	13	13		6	7		2.68	
A 7007	NP	0						----	
A 7008	P	13	9		3	6	4	1.93	
A 7009	P	9	3		1	2	6	1.89	
A 7010	P	13	12		5	7	1	2.49	
A 7011	P	12	9		4	5	3	2.61	
A 7012	P	12	12		8	4		2.02	
A 7013	P	11	2		1	1	9	2.03	
A 7014	P	10	10		7	3		3.13	
A 7015	P	7	2		0	2	5	1.50	
A 7016	P	11	11		2	9		2.08	
A 7017	P	13	13		6	7		2.68	
A 7018	NP	0						----	
A 7019	P	9	9		1	8		2.51	
A 7020	P	12	12		4	8		2.44	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 317

Material FDA 71-70

Dose 3.68 mg/kg

Appendix II

Reproduction Data in Rats (Individual)

Date September 25, 1973

Laboratory No. 1766 e

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
E 6001	P	11	11		7	4		4.06	
E 6002	P	12	12		4	8		3.85	
E 6003	P	13	13		6	7		3.65	
E 6004	--	--	--		--	--		----	Sacrificed Day 9
E 6005	NP	0						----	
E 6006	P	10	10		7	3		3.76	
E 6007	P	13	13		6	7		3.92	
E 6008	P	11	11		4	7		3.96	
E 6009	P	11	11		5	6		3.50	
E 6010	P	15	15		10	5		3.78	
E 6011	P	13	12		6	6	1	3.80	
E 6012	P	11	11		7	4		4.06	
E 6013	P	7	5		5	0	2	3.28	
E 6014	P	12	12		7	5		3.73	
E 6015	P	13	13		8	5		3.44	
E 6016	P	13	13		7	6		3.84	
E 6017	P	10	10		6	4		3.70	
E 6018	P	12	12		6	6		3.52	
E 6019	P	9	9		5	4		3.75	
E 6020	P	11	11		5	6		3.43	
E 6021	P	10	10		6	4		3.95	
E 6022	P	11	11		4	7		3.41	
E 6023	NP	0						----	
E 6024	NP	0						----	
E 6025	P	9	9		6	3		4.02	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 318

Material FDA 71-70

Dose 17.1 mg/kg

Appendix II

Reproduction Data in Rats (Individual)

Date September 25, 1973

Laboratory No. 1766 e

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
E 6031	P	13	13		3	10		3.88	
E 6032	P	13	13		6	7		3.57	
E 6033	P	11	11		4	7		3.68	
E 6034	P	9	9		6	3		3.86	
E 6035	P	11	10		5	5	1	3.91	
E 6036	P	11	11		5	6		3.37	
E 6037	P	3	3		0	3		4.03	
E 6038	P	12	12		5	7		3.30	
E 6039	NP	0						----	
E 6040	P	13	13		7	6		3.60	
E 6041	P	15	15		6	9		3.59	
E 6042	P	14	14		5	9		3.42	
E 6043	P	13	13		5	8		3.33	
E 6044	NP	0						----	
E 6045	P	13	13		5	8		3.62	
E 6046	P	11	11		7	4		3.89	
E 6047	P	10	10		5	5		3.81	
E 6048	P	9	9		6	3		3.89	
E 6049	P	10	10		4	6		3.78	
E 6050	P	8	8		4	4		3.87	
E 6051	P	12	12		6	6		3.84	
E 6052	P	1					1	----	
E 6053	NP	0						----	
E 6054	P	9	9		3	6		3.79	
E 6055	P	11	11		5	6		3.64	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 319

Material FDA 71-70

Dose 79.4 mg/kg

Appendix II

Reproduction Data in Rats (Individual)

Date September 25, 1973

Laboratory No. 1766 e

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
E 6061	P	11	11		6	5		3.97	
E 6062	P	13	13		9	4		3.99	
E 6063	P	16	16		9	7		3.79	
E 6064	NP	0						----	
E 6065	P	12	12		6	6		3.25	
E 6066	P	10	10		3	7		3.51	
E 6067	P	11	11		11	0		3.91	
E 6068	P	12	12		6	6		4.17	
E 6069	P	9	9		5	4		3.17	
E 6070	P	12	12		6	6		3.60	
E 6071	P	12	12		2	10		3.60	
E 6072	P	12	12		7	5		4.12	
E 6073	P	12	12		9	3		3.87	
E 6074	P	11	11		6	5		3.17	
E 6075	P	12	12		9	3		4.49	
E 6076	P	14	14		7	7		3.58	
E 6077	P	6	6		4	2		3.71	
E 6078	P	12	11		6	5	1	3.53	
E 6079	P	10	10		5	5		3.27	
E 6080	NP	0						----	
E 6081	P	12	12		8	4		3.89	
E 6082	P	11	11		6	5		3.56	
E 6083	P	8	7		3	4	1	4.16	
E 6084	P	11	9		8	1	2	3.86	
E 6085	P	11	11		5	6		3.93	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 320

Material FDA 71-70

Dose 368.0 mg/kg

Appendix II

Reproduction Data in Rats (Individual)

Date September 25, 1973

Laboratory No. 1766 e

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
E 6091	P	14	14		9	5		3.97	
E 6092	P	14	14		6	8		3.75	
E 6093	P	13	13		7	6		3.74	
E 6094	P	12	11		4	7	1	3.79	
E 6095	P	11	11		6	5		3.59	
E 6096	NP	0						----	
E 6097	P	11	11		5	6		3.72	
E 6098	P	12	12		4	8		3.57	
E 6099	NP	0						----	
E 6100	NP	0						----	
E 6101	NP	0						----	
E 6102	P	15	14		8	6	1	3.51	
E 6103	P	13	13		10	3		4.81	
E 6104	P	11	11		5	6		3.60	
E 6105	P	12	12		6	6		3.54	
E 6106	P	12	12		4	8		3.67	
E 6107	P	13	13		9	4		3.69	
E 6108	P	9	9		6	3		4.18	
E 6109	NP	0						----	
E 6110	P	11	10		7	3	1	3.65	
E 6111	NP	0						----	
E 6112	P	12	12		6	6		3.64	
E 6113	P	9	9		5	4		3.57	
E 6114	P	10	10		6	4		3.81	
E 6115	P	10	10		3	7		3.26	

* P = Pregnant; NP = Not Pregnant

THE EFFECTS OF DEFICIENCY OF B VITAMINS ON SALT TOXICITY IN THE RAT

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Although excessive intake of sodium has long been suspected as an important factor in the production of arterial hypertension in man (Allen, '20), little work has been reported on the effects of sodium chloride toxicity. Meneely et al. ('52) reported that rats fed diets containing from 2.8 to 9.8% of sodium chloride grew more slowly than those fed a similar diet containing 0.15% of sodium chloride. In further studies, Meneely et al. ('53) observed sustained arterial hypertension in rats fed excessive amounts of sodium chloride for 9 months, and noted a linear relationship between the level of sodium chloride in the diet and the systolic blood pressure. Meyer ('54, '55) studied interrelationships between sodium chloride and protein levels in diets for growing rats. He concluded that in animals fed ad libitum, the growth depression caused by the addition of excessive sodium chloride to the diet was independent of the dietary protein level. However, increasing the dietary protein level resulted in much greater renal hypertrophy in animals receiving 15.5% of sodium chloride, than in those receiving only 0.5% of sodium chloride.

Schaefer ('57) presented evidence, from preliminary experiments, that the toxicity of excessive sodium chloride is influenced by the intake of B vitamins and reported sea salt to be somewhat less toxic than an equivalent amount of sodium chloride. In the present studies, two experiments, one of 6 weeks and the other of 17 weeks duration were conducted to study the effects of deficiency of B vitamins on salt toxicity

in the rat. The results of both experiments show that excessive amounts of either sodium chloride or sea salt are much more toxic to rats fed diets deficient in B vitamins than to those animals receiving adequate amounts of B vitamins. Evidence was also obtained indicating that the cardiac hypertrophy brought about by administration of excessive sodium chloride to animals fed an otherwise adequate diet is partially overcome by addition of potassium chloride to the diet. Since the results of both experiments were in close agreement, only the results of the longer-term study are reported in detail herein.

EXPERIMENTAL

The basal 18% casein diet used in the experiment was similar to that of Sarett and Snipper ('54), except that sucrose was used as the carbohydrate, and certain changes were made in the amounts of B vitamins added to the diet. Twelve comparable groups of 10 male weanling rats each (McCollum-Wisconsin strain) were selected on the basis of litter origin and body weight. Six of the groups received the basal diet alone or supplemented with 3 or 6% of sodium chloride, 4 or 8% of sea salt (containing approximately 75% of sodium chloride) or 6% of sodium chloride and 2% of potassium chloride. These groups received the following amounts of B vitamins per 100 gm of diet: thiamine hydrochloride, 0.25 mg; riboflavin, 0.5 mg; niacinamide, 5.0 mg; calcium pantothenate, 2.0 mg; pyridoxine hydrochloride, 0.25 mg; folic acid, 0.2 mg; biotin, 0.02 mg; vitamin B_{12}, 0.01 mg; choline bitartrate, 200.0 mg; inositol, 100.0 mg; and *p*-aminobenzoic acid, 10.0 mg. The levels of thiamine, riboflavin, pyridoxine, pantothenate and choline used were twice those listed by Brown and Sturtevant ('49) as the levels required by the growing rat. The remaining 6 groups received similar diets containing 30% of the levels of the water-soluble vitamins given to the other animals. The sea salt was donated [1] and the sodium chloride and potassium chloride were reagent grade chemicals.

[1] Trace Elements Corporation, Houston, Texas.

TABLE 1

Data on weight gains, ad libitum food and water intakes and caloric efficiencies of male weanling rats fed otherwise adequate diets containing different levels of B vitamins, sodium chloride, potassium chloride and sea salt for 17 weeks

DIET NO. AND DESCRIPTION	NO. OF SURVIVORS [1]	INITIAL WEIGHT	GAIN IN WEIGHT	FOOD INTAKE	WATER INTAKE		CALORIC EFFICIENCY [2]
		gm	*gm*	*gm*	*ml*	*ml/100 gm food*	
Inadequate B vitamins							
1 Control	10	45	149 ± 42 [3]	1057	1331	126	32.2 ± 7.1
2 + 3% NaCl	9	45	118 ± 54	930	1997	215	30.1 ± 10.8
3 + 6% NaCl	10	45	92 ± 33	941	2861	304	25.2 ± 8.5
4 + 4% sea salt	9	45	78 ± 31	760	1675	220	25.3 ± 7.9
5 + 8% sea salt	8	45	111 ± 68	975	3854	395	28.3 ± 9.8
6 + 6% NaCl + 2% KCl	6	45	92 ± 35	972	3221	331	24.9 ± 9.5
Adequate B vitamins							
7 Control	10	47	320 ± 32	1753	2068	118	44.0 ± 5.0
8 + 3% NaCl	10	48	307 ± 37	1776	4181	235	42.9 ± 3.5
9 + 6% NaCl	10	47	305 ± 62	1806	6166	341	42.8 ± 8.3
10 + 4% sea salt	9	47	319 ± 28	1686	3872	230	47.0 ± 2.4
11 + 8% sea salt	10	47	290 ± 34	1783	7391	415	42.9 ± 5.8
12 + 6% NaCl + 2% KCl	10	48	294 ± 27	1859	6746	363	41.3 ± 4.3

[1] Each group contained 10 animals initially.
[2] Grams gain per 1000 Cal.
[3] Standard deviation.

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received the control diet deficient in B vitamins (diet 1) gained only 149 gm, with a significantly lower caloric efficiency of 32.2 ($P < 0.01$, by "t" test). These adverse effects of vitamin deficiency on weight gain and caloric efficiency were also observed during the earlier weeks of the experiment (see fig. 1).

In the animals which received adequate amounts of B vitamins (diets 7 to 12), sodium chloride, sea salt or the combina-

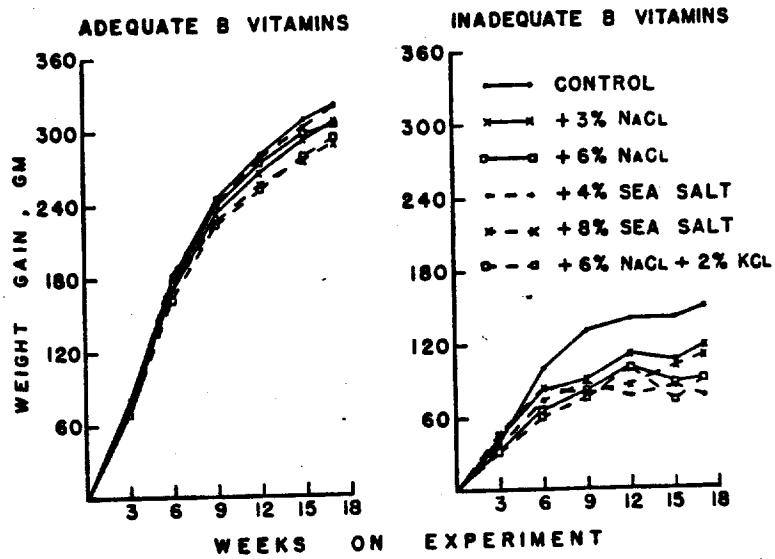

Fig. 1 Effect of excess salt on weight gains of male weanling rats fed diets containing adequate or inadequate levels of B vitamins.

tion of sodium and potassium chlorides had no significant effects on weight gain or caloric efficiency. However, addition to the vitamin-deficient control diet of 3 or 6% of sodium chloride (diets 2 and 3), or equivalent amounts of sea salt (diets 4 and 5), resulted in significantly reduced weight gain ($P < 0.01$) and somewhat lower caloric efficiency. The adverse effects of 6% of sodium chloride on the weight gain and caloric efficiency of the vitamin-deficient animals, were not influenced by addition of 2% of potassium chloride to the diet (diet 6).

Polydipsia and polyuria were observed in the animals which received the diets containing added salt. The animals which received diets containing 3% of sodium chloride (diets 2 and 8) consumed amounts of water per 100 gm of food intake comparable to those which received similar diets containing 4% of sea salt (diets 4 and 10). However, the animals fed the two diets containing 8% of sea salt (diets 5 and 11) consumed more water per 100 gm food intake than those fed the corresponding diets containing 6% of sodium chloride (diets 3 and 9), or 6% of sodium chloride and 2% of potassium chloride (diets 6 and 12).

The data on organ weights are summarized in table 2. The livers, kidneys, and hearts of the animals which received the vitamin-deficient control diet (diet 1) were somewhat larger per unit of body weight, than those of the animals fed the adequate control diet (diet 7). These differences are probably related, in part, to the slower rate of growth and smaller body size of the animals fed the vitamin-deficient diet. The marked adrenal hypertrophy observed in the vitamin-deficient animals is also probably a reflection of the stress of vitamin deficiency.

In the vitamin-deficient animals, but not in those fed adequate amounts of B vitamins, addition to the control diet of sodium chloride, sea salt or the mixture of sodium chloride and potassium chloride, resulted in somewhat larger livers and adrenals, per unit of body weight.

Addition to the vitamin-deficient control diet of 3 or 6% of sodium chloride, equivalent amounts of sea salt, or 6% of sodium chloride and 2% of potassium chloride, resulted in similar significant increases in the relative weight of the kidneys ($P < 0.01$). However, in the animals which received adequate B vitamins, the lower levels of sodium chloride and sea salt had no significant effect on kidney size, although the higher levels of the two substances, and the mixture of 6% of sodium chloride and 2% of potassium chloride, significantly increased the relative size of the kidneys ($P < 0.01$). Histological examination of the kidneys of two animals fed each

TABLE 2

Data on liver, heart, kidney and adrenal weights of male weanling rats fed otherwise adequate diets containing different levels of B vitamins, sodium chloride, potassium chloride and sea salt for 17 weeks

DIET NO. AND DESCRIPTION	NO. OF SURVIVORS [1]	LIVER		HEART		KIDNEY		ADRENAL	
		gm	*gm/100 gm body weight*	*mg*	*mg/100 gm body weight*	*gm*	*gm/100 gm body weight*	*mg*	*mg/100 gm body weight*
Inadequate B vitamins									
1 Control	10	5.3	2.9 ± 0.2 [2]	612	337 ± 33	1.65	0.90 ± 0.11	35.8	20.1 ± 4.2
2 + 3% NaCl	9	4.8	3.2 ± 0.5	576	392 ± 59	1.62	1.12 ± 0.23	31.2	22.2 ± 6.1
3 + 6% NaCl	10	4.2	3.3 ± 0.3	512	409 ± 46	1.53	1.23 ± 0.13	27.6	22.5 ± 4.8
4 + 4% sea salt	9	3.7	3.3 ± 0.6	479	426 ± 70	1.38	1.24 ± 0.19	27.9	25.5 ± 6.8
5 + 8% sea salt	8	4.8	3.3 ± 0.3	598	411 ± 47	1.63	1.17 ± 0.17	28.9	21.6 ± 5.7
6 + 6% NaCl + 2% KCl	6	4.1	3.4 ± 0.6	504	414 ± 81	1.49	1.23 ± 0.21	25.4	21.5 ± 6.5
Adequate B vitamins									
7 Control	10	9.3	2.7 ± 0.2	995	286 ± 27	2.35	0.68 ± 0.09	42.4	12.2 ± 1.7
8 + 3% NaCl	10	9.1	2.7 ± 0.3	1028	298 ± 34	2.38	0.75 ± 0.07	43.2	13.1 ± 2.0
9 + 6% NaCl	10	9.3	2.8 ± 0.3	1156	361 ± 93	2.71	0.83 ± 0.09	44.8	14.4 ± 6.5
10 + 4% sea salt	9	9.2	2.7 ± 0.2	1020	298 ± 31	2.52	0.73 ± 0.07	39.9	11.6 ± 0.8
11 + 8% sea salt	10	9.3	3.0 ± 0.4	1256	402 ± 77	2.52	0.80 ± 0.08	40.6	13.1 ± 3.5
12 + 6% NaCl + 2% KCl	10	8.4	2.6 ± 0.2	993	311 ± 31	2.56	0.80 ± 0.06	39.7	12.4 ± 1.2

[1] Each group contained 10 animals initially.

[2] Standard deviation.

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diet[3] failed to reveal any marked abnormalities of structure. No evidence of tubular swelling, necrosis or degenerative changes was noted. The kidney tubules were free of epithelial debris, casts and cellular exudates.

In the vitamin-deficient animals, addition to the diet of 3 or 6% of sodium chloride, equivalent amounts of sea salt, or 6% of sodium chloride and 2% of potassium chloride, resulted in similar increases in the relative weight of the heart. These results are in contrast to the findings with the animals fed adequate B vitamins, in which only the higher levels of sodium chloride and sea salt caused significant cardiac hypertrophy. Cardiac hypertrophy has been observed previously, by other investigators, in rats fed high levels of sodium chloride (Tucker et al., '54; Ball and Meneely, '57). In the animals fed adequate B vitamins, the mixture of 6% of sodium chloride and 2% of potassium chloride resulted in less cardiac hypertrophy than was found with 6% of sodium chloride alone. These results, which indicate an ameliorating effect of potassium chloride on sodium chloride toxicity, are in line with the observations of Meneely et al. ('56), that addition of potassium chloride to the diet increased the survival time of rats fed toxic levels of sodium chloride.

When calculated as a percentage of the fresh weight, the level of carcass moisture (table 3) tended to be greater in the animals fed inadequate amounts of B vitamins (diets 1 to 6), than in those which received adequate amounts of B vitamins (diets 7 to 12), but was not significantly influenced by the level of salt in the diet. However, when calculated on the basis of the fat-free fresh weight, the level of carcass moisture was apparently uninfluenced by the dietary B vitamin or salt intakes of the animals, and tended to be a constant value. These latter results are in agreement with those of Pace and Rathbun ('45) who advanced the concept of a fat-free body mass of relatively constant gross chemical composition.

[3] This was done by Dr. C. C. Erickson, University of Tennessee, Knoxville.

TABLE 3

Data on fasting weight losses and carcass composition of male weanling rats fed otherwise adequate diets containing different levels of B vitamins, sodium chloride, potassium chloride and sea salt for 17 weeks

DIET NO. AND DESCRIPTION	NO. OF SURVIVORS [1]	CARCASS COMPOSITION				
		MOISTURE		Ash	Fat	Protein
		Wet weight	Fat-free weight			
		%	%	%	%	%
Inadequate B vitamins						
1 Control	10	65.7 ± 1.8 [2]	71.0 ± 0.7	4.8 ± 0.4	7.5 ± 2.6	23.5 ± 1.0
2 + 3% NaCl	9	66.7 ± 1.2	70.9 ± 0.7	5.1 ± 1.0	5.9 ± 2.3	24.4 ± 0.6
3 + 6% NaCl	10	67.2 ± 1.0	70.9 ± 1.1	5.2 ± 0.7	5.3 ± 1.4	23.9 ± 0.7
4 + 4% sea salt	9	67.4 ± 1.1	70.7 ± 1.6	5.6 ± 0.6	4.8 ± 1.4	24.2 ± 0.6
5 + 8% sea salt	8	66.4 ± 2.1	70.8 ± 1.5	5.3 ± 0.8	6.2 ± 3.3	23.9 ± 0.9
6 + 6% NaCl + 2% KCl	6	66.1 ± 1.1	70.8 ± 0.9	5.3 ± 0.7	6.1 ± 2.0	23.9 ± 1.0
Adequate B vitamins						
7 Control	10	61.7 ± 2.7	71.2 ± 0.7	3.7 ± 0.3	13.4 ± 3.3	22.7 ± 0.9
8 + 3% NaCl	10	61.7 ± 1.3	71.0 ± 0.4	3.8 ± 0.2	13.1 ± 1.7	23.0 ± 0.6
9 + 6% NaCl	10	62.2 ± 3.0	71.0 ± 0.4	4.0 ± 0.6	12.3 ± 4.5	23.1 ± 1.3
10 + 4% sea salt	9	60.0 ± 2.5	71.1 ± 0.4	3.7 ± 0.4	15.6 ± 3.4	22.2 ± 1.0
11 + 8% sea salt	10	63.5 ± 2.0	71.1 ± 1.0	3.8 ± 0.2	11.0 ± 2.3	23.0 ± 0.7
12 + 6% NaCl + 2% KCl	10	61.0 ± 1.8	70.9 ± 0.5	3.7 ± 0.2	14.0 ± 2.1	22.7 ± 0.5

[1] Each group contained 10 animals initially.

[2] Standard deviation.

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The level of carcass fat was approximately twice as high in the animals which received adequate amounts of B vitamins as in those which received the vitamin-deficient diets and was not significantly influenced by the level of salt in the diet.

Carcass ash values were significantly higher in the animals fed the vitamin deficient control diet (diet 1) than in those fed the control diet containing adequate B vitamins ($P < 0.01$). This difference is probably related to the greater skeleton to body weight ratio found in the smaller vitamin-deficient animals. No effects of the salt level of the diet on carcass ash values were seen in the animals which received sufficient B vitamins, whereas in those receiving vitamin-deficient diets, the carcass ash values were slightly, but not significantly, increased by the addition of sodium chloride, sea salt or the mixture of sodium and potassium chlorides to the diet.

The results of the present experiment indicate that the toxicity of excess sodium chloride or sea salt is markedly influenced by the B vitamin intake. The small vitamin-deficient animal is evidently less able to withstand the additional stress of high dietary salt levels than is the animal which has received adequate B vitamins. Other investigators have also shown that nutritional factors are involved in the ability of animals to withstand stress (Dumm and Ralli, '50; Ershoff, '52).

SUMMARY

An experiment was conducted to determine the effects of B vitamin deficiency on the toxicity of excess salt for the growing rat.

During a 17-week experimental period, the weight gain and caloric efficiency of animals which received an otherwise adequate diet deficient in B vitamins were significantly less than those of animals fed a similar diet containing adequate amounts of B vitamins. In the vitamin-deficient animals, but not in those which received adequate B vitamins, addition to the diet of 3 or 6% of sodium chloride, or equivalent amounts of sea salt (4 and 8%) resulted in significantly decreased weight gain and somewhat lower caloric efficiency.

Addition to the vitamin-deficient diet of 3 or 6% of sodium chloride, or equivalent amounts of sea salt, resulted in similar significant increases in the relative weight of the kidneys, adrenals and hearts of the animals. However, in the animals which received adequate B vitamins, only the higher levels of sodium chloride and sea salt caused significant renal and cardiac hypertrophy. The cardiac hypertrophy brought about in the normal animals by addition of 6% of sodium chloride to the diet was prevented to a large extent by concomitant administration of 2% of potassium chloride.

The level of carcass moisture (calculated on a fat-free basis) tended to be a constant value and was uninfluenced by the dietary B vitamin or salt intake.

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Hypothalamic regulation of spontaneous salt intake in the rat

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NOVÁKOVÁ, A., AND J. H. CORT. Hypothalamic regulation of spontaneous salt intake in the rat. Am. J. Physiol. 211(4): 919–925. 1966.—Adult male rats, maintained in metabolism cages, were depleted of Na by hydrochlorothiazide administration and a salt-free synthetic diet that contained sufficient K to balance urinary losses of the latter cation. Before and after the depletion period the rats were presented with a free choice of tap water, physiological saline, and a saline containing 20 g/liter NaCl. During administration of the diuretic only tap water was available. Normal rats reacted to the depletion by markedly increasing spontaneous salt intake and regaining salt balance, with some overshooting, within 3 days. The same animals were then subjected to hypothalamic electrolytic lesions at various sites in the midhypothalamus, and the response to a new depletion period was again tested. With lesions in the region of the ventromedial nucleus of the hypothalamus the animals failed to increase salt intake after depletion—with other lesions, even in the close vicinity of the ventromedial nucleus, this effect was not observed.

regulation of salt intake; ventromedial nucleus of hypothalamus; reaction to salt depletion

DESPITE SEVERAL DECADES of intensive study of the regulation of salt balance, most attention has been given only to excretory pathways, in particular those involving the kidneys. Renal salt excretion shows a high degree of precision in elimination of administered salt loads under normal conditions, despite the limiting factor of glomerular filtration, but in the presence of a deficit the most efficient renal response can only decrease further losses. Because of the obvious clinical and physiological significance of the other half of the balance equation, spontaneous salt intake has been studied and is reported here.

Observations on intake regulation in human subjects are complicated by cultural, social, and individual variations, but despite this it is common clinical experience, with reference to the present study, that Addisonian patients prefer a high-salt intake. This same effect has been more quantitatively documented in adrenalectomized rats (1, 2, 11, 13, 16–18), a preparation that under these circumstances can differentiate between Li and Na in terms of intake preference (14). Although such observations suggest that the regulation of salt intake does exist, it is difficult to state exactly what is being regulated, since hypoadrenalism results in a large number of physiological changes.

There are a number of observations implicating the hypothalamus in regulation of salt intake, although no clear picture has as yet emerged. Anterior lesions in the region of the supraoptic and paraventricular nuclei have been reported to be followed by either an increase or decrease (9) of hypertonic saline intake, and these observations served as the basis of a hypothesis on the existence of a "salt center" in this region of the brain that supposedly functioned in relation to oxytocin elaboration and subsequent alterations in urinary salt loss; however, the hypothesis was not accompanied by balance or urinary excretion data. Lesions in the lateral hypothalamus are followed by a marked decrease in saline intake (12, 20) but are also followed by hypodipsia in general, so that it is difficult to state how specific these changes are. Lesions in the posterior hypothalamus, about the posterior nucleus, are followed by marked urinary salt losses, similar to the clinical state of "cerebral salt wasting" (3, 4, 15). However, if such animals are allowed to go into negative external balance due to the urinary loss, and are then offered a choice of physiological or hypertonic saline in addition to tap water, they promptly increase their intake primarily of isotonic saline and rapidly repair the loss (6). In other words, whatever it is that rats "recognize" during salt depletion that stimulates them to increase spontaneous salt intake, this recognition is intact after a posterior hypothalamic lesion that apparently results in changes only in salt excretion. The region about the ventromedial nucleus of the hypothalamus has been studied mainly with reference to food intake and hyperphagic obesity (8) but here, as well, there are some references to a possible role of salt intake. Obesity lesion animals have been found to be in a state of chronic hypernatremia (19) but this may be

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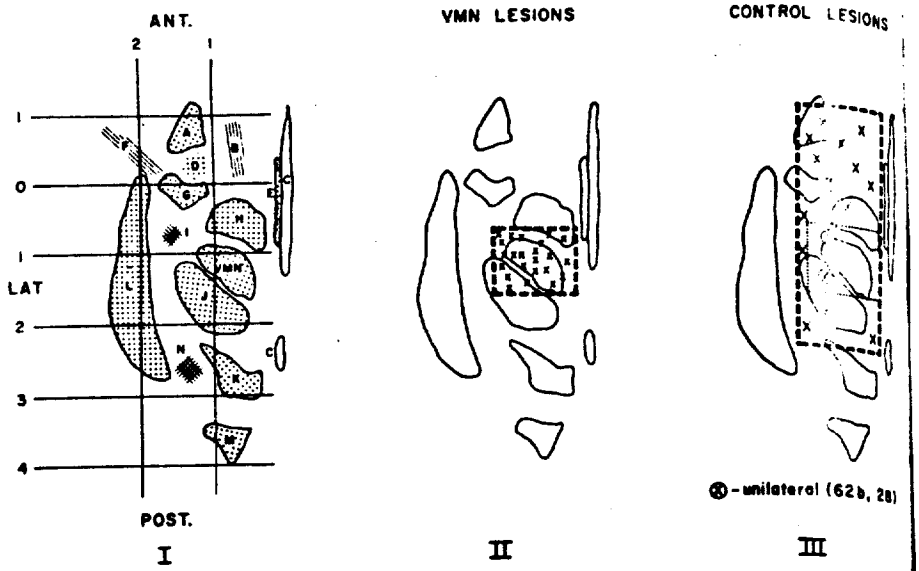

FIG. 1. Diagram of a horizontal section of rat hypothalamus, taken parallel to base of the brain and 1.0 mm above it, showing in all three cases the left side only. *Diagram I* gives the topographical anatomy in reference to a 1.0-mm coordinate grid in which the anteroposterior 0 is the bregma and the lateral 0 the saggital suture (4). This plane of sectioning has been found far more convenient for locating lesion sites than the more usual coronal plane. *Diagram II* presents the sites of the needle holes for the entire VMN group of animals —all lesions bilateral. It should be stressed that the extent of the gliotic reaction about the hole usually amounts to at least 0.5 mm in diameter. *Diagram III* presents the sites of the needle holes in the control, or non-VMN, lesion group, the VMN area being shown in the middle. The circled crosses refer to two animals (*62b* and *28*) where the needle hole on the left side was in the VMN area, but the right side showed sites outside the VMN area, and the animals behaved in the same manner as the other control animals in their response to salt depletion. A = preoptic nucleus; B = stria terminalis; C = 3rd ventricle, invariably appearing as two separate openings in any section at this level; D = anterior hypothalamic area; E = paraventricular nucleus; F = anterior lemniscus; G = anterior hypothalamic nucleus; H = filiform nucleus; I = fornix; J = dorsomedial nucleus; K = posterior nucleus; L = lateral nucleus; M = supramammillary nucleus N = mammillohypothalamic tract, VMN = ventromedial nucleus.

related to thirst perception rather than salt per se. Salt loads decrease food intake in normal animals (8) and this type of inhibition is partly to completely eliminated after destruction of the ventromedial nucleus (VMN) (7). In other words, there is the suggestion that some physiological factor related to external salt balance is "perceived" or recognized, or the afferent signal is "integrated," in this region of the diencephalon. The present experiments are a more direct test of this latter possibility.

METHODS

Adult Wistar-strain male rats, initial body wt 250–290 g, were maintained in individual metabolism cages with a loose-mesh, wire-net bottom, enabling urine to be collected in shallow, flat pans under mineral oil. The animals were fed a constant synthetic diet which contained only traces of Na, in amounts of 5 g diet/100 g body wt per day (the animals were trained fed, i.e., they were given only 2 hr in which to eat their rations, always at noon). Each lot of diet was made up as follows: 250 g hydrogenated vegetable fat, 250 g Na-free casein, 500 g sucrose, 11.4 mg KCl, 14.9 g $CaCO_3$, 0.07 g KI, 30 mg thiamine, 12 mg riboflavin, 9 mg pyridoxine, 15 mg pantothenic acid, and 120 mg nicotinamide. The amount of K was greater than usual to compensate for urinary losses during the depletion period. Between periods of balance measurement, the animals were put back onto stock diet to prevent possible inadequacies of the experimental diet from showing any cumulative effects. The only source of Na was in the drinking fluids offered, which consisted of tap water, physiological saline, and 2.0 g NaCl/100 ml water, contained in three separate, drip-proof, calibrated drinking tubes (50 ml total volume) attached to the side of the cage. The spatial sequence of the three drinking tubes was kept constant throughout the experiment. Intake of food and of each of the three drinking fluids was measured daily. Urine was collected cumulatively for each 3- to 5-day balance period, and Na and K concentrations were determined on a Zeiss flame photometer. All fluid and electrolyte intake and output data were calculated per 100 g body wt. Intake, output, and balance data were then analyzed using the Fisher *t* test, such that for equivalent balance periods at different stages of the experiment, differences in values were calculated for each animal separately between the separate balance days (i.e., 1st day of balance period 1 – 1st day of balance period 2; 2nd day of balance period 1 — 2nd day of balance period 2, etc.). In other words, each animal served as its own control. This approach was selected because absolute balance data per se show a marked interindividual variation.

The arrangement of the experiment was as follows: Balance period 1—3 days of base-line values of Na, K and water turnover, with three choices of drinking fluid. Balance period 2—3 days of Na depletion, with daily im injection of 1.0 mg hydrochlorothiazide in aqueous solution, tap water only available as drinking fluid. Balance period 3—3 days of recovery from depletion, with free choice again of water and two salines.

On several occasions, the three balance periods were repeated on the same rats.

The interval period was 3-5 days with no balance measurements, on stock diet and tap water.

All the animals were then anesthetized with 30 mg/kg body wt pentobarbital, given ip; the heads were affixed in a stereotaxic instrument of our own design (5), and stainless steel electrodes, 0.2 mm in diameter, insulated with transformer lacquer except for 0.5 mm at the rounded tip, were inserted into the hypothalamus and lesions were made by passage of 3 ma × 5 sec of d-c current. Bilateral lesions were made by reinsertion of the same electrode at the contralateral site. Stereotaxic coordinates were taken from published atlases for the rat (10) using the bregma as an anteroposterior null point. The coordinates by this system for the ventromedial nucleus of the hypothalamus (VMN) are: 1.0-1.5 mm posterior to the bregma, bilateral at 0.75 mm from the midline, at a depth of 9 mm from the cortical surface of the brain, or at a height of 1.0 mm above the base of the brain.

Interval period A was 14 days without balance measurements, on stock diet, to allow for postoperative recovery.

Balance periods 1A, 2A, 3A were repetitions of the prelesion periods under identical conditions as before, and further repeated either once or twice.

To test whether the organization of the experiment per se might result in changes in spontaneous salt intake, a "dry run" was carried out with 1 group of 10 rats, i.e., the entire procedure was repeated as above, but no hydrochlorothiazide was administered, and no electrolytic lesion was made.

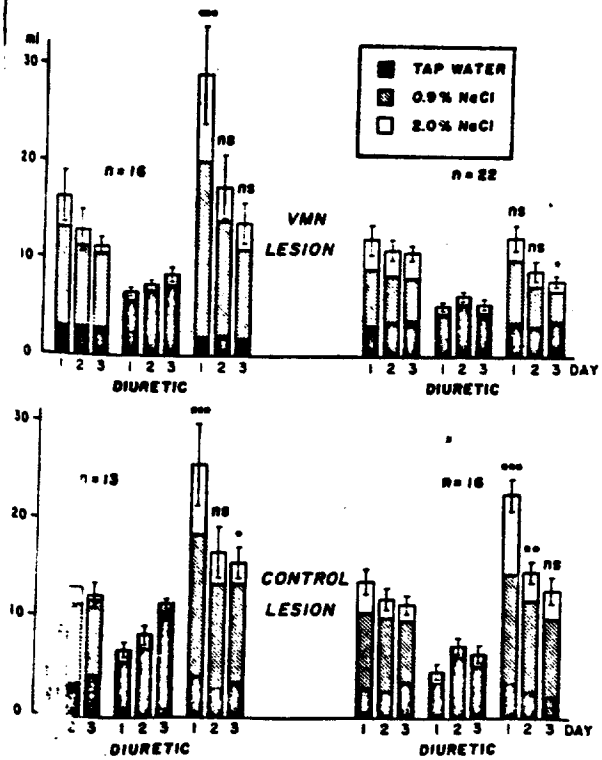

FIG. 2. Fluid intake per 100 g body wt in VMN and control lesion rats. Each column is subdivided for a particular fluid, as indicated. For sequence of experimental procedures, see text; n is the number of observations in each group. Each column shows a S.E. distribution, and *** is a P value of <0.001, ** is <0.01 and * is <0.05 (ns = not significant).

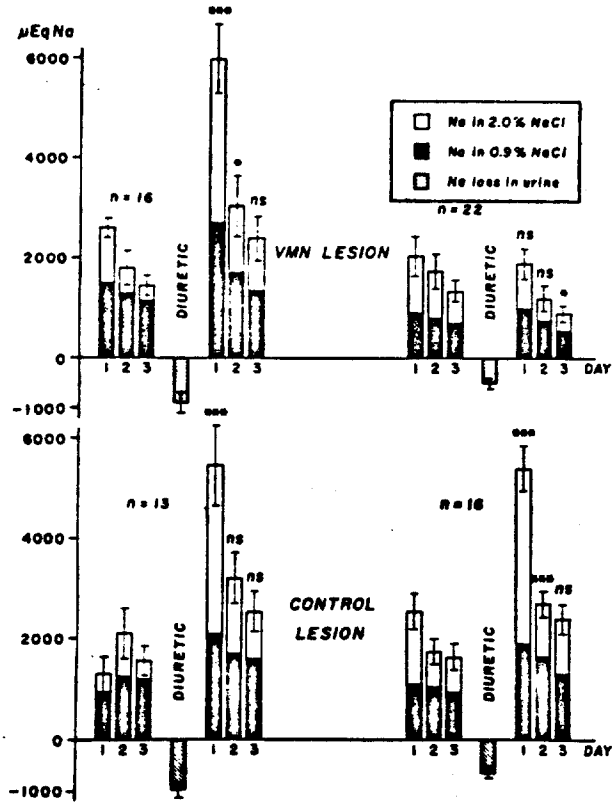

FIG. 3. Na intake and output in μEq/100 g body wt in VMN and control lesion rats. For explanation of symbols see Fig. 2.

At the end of the entire series of balance periods, the rats were decapitated, the heads placed in 40% formol for 10 days, the brains then dissected out, and placed again in 40% formol. The hypothalamic region was cut out of the base of the brain, and sections were taken in a plane horizontal to the base of the brain and 1 mm above it to facilitate localization of the lesion (7). The sections were stained with hematoxylin and eosin, toluidine blue, or cresyl violet.

Localization of the lesions was estimated independently by each author without previous knowledge of the balance data. Animals with gross pathology after operation, e.g., damage to the vestibular system, and animals where both observers did not agree on localization were excluded from the material presented here. According to localization, the rats were divided into two groups: 1) with a clear bilateral lesion in the VMN region, and 2) with lesions outside of this area, or when only a unilateral lesion at the correct site could be found. The VMN area was judged by position relative to the horizontal section of the anterior portion of the third ventricle, as shown in Fig. 1, which presents the lesion coordinates for all ani-

TABLE 1. *Total spontaneous sodium intake (μEq/100 g body wt)*

Rat No.	Before Lesion						After Lesion					
	1st Day		2nd Day		3rd Day		1st Day		2nd Day		3rd D	
	B	A	B	A	B	A	B	A	B	A	B	A
						A. Bilateral VMN lesions						
19	1,903	3,117	2,192	2,277	2,393	2,569	5,045	6,045	3,337	2,903	2,469	588
32	1,079	4,105	1,420	2,888	1,934	2,156	2,016	1,752	1,929	2,006	1,923	173
36a	1,802	4,962	1,330	1,994	1,777	501						
36b	1,802	5,966	1,330	4,117	1,777	3,432	3,138	4,488	2,147	1,958	2,345	930
38a	2,143	3,880	1,665	2,932	1,921	1,117	962	1,602	612	670	644	474
38b	2,143	3,107	1,665	1,364	1,921	1,803	962	1,447	612	719	644	648
56	2,940	3,987	1,289	645	466	241	2,452	2,268	2,334	961	1,768	284
57a	1,466	3,798	428	1,190	738	2,121	678	639	158	451	420	637
57b							651	408	171	44	383	243
58a	9,674	12,396	4,916	6,909	2,311	5,659	5,699	2,830	5,357	1,279	3,193	797
58b							3,759	1,181	2,701	589	2,099	2,954
59a	43	5,713	427	2,797	313	1,340	61	161	261	136	401	61
59b							188	253	376	58	115	246
63a	782	4,911	706	1,968	646	2,076	746	1,302	592	152	453	358
63b							1,183	2,031	651	830	557	253
65a	3,113	6,820	2,808	2,183	1,547	2,275	4,935	3,250	5,656	2,469	3,302	1,836
65b							4,889	2,705	2,839	4,332	3,347	1,562
66	315	5,334	173	1,748	359	1,994	748	1,403	737	700	718	214
67a	6,602	9,584	3,327	9,584	1,306	7,092	1,367	1,722	1,170	702	1,286	572
67b							746	772	734	670	516	643
68a	2,236	4,395	956	1,906	1,674	1,123	1,056	1,245	574	1,012	574	323
68b							999	781	1,116	919	476	673
69	4,196	11,038	4,765	4,453	2,302	2,704	2,794	3,124	3,766	3,309	2,168	1,014
Avg.	2,646	5,819	1,837	3,060	1,461	2,388	2,049	1,882	1,721	1,221	1,354	886
SE	243	700	360	575	182	440	376	304	348	244	229	174
	$P < 0.001$		$P < 0.025$		$P < 0.1$		NS		$P < 0.1$		$P < 0.025$	
						B. Control lesions						
18	2,059	5,410	4,068	4,547	1,031	3,305	5,272	7,857	2,834	3,749	2,098	3,783
20	0	2,490	656	3,178	749	1,899	2,117	4,303	1,698	2,502	872	2,222
21	1,469	4,265	2,360	1,201	1,094	1,052	1,911	5,011	2,087	2,553	1,186	1,099
22	186	530	1,723	3,109	2,037	1,971	3,139	5,427	2,508	2,490	2,345	1,921
23	939	6,831	2,220	4,311	2,163	3,383	4,053	7,881	1,837	4,786	1,477	4,057
24	2,140	6,186	3,991	66	2,884	2,610	3,988	7,780	2,217	3,194	1,892	2,984
26	0	3,555	264	3,979	220	3,259	1,629	5,665	805	805	2,238	1,036
28	502	5,445	535	1,806	608	1,696	1,178	5,142	676	1,568	897	1,950
30	2,249	2,182	3,867	3,571	3,284	6,010	2,922	6,336	2,319	2,553	2,085	2,069
31	961	5,388	1,886	2,653	2,033	2,150	4,008	6,980	3,260	2,866	2,428	2,393
61a	4,572	10,765	5,424	6,320	3,429	3,777	3,508	5,326	2,344	2,617	2,506	4,891
61b							2,750	5,488	2,383	4,188	2,670	1,902
62a	772	7,822	487	2,251	532	1,491	657	5,651	100	1,514	381	590
62b							471	1,162	312	1,795	106	460
64a	713	10,383	526	5,265	362	834	619	3,291	376	2,645	116	3,472
64b							2,686	2,814	2,686	2,989	2,414	3,600
Avg.	1,274	5,481	2,154	3,251	1,571	2,572	2,557	5,382	1,778	2,676	1,669	2,402
SE	349	837	472	471	314	385	355	465	250	252	240	324
	$P < 0.001$		NS		NS		$P < 0.001$		$P < 0.0025$		$P < 0.1$	

B = before Na depletion. A = after Na depletion.

mals in both groups. In essence, group 2 served as a control to group 1 far better than sham-operated controls would have done. The non-VMN lesions extended from the region of the dorsomedial nucleus to the mammillothalamic tract area, but did not include either the lateral or posterior nuclei (hypophagic, hypodipsic, and salt-wasting lesions were discarded from the series).

RESULTS

Figure 2 shows the spontaneous intake of the three drinking solutions offered, before and after placing hypothalamic lesions, as ml/100 g body wt, for both VMN and "control" lesions. In both of the latter groups before the lesions were placed, there was a highly significant

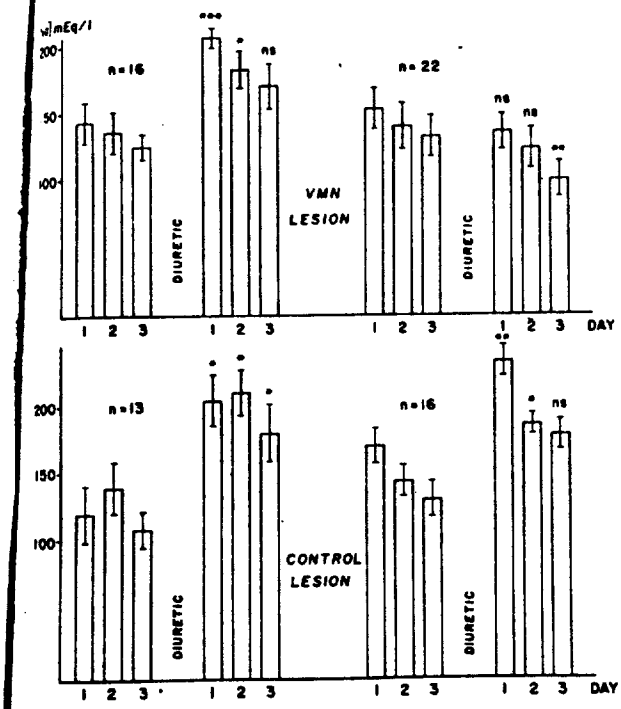

FIG. 4. Apparent osmolarity of fluid drunk by VMN and control lesions rats. For explanation of symbols see Fig. 2.

increase in total fluid intake ($P < 0.001$) on the 1st day after hydrochlorothiazide administration, as compared to the first balance day before Na depletion. This temporary increase rapidly disappeared over the next two balance days, and involved an intake of salines; tap water intake remained unchanged. The increases in physiological and hypertonic saline intakes were both significant prelesion in both VMN and non-VMN groups ($P < 0.005$ for 0.9% saline, $P < 0.001$ for 2.0% saline). The intake of hypertonic saline actually remained above base-line values for the entire 3-day postdepletion period.

Following a VMN lesion, Na depletion did not result in a significant rise in intake of any of the three solutions, although there was a slight preference for physiological saline. During the actual depletion periods, when only tap water was available, the animals increased fluid intake and tended to increase still further with each successive day on hydrochlorothiazide, but total fluid intake did not come up to total fluid intake in the initial control periods. Following the non-VMN lesions, the animals reacted to salt depletion in the same manner as in the prelesion periods.

Figure 3 presents the data as total salt intake (in μEq Na/100 g body wt) in the same groups of animals as above. In the prelesion periods of the VMN group, the increase in spontaneous salt intake after depletion was significant not only for total intake ($P < 0.001$) but also for salt intake in each of the salines separately ($P < 0.01$ for 0.9% saline and $P < 0.001$ for 2% saline). Following a VMN lesion, the animals failed to increase salt intake after depletion. These animals lost less Na in the urine than the non-VMN control group, but this difference was not significant. In the non-VMN lesion group, the animals increased salt intake following depletion to the same degree after the lesion as before it. The detailed salt intake data are given in Table 1.

The apparent osmolarity of total fluid intake was calculated as total Na intake-to-total water intake, and Fig. 4 shows that non-VMN lesion animals increased "intake osmolarity" after depletion to the same degree after the lesion as before, whereas the VMN group failed to show this increase with the lesion in place. The post-lesion difference between the VMN and non-VMN groups was significant at a P level of 0.005.

Figure 5 shows the average daily Na excretion in the separate experimental periods. In non-VMN lesion

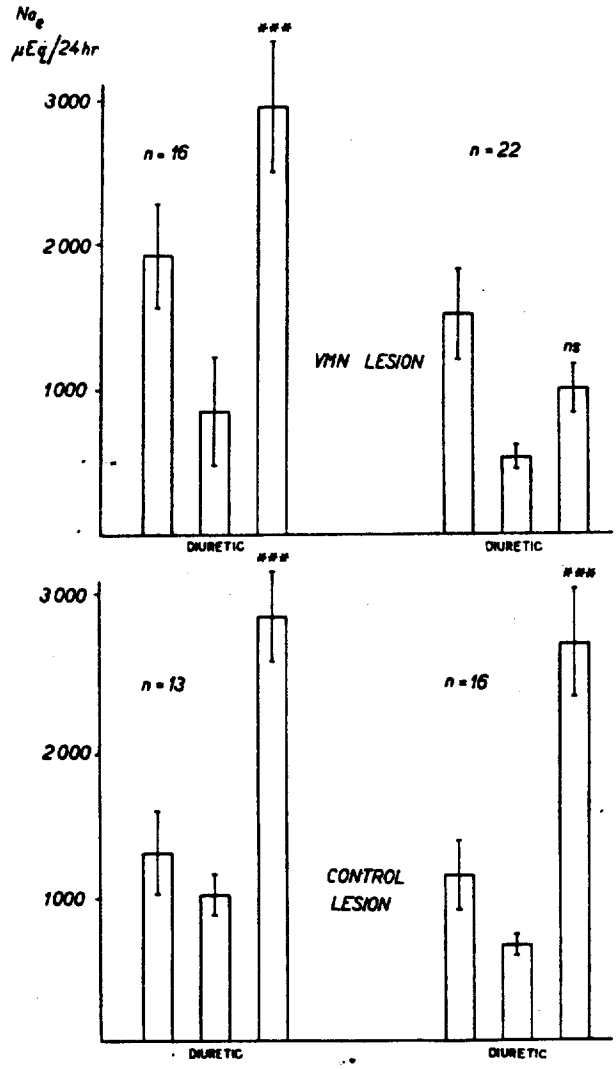

FIG. 5. Na urinary excretion in μEq/100 g body wt per 24 hr in VMN and control lesion rats. Since urine samples were pooled for each 3-day balance period, only one column is shown for each, so that the pre- and postlesion sequence is: before depletion, during depletion, after depletion. As a comment on the lower excretion values during the diuretic period, it should be stressed that the rats were without any salt intake whatsoever during these 3 days. For other symbols see Fig. 2.

Appendix I

Teratology Study in Rats

Virgin adult female albino rats (Wistar derived stock) were individually housed in mesh bottom cages in temperature and humidity-controlled quarters with free access to food and fresh tap water. They were mated with young adult males, and observation of the vaginal sperm plug was considered Day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, the females were dosed with the indicated dosages by oral intubations. The controls were sham treated with the vehicle at a level equivalent to the group receiving the highest test dose. The test material was prepared and doses calculated according to the following table:

Dosage (mg/kg)	Dose (ml/kg)	Concentration (mg/ml)
≤ 250	1	≤ 250
251 - 500	2	125 - 250
501 - 750	3	133 - 250
751 - 1000	4	187 - 250
1001 - 1250	5	200 - 250
1251 - 1500	6	208 - 250
1501 - 1600	6.4	235 - 250

Body weights were recorded on Days 0, 6, 11, 15, and 20 of gestation. All animals were observed daily for appearance and behavior with particular attention to food consumption and weight, in order to rule out any abnormalities which may have occurred as a result of anorexic effects in the pregnant female animal.

On Day 20 all dams were subjected to Caesarean section under surgical anesthesia, and the numbers of implantation sites, resorption sites, and live and dead fetuses were recorded. The body weights of

the live pups were also recorded. The urogenital tract of each dam was examined in detail for anatomical normality.

All fetuses were examined grossly for the presence of external congenital abnormalities. One-third of the fetuses of each litter underwent detailed visceral examinations employing the Wilson technique. The remaining two-thirds were cleared in potassium hydroxide (KOH), stained with alizarin red S dye and examined for skeletal defects.

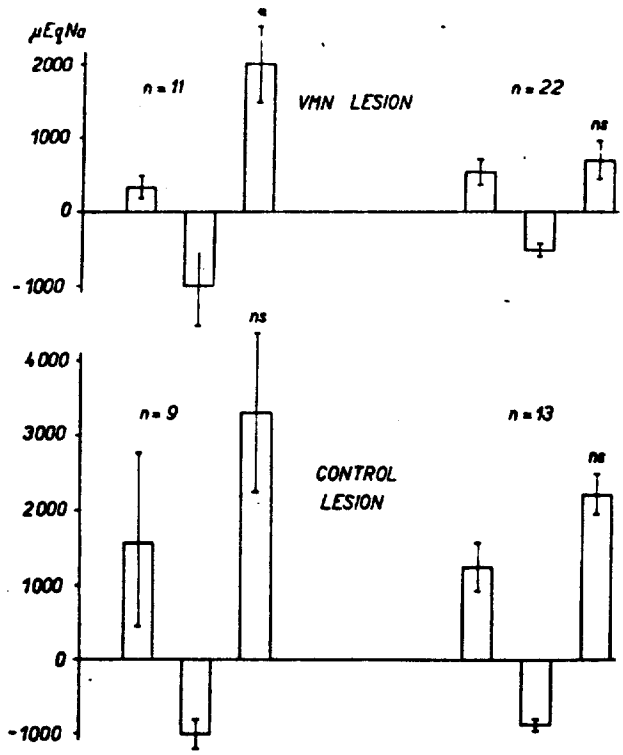

FIG. 6. External cumulative Na balance in VMN and control lesion rats. For explanation of columns and symbols see Fig. 5.

animals pre- and postlesion, and in VMN lesion animals previous to the lesion, urinary Na excretion increased during the repletion period, suggesting that the reactive increase in Na intake tended to overshoot the mark. Following Na depletion after a VMN lesion, however, Na excretion reached the lowest observed values in the experiment, apparently as a reflection of the lack of intake response to correct the deficit.

Cumulative external balances (Fig. 6) are, of course, associated with considerable collection errors and losses of urinary constituents on the cage structure, but despite this the difference in positive balance in the VMN lesion group in the repletion period was significant when comparing the postlesion period with the equivalent prelesion one, whereas there was no statistical difference in the non-VMN control lesion animals in the same comparison.

There were no significant differences in serum Na and K concentrations in any of the groups of animals in any of the above experimental periods. The dry run control series without either diuretic or lesion placement showed a slight increase in spontaneous salt intake in periods 3 and 3A, as above, due to the fact that in preceding periods 2 and 2A no salt was available to the animals, but there was a marked difference to the increases recorded after diuretic administration ($P < 0.001$) so that the organization of the experiment did not per se influence the results.

DISCUSSION

It has long been known that states of hyponatremia and extracellular fluid contraction can be associated with an increased spontaneous intake of water, accompanied, in humans, by a sensation of thirst (7). The depletion periods of the present experiment, during diuretic administration when only tap water was available, showed the same phenomenon. In the present case, however, interpretation is complicated by the fact that prior to these periods, the only source of salt intake was as drinking fluid.

The finding of principal physiological interest in the prelesion periods of all groups is that rats are clearly able to recognize a Na deficit and react to it by increasing spontaneous salt intake. The degree of intake regulation is not as precise as in the case of a pure water deficit and thirst, since, as shown in Table 1, the intake response after the deficiency period tended to overshoot in the 1st day of repletion, the excess spilling out into the urine. What the precise nature of this "recognition" of salt deficiency involves cannot be stated at present. Previous experiments in which a posterior hypothalamic lesion was the actual mechanism of salt depletion showed that spontaneous repletion was practically exclusively by means of an increased intake of physiological saline, the suggestion from this being that volume, or some hemodynamic function thereof, was the actual afferent signal, rather than extracellular Na concentration (6). These rats were not an intact physiological preparation, however. In the present experiments, Na concentration cannot be easily incriminated as an afferent signal, since there were no changes in this parameter throughout the various experimental stages. In the present work, the reactive increase in salt intake involved both physiological and hypertonic salines, so that no clear differentiation can be suggested in terms of an afferent signal. The fact remains, however, that the depleted animals repleted themselves of at least half of the deficit by drinking physiological saline, despite the fact that the most effective repletion would have been to drink the hypertonic saline. In other words, the suggestion of volume as at least part of the afferent signal remains here as well, and there is no a priori reason to exclude the possibility that both volume and Na concentration serve as sources of afferent information to the diencephalon.

Of all the possible statistical tests, comparison of VMN with non-VMN lesions proved to be that subdivision which showed highly significant differences from prelesion behavior, i.e., only in the case of a VMN lesion was there a clear loss of response in spontaneous salt intake to salt deficiency. No other type of postlesion control was attempted, such as mock operation, since noneffective lesions, of the same anatomical extent but different hypothalamic localization, appeared to be the best possible control. As opposed to more lateral hypothalamic lesions at the same anteroposterior site, VMN lesions are not associated with hypodipsia, which would per se

complicate interpretation of the specificity of the loss of the repletion reaction. In other words, it would appear that the VMN region of the hypothalamus is specifically involved in the recognition of salt deficits, although no statement can be made as to whether such participation involves direct perception of an afferent signal—volume, hemodynamic, or osmotic—or processing of a signal pattern reflected from another central nervous site. It is, however, very interesting in this regard that in studies of food intake in animals with VMN lesions (7, 8) one of the few qualitative differences in response, as opposed to control, pair-fed animals, was a failure to decrease food intake after an oral or subcutaneous salt load, i.e., a further suggestion that this region of the hypothalamus is involved in processing of afferent information relating to the extracellular content of sodium.

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OBSERVATIONS ON SALT IN VASCULAR HYPERTENSION *

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Any therapeutic measure claiming efficacy in a given disease or condition should fulfil at least this primary condition: It should be proved beyond a reasonable doubt that this measure, and this alone, produced the given result. As a corollary to the main proposition, it may be fairly demanded that any maneuver claiming to be more effective than another should show that the former measure is definitely and measurably superior to the latter.

In 1920, Allen [1] published a paper in which he asserted that a greater restriction in salt intake in cases of hypertension would produce a much greater reduction in pressure, a greater relief in symptoms, a speedier clearing up of retinal hemorrhages, etc., without other restriction in diet, exercise, etc., than other methods of treatment. He advocated limiting the intake of salt to 0.5 gm. a day instead of the usual 2 gm. His claim, if true, would have been a welcome advance in our therapy of a troublesome and apparently increasing disease.

This seemed, however, rather hard to believe for several reasons. In the first place, there is no good foundation for the restriction of salt in cases of hypertension with the exception of those associated with nephritic retention. The only reason given for the almost universal restriction of salt in treating hypertension is based on the assumed decreased viscosity of the blood resulting therefrom. But this is mere assumption and not proved or accepted. Perhaps the close relation between chronic nephritis (with salt retention) and vascular hypertension is responsible for the salt reduction treatment. Perhaps, it is pure empiricism or a clever method to prevent overeating by rendering the food less palatable.

In the second place, it was hard to believe that a reduction in salt intake from 2 gm. to 0.5 gm. would prove so much more effective than the restriction from 10 gm. to 2 gm., as had been tried frequently.

Then, too, it seemed hard to understand the comparatively low pressure of subacute nephritis and chronic nephritis with edema. Only

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* From the Medical Clinic of the Peter Bent Brigham Hospital.

* This paper was read by title at the meeting of the American Society for Clinical Investigation in Washington, May, 1922.

* This paper is the second one of a series of studies in metabolism from the Harvard Medical School and allied hospitals. The expenses of this investigation have been defrayed in part by a grant from the Proctor Fund of the Harvard Medical School for the Study of Chronic Diseases.

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rarely is hypertension seen in these conditions, and yet they show the greatest retention of salt.

Furthermore, if the salt is such a determining factor, we should expect to find the blood chlorid increased in our hypertension cases and a definite relation existing between the level of this substance and that of the blood pressure. Charts 1, 2 and 3 throw some light on this subject.

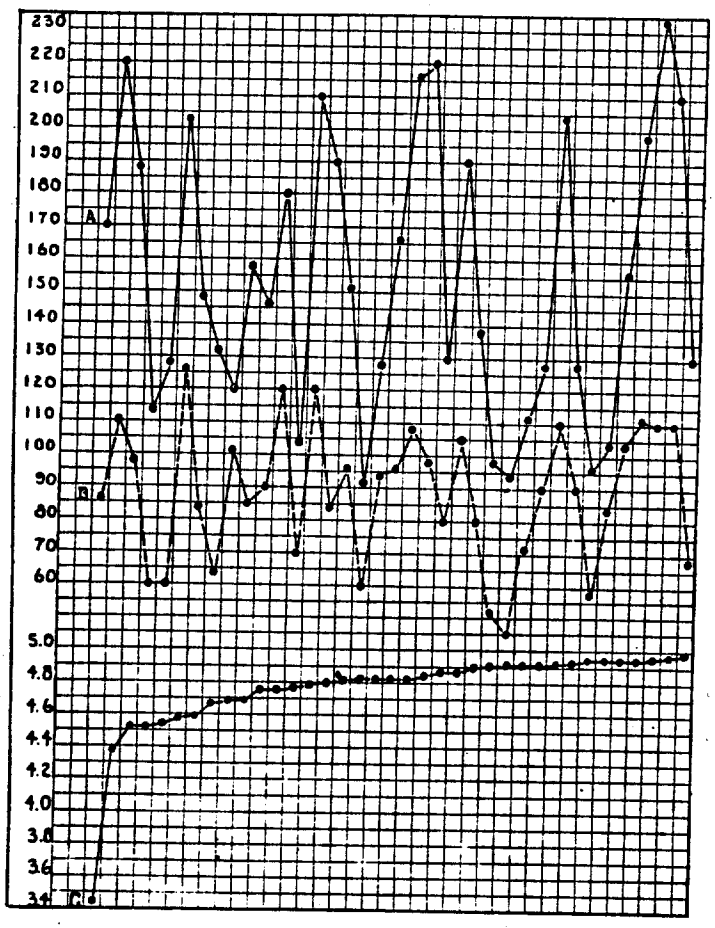

Chart 1.—Thirty-seven determinations of blood pressure and whole blood chlorid on thirty-three patients. Pressure and chlorid determined on the same day. Twenty of the thirty-seven had hypertension. The arrangement is according to an ascending value of the blood chlorid. All of these except the lowest are within the accepted limits of normal. The commonly accepted limits for normal according to Myers, McLean and Berglund are 4 to 5 gm. per liter of whole blood and 5.62-6.25 gm. per liter of plasma. Berglund feels that even 5.2 gm. per liter of whole blood is normal. In the chart, *A* represents systolic blood pressure; *B* diastolic blood pressure; *C* whole blood chlorid in grams per liter.

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BLOOD PRESSURE AND WHOLE BLOOD CHLORIDS IN THIRTY-THREE PATIENTS

Chart 1 demonstrates some significant facts. Although none of the blood chlorids is above normal, twenty of the thirty-seven pressures are above normal. Eight patients had systolic pressures of over 200 and three had diastolic pressures of 120 or over. More significant even

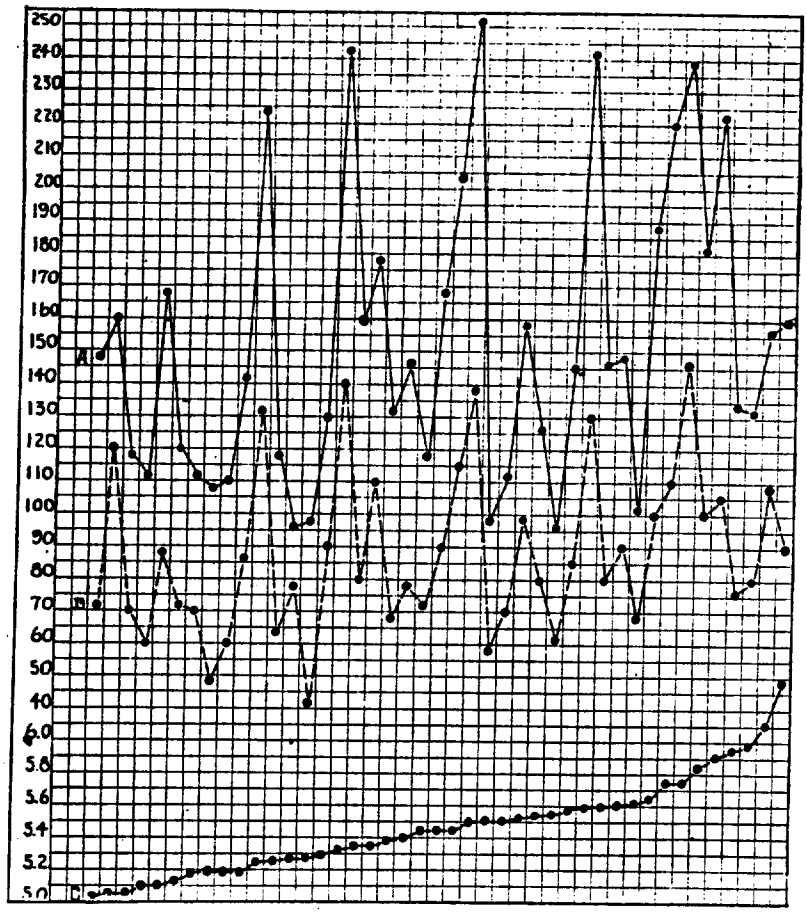

Chart. 2.—Forty-three determinations on twenty-four patients. Twenty-three had hypertension. Arrangement is the same as in Chart 1. *A* represents systolic blood pressure; *B*, diastolic blood pressure; *C*, whole blood chlorid in grams per liter. The whole blood chlorids are all above normal.

is the contrast between the curve representing the blood chlorids and those of the pressures. The pressures are represented by absolutely irregular types of curves without any relation to the blood chlorid curve,

which steadily climbs. High systolic and diastolic pressures may be found corresponding to low values of chlorid and normal or low pressures associated with the higher chlorids in this chart. The chart demonstrates that hypertension is compatible with normal or low blood chlorid, and that there is no relation between the levels of the chlorid and the pressures.

Chart 2 is similar to Chart 1. However, all of the blood chlorids in this chart are above normal. Despite this fact, twenty of the forty-three pressures are normal or low. The contrast between the steadily rising curve of the blood chlorids and the absolutely irregular, jagged curves of the pressures is as marked as in Chart 1. It may be pointed out that there are many higher pressures, both systolic and diastolic, in this group than in the one preceding. On the other hand, there are many lower. In our first chart, there are only two systolic pressures as low as 120, whereas in the second chart there are fourteen. Chart 2 indicates that a normal or low blood pressure is compatible with a high blood salt content, and that there is no relation between the levels of chlorid and pressure.

BLOOD PRESSURE AND BLOOD PLASMA CHLORIDS IN VASCULAR HYPERTENSION

Chart 3 illustrates the facts brought out in the two previous ones. The single high plasma chlorid in so many patients with hypertension is especially noteworthy.

These three charts demonstrate that in different persons: (1) hypertension is compatible with normal blood chlorid; (2) normal tension is compatible with high blood chlorid; (3) there is no relation between the height of the blood pressure and that of the blood chlorid.

Such conclusions apply also to the same determinations in the same patient.

The figures in Table 1 speak for themselves and indicate that in the same patient, as well as in different patients, there is absolutely no relation between the level of the plasma chlorid and the blood pressure. In the fourth, fifth and sixth patients in this group, it is interesting to note the pressures corresponding to the lowest and highest chlorids. They are almost alike.

Another doubt is cast on the relation between the blood pressure and the chlorid metabolism. It seems difficult to imagine that changes in the salt metabolism of the body could take place rapidly enough to bring about the sudden and often marked changes that take place in the blood pressure from second to second and from minute to minute.[2]

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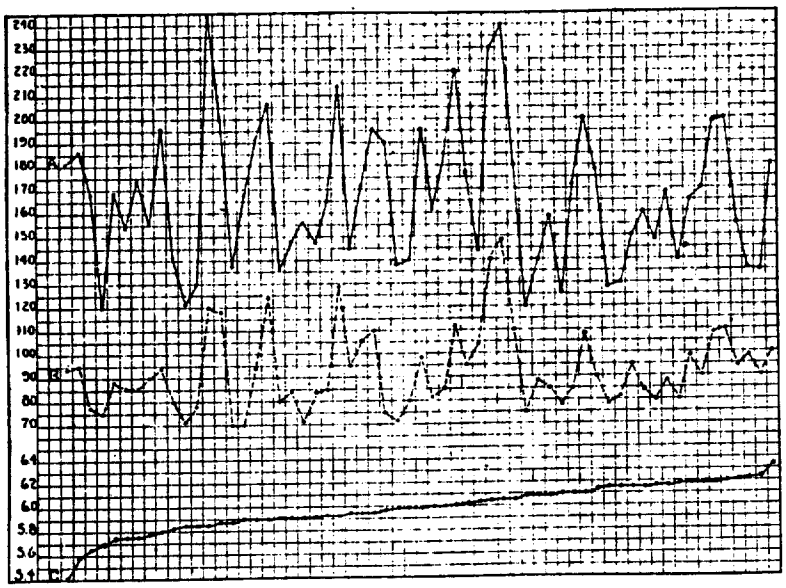

Chart. 3.—Sixty-one determinations of blood pressure and plasma chlorids on thirteen patients. All cases but the first have been diagnosed as vascular hypertension, although seventeen at the time showed a normal pressure. The plasma chlorids are all within the accepted limits of normal, except the first two which are low, and the last which is high. The arrangement is the same as in Chart 1. *A* represents systolic blood pressure; *B*, diastolic blood pressure; *C*, plasma chlorid in grams per liter.

TABLE 1.—*Cases in Which Plasma Chlorids and Blood Pressures Have Been Repeated*

Medical No.	Plasma Chlorid	Blood Pressure		Medical No.	Plasma Chlorid	Blood Pressure	
		Systolic	Diastolic			Systolic	Diastolic
12,459	5.83	142	80	15,402	5.91	156	72
	5.85	122	72		5.91	148	84
	5.86	130	78		5.93	165	85
	5.89	138	70		5.96	196	110
	5.99	138	72		5.99	140	80
	6.09	120	75		6.00	162	82
	6.18	140	80		6.10	172	85
	6.14	178	90		6.18	165	98
	6.15	150	95		6.20	200	110
	6.18	170	90	15,811	5.95	145	99
17,285	5.41	182	94		6.04	144	104
	5.58	186	95		6.06	180	110
	5.65	168	78		6.15	128	78
	5.75	168	88		6.23	136	98
	5.76	174	86		6.24	136	90
	5.76	154	86	17,477	5.86	244	120
	5.78	156	90		5.88	200	118
	5.80	196	94		5.90	206	124
	5.91	136	80		5.93	214	128
	5.99	196	98		6.06	230	140
	6.01	220	112		6.08	240	148
	6.09	140	88				
	6.09	158	86	16,202	6.03	177	95
	6.10	126	78		6.16	168	88
	6.15	180	82		6.16	148	80
	6.15	160	84		6.18	198	108
					6.21	155	94
					6.34	180	100

TABLE 2.—*Effect of Alternate Low Salt and Higher Salt Intake*

Case	Period	Duration in Days	Systolic and Diastolic Pressures at Onset	Highest Systolic Pressure	Lowest Systolic Pressure	Variation in Systolic Pressure	Average Systolic Pressure	Difference in Average Systolic Pressure	Highest Diastolic Pressure	Lowest Diastolic Pressure	Variation in Diastolic Pressure	Average Diastolic Pressure	Difference in Average Diastolic Pressure
1	0.5	16	190/100	195	140	55	168	+14	110	80	30	92	+3
	4.0	18	145/ 85	185	135	50	154		100	75	25	89	
2	4.0	9	124/ 64	130	124	6	127	+12	70	64	6	67	+10
	0.5	15	134/ 66	150	122	28	139		88	64	24	77	
3	0.5	8	180/110	180	120	60	137	+9	110	80	20	88	+2
	4.0	7	105/ 80	140	105	35	126		90	60	10	86	
4	0.5	15	204/110	208	173	35	193	+3	106	92	14	99	0
	4.0	15	182/ 94	220	174	46	190		112	90	22	99	
	0.5	64	196/ 98	196	136	60	165	(25)	106	78	28	84	15
5	0.5	13	168/ 60	172	156	16	165	0	70	58	12	62	+2
	4.0	8	156/ 54	174	156	18	165		70	54	16	60	
	0.5	11	162/ 60	170	152	18	150	(6)	70	56	14	63	+3
6	4.0	10	150/ 78	150	134	16	141	1	83	64	19	73	+5
	0.5	12	138/ 74	152	128	24	140		84	66	16	78	
7	4.0	6	230/140	266	230	36	247	1	148	130	18	142	10
	0.5	38	254/150	256	192	64	246		164	122	42	132	
8	0.5	16	178/ 94	200	140	60	162	4	98	74	24	88	+2
	4.0	15	162/ 82	180	156	24	166		98	80	18	86	
	0.5	15	168/ 88	182	148	34	164	(2)	102	72	30	89	+3
9	4.0	7	188/135	188	160	28	175	5	135	110	25	124	13
	0.5	25	153/110	200	150	50	170		132	104	28	111	
10	4.0	8	200/130	220	195	25	204	7	155	125	30	131	3
	0.5	18	220/140	220	170	50	197		140	105	35	129	
11	4.0	15	225/ 95	255	215	40	229	11	110	85	25	98	4
	0.5	31	225/100	243	140	103	218		120	70	50	94	
	4.0	18	215/ 85	240	200	40	218	(0)	110	85	25	90	+4
12	4.0	9	214/130	214	180	34	201	13	130	105	25	119	5
	0.5	20	196/110	202	160	42	188		120	94	26	114	
13	4.0	7	170/ 90	205	162	43	185	18	105	80	25	91	0
	0.5	46	178/ 90	195	145	50	167		105	80	25	91	
14	4.0	8	180/ 90	210	180	30	194	19	120	90	30	106	14
	0.5	38	185/ 95	260	135	125	175		140	75	65	92	
15	4.0	8	193/ 98	220	150	70	200	22	110	80	30	99	0
	0.5	14	212/100	215	150	65	178		110	90	20	99	
16	4.0	13	250/130	250	220	30	234	22	142	116	26	129	10
	0.5	11	228/114	232	194	38	212		138	108	30	119	
17	4.0	6	206/100	244	206	38	233	24	130	100	30	118	16
	0.5	22	224/122	224	190	34	209		112	90	22	102	
18	4.0	10	200/122	240	185	55	205	40	150	105	45	128	19
	0.5	35	198/118	220	132	88	165		140	90	50	107	
	10.0	6	148/ 90	184	145	39	161	(+4)	110	90	20	106	+1

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It is hard to believe that the salt has anything to do with such a marked change in pressure in such a short period of time as is illustrated in Chart 9. Note particularly the change of over 100 mm. systolic and 55 mm. diastolic pressure in twenty-four hours on March 1. Such a tremendous change, one would feel, must assume an equivalent change in chlorid metabolism. The urine and plasma chlorids indicate no such change. Furthermore, we know that any such marked change would be extremely dangerous to life. It is certain in this case that nervous influences were responsible, and it is hard to believe that these act on the vessels through sudden changes in salt metabolism.

In spite of the doubts that crept into our mind, it seemed desirable to check up the very low salt regimen to discover whether it fulfilled the requirements mentioned in the introduction of this paper. At first thought this might seem a simple, straightforward piece of work. Our original plan was to place patients with hypertension, on alternate weeks, on a 0.5 gm. and 4 gm. salt regimen. Many practical difficulties were soon encountered. The chief of these was our inability to get, in one week, dechloridization sufficient to bring the case within the accepted limits of an excretion of salt of 0.5 gm. or less a day. This necessitated, as a rule, the use of long periods of low salt intake and shorter control periods. Rarely could our patients stay in the hospital long enough to allow a period of higher salt intake comparable in length to the low salt period. This time variable should be borne in mind in the interpretation of the results.

Since the reduction in pressure was the only measurable criterion, we felt that we should use cases of pure hypertension and exclude those complicated by nephritis. Eighteen cases of vascular hypertension, therefore, were selected. Our aim was to have these patients under as constant conditions as possible during the experiment. They were, therefore, all kept at rest in bed during the entire period of observation. All were given a fixed diet containing 60 gm. of protein, 2,000 calories, and 1,800 c.c. of fluid. The only (controllable) variation was in the salt intake, which was alternately 0.5 gm. and 4 gm. (The latter amount was selected instead of 2 gm. to exaggerate any differences due to the low salt content.) Practically the only variable over which we had no control was nervous influences. These played, however, an important part.

Blood pressure readings were taken at the same time each day. The chlorid in the urine was quantitated daily to control the intake. The plasma chlorid was determined, as a rule, once a week.

The only good way to view the results of this study is to see the charts. For obvious reasons, only a few can be included here. A table such as Table 2 is the most easily available way to present the data in print. Necessarily, it is somewhat imperfect.

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The arrangement of patients is in order of differences in the average systolic pressures. A plus sign indicates that the pressure was lower with the 4 gm. than with the 0.5 gm. intake. In the first column is indicated the salt period—either 0.5 gm. or 4 gm. In the given case, the arrangement of the periods is chronologic. The second column indicates the duration of the periods in days. (It will be noted that the 0.5 gm. period is often considerably longer than the control period. It has, therefore, the added advantage of longer rest in bed, etc.) In the next column is given the systolic and diastolic pressures at the onset of the experiment. The next two columns contain the highest and lowest systolic pressures and the following one the difference between these two. These three give us the range of fluctuation. The next column

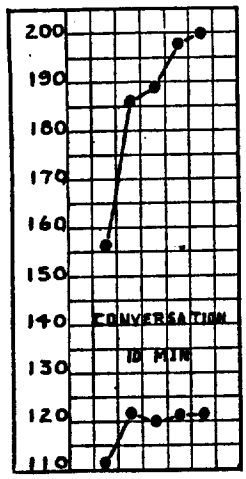

Chart. 4.—Effect of conversation. Pressures taken every two minutes.

gives us the average systolic pressure. The one following indicates the difference between the average systolic pressures in the periods. The remaining columns are the same for the diastolic pressure.

Before analyzing these figures in detail, let us see what previous experience with patients with hypertension has taught us. This will enable us to judge our results more correctly and to avoid the extremely common error of misinterpretation.

This experience has shown clearly that the pressure is extremely variable from minute to minute.[2] Systolic changes of 25 mm. to 40 mm. are not uncommon.

Chart 4 illustrates a striking effect of ordinary conversation in one of our nervous patients with hypertension. In her, the systolic pressure jumped 30 mm. within two minutes of the beginning of a simple conversation. In ten minutes, this pressure had climbed from 156 to 200.

Another fact brought out previously is that the pressures not infrequently fall materially (40 or more millimeters, systolic) without any special treatment except physical and mental rest. Note the decrease of 68 mm. and 56 mm. in systolic pressure and 48 mm. and 20 mm. in diastolic, in the first patient in Chart 5.

This patient was given no special treatment except rest in bed. The diet was "house diet," with no restrictions in salt. The second patient in this chart showed a drop of 35 mm. systolic and 25 mm. diastolic pressure, in spite of the fact that the patient was getting 4 gm. of salt.

If the 0.5 gm. salt regimen is effective, it seems to me that we should expect: (1) a definite decrease in the variations, and (2) a material lowering of the pressure, about which there can be no question. With regard to the second point, we think it is fair to demand an average decrease in systolic pressure of 25 mm. and, perhaps, somewhat less in the diastolic. Anything less is doubtful and can easily be accounted for by the physical rest or mental ease obtained.

Let us now return to Table 2. An analysis of the columns headed "Variation in Systolic" indicates that there were wider variations in both systolic and diastolic pressures during the 0.5 gm. regimen. Seventeen times during the low salt regimen, the degree of fluctuation was greater. It was less only four times and the same once. As for the diastolic pressure, the figures are about the same: greater fluctuation during the low salt regimen fifteen times, less six times and the same once. These are brought out better in the charts than in this table. Certainly the low salt regimen does not prevent the wide swings in pressure so common in these patients.

Analysis of the columns headed "Difference in Average Systolic" and "Difference in Average Diastolic" shows that only Cases 4 and 18 can reasonably be assumed to show sufficient change in pressure during the 0.5 gm. regimen to admit the possibility of the low salt ingestion being effective. While such is a possible interpretation, certain doubts are cast on the truth of such interpretation. In Case 4, it is only in the prolonged sixty-four day second period that the pressure fell so much. In the first low salt ingestion period, in which the duration was identical with the control period, there was actually a slight difference in favor of the latter. Furthermore, when this patient was allowed much larger amounts of salt, there was no evident relation between the pressure and the amount of salt excreted (see Chart VI). In Case 18, too, there is some doubt because on an intake of 10 gm. of salt per day for six days, this patient had an average systolic pressure that was actually 4 mm. less than during the low salt period. This table does not indicate that the 0.5 gm. intake is of greater value than the 4 gm. salt diet.

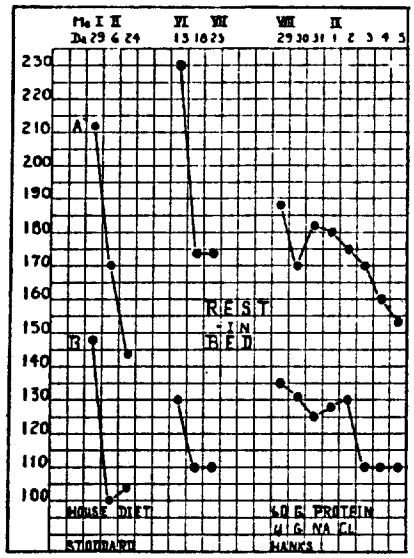

Chart 5.—Effect of rest in bed. At the top of the chart is indicated the month and just below it the day of the month. *A* represents systolic blood pressure; *B*, diastolic blood pressure.

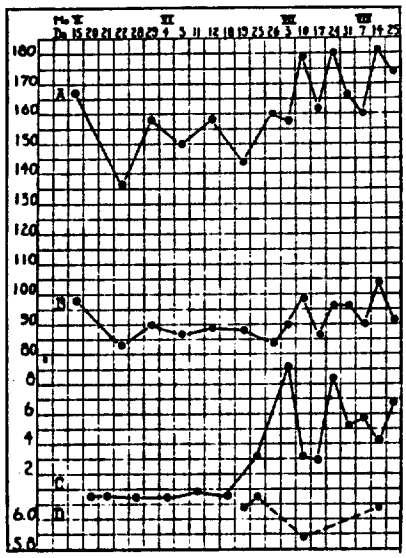

Chart 6.—Excerpt from the record of Case 4, illustrating the result of increased salt intake. *A* represents systolic blood pressure; *B*, diastolic blood pressure; *C*, twenty-four hour urine chlorid in grams; *D*, plasma chlorid in grams per liter.

Further evidence of the lack of relation between salt and hypertension is illustrated by four of our cases in which the patients took large amounts of salt, either at our suggestion or of their own free will. Case 18, Table 2, I have mentioned. This patient took 10 gm. of salt a day for six days. The table shows that during this period the fluctuations in pressure were less and the average pressures somewhat lower than during the 0.5 gm. salt regimen. Three more patients were

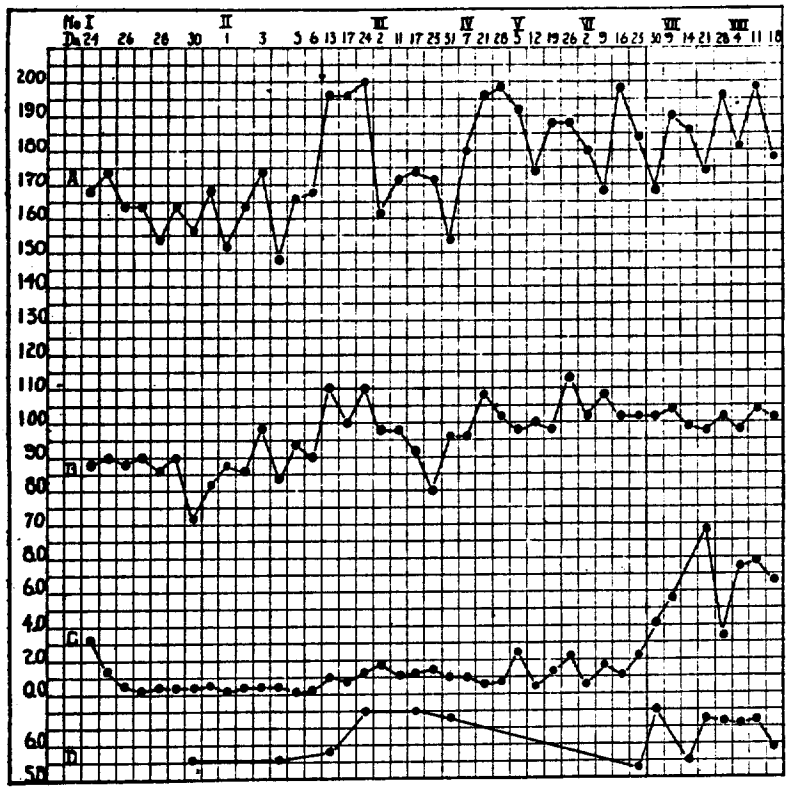

Chart 7.—Excerpt from record of Case 8. Patient discharged from hospital on February 7. *A* represents systolic blood pressure; *B*, diastolic blood pressure; *C*, twenty-four hour urine chlorid in grams; *D*, plasma chlorid in grams per liter.

followed after their discharge from the hospital, and the effect of increased salt ingested was noted.

It will be noted in Chart 6 that up to June 18 this patient was taking only enough salt to excrete less than 0.5 gm. After that date she took a greater and variable amount, as indicated by the urine chlorid. As one looks at the chart, the first impression is that with the increased salt intake, the pressure, especially the systolic, rose. However, a little

closer inspection of this period will show that while this is true, there is really no relation between the salt and the blood pressure. For, on July 3, the output of salt registered over 9 gm., and yet the blood pressure was the lowest in that period and just about what it had been in the 0.5 gm. period. On July 10, with a fall in the urine salt to 3 gm. and a fall also in the plasma chlorid, the systolic pressure rose 22 mm. and the diastolic 9 mm. Such observations make me feel certain that the general rise in pressure was due to factors other than salt.

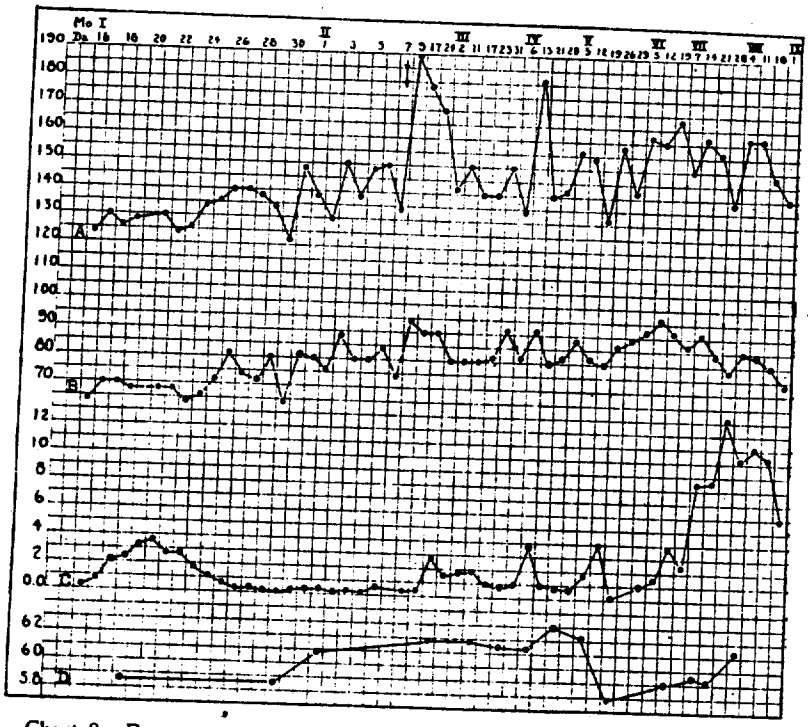

Chart 8.—Excerpt from record of Case 2. Patient discharged from the hospital on February 7. *A* represents systolic blood pressure; *B*, diastolic blood pressure; *C*, twenty-four hour urine chlorid in grams; *D*, plasma chlorid in grams per liter.

Case 8 is similar in many respects to the previous one. One may note in the chart that after the patient's discharge from the hospital and with her return to home conditions, there was a sharp rise in both systolic and diastolic pressure. There is no comparable change in salt excretion, which shows only a slight rise. It is not possible to believe that the latter had anything to do with the rise in pressure. If so, why should the latter continue to stay at approximately the same level when the salt excretion rose subsequently to seven or eight times its former

level? A point brought out in the previous chart is illustrated again here. On July 21, this patient's salt output was almost 10 gm., and yet the systolic pressure was much lower than on several other days when the excretion was between 0.5 gm. and 1 gm. On July 28, a

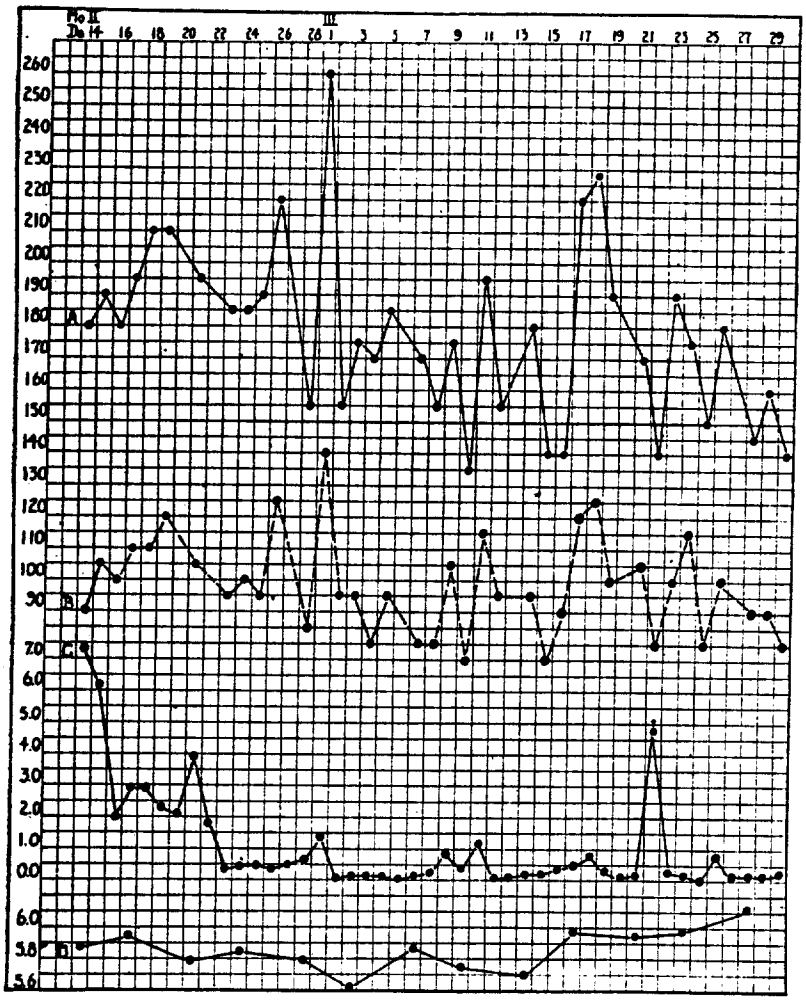

Chart 9 (Case 14).—This illustrates the effect of nervous influences. *A* represents systolic blood pressure; *B*, diastolic blood pressure; *C*, twenty-four hour urine chlorid in grams; *D*, plasma chlorid in grams per liter.

fall in salt to almost 3 gm. was accompanied by a rise of 22 mm. systolic pressure. The diastolic pressure was fairly constant.

This chart is almost similar to the previous two, except that it is somewhat more striking. There are four things that are especially

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worthy of note. The first is the gradual rise in pressure, in spite of a low salt excretion, before the patient was sent home. The second is the sharp rise of 58 mm. systolic and 20 mm. diastolic pressure two days after leaving the hospital. At this time, the salt excretion was 0.5 gm. Such a rise was undoubtedly the result of conditions at home. The excretion on July 28 of over 13 gm. of salt with a pressure that fell to normal is especially noteworthy. Last but not least is the tendency for the systolic and especially the diastolic pressure to fall slightly during the very high salt feeding.

It is hard to believe from these experiences with high salt intake that the salt has much to do with the level of the blood pressure.

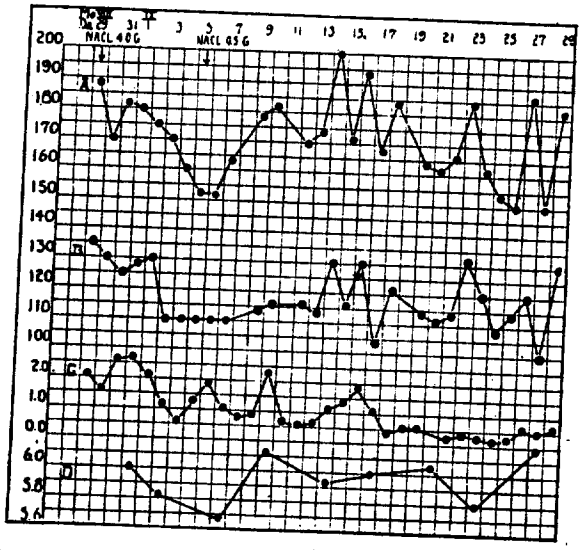

Chart 10 (Case 9).—The patient was on a 4 gm. salt intake diet up to September 5. From that date he was taking only 0.5 gm. *A* represents systolic blood pressure; *B*, diastolic blood pressure; *C*, twenty-four hour urine chlorid in grams; *D*, plasma chlorid in grams per liter.

Two charts are appended which illustrate various obvious difficulties in accepting the idea that low salt intake is effective in hypertension. Chart 9 demonstrates the extreme variability in pressures that, to say the least, is not prevented by the very low salt diet. Note particularly the sharp changes in pressure on March 1 and 17, with no corresponding changes in salt excretion. An increase of 105 mm. and 80 mm. systolic and 55 mm. and 30 mm. diastolic pressure in twenty-four hours is difficult to attribute to the changes that took place in the salt at those times. In Chart 10, note particularly the primary fall in pressure during the 4 gm. salt regimen and the paradoxical marked rise in

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pressure following the decrease to an 0.5 gm. intake. One may point, by way of objection, to the rise in plasma chlorid with this rise in pressure. The former, however, is still within normal limits. Furthermore, we have previously demonstrated the lack of relation between the plasma chlorid and blood pressure. Lastly, this does not at all influence our main contention that the 0.5 gm. salt intake does not materially decrease the pressure.

CONCLUSION

The experiences detailed above, with the extremely low salt intake advocated for the treatment of hypertension, do not indicate any special advantage over the older less rigid restriction. The former regimen has certainly not fulfilled in our patients the conditions mentioned in the introduction. This very low salt diet is, furthermore, difficult to carry out at home, is monotonous and expensive. This paper also suggests that salt plays little part in vascular hypertension.

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BRAIN-DAMAGING POTENTIAL OF PROTEIN HYDROLYSATES

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Abstract Casein and fibrin hydrolysates commercially available for use in human parenteral alimentation therapy were administered subcutaneously to 90 10-day-old mice at five doses (20, 40, 60, 80 and 100 μl per gram of body weight). Casein hydrolysate at each dose tested and fibrin hydrolysate at 80 and 100 μl per gram induced acute degeneration of neurons in the developing hypothalamus. Hypothalamic lesions were identical to those occurring in various animal species after oral or parenteral administration of acidic amino acids such as glutamic, aspartic and cysteic acids. A control amino acid mixture excluding glutamic, aspartic and cysteic acids induced no brain damage in similar experiments on 30 additional mice. A relatively low combined concentration of acidic amino acids thus appears to be one of the criteria that a parenteral alimentation preparation should meet to be maximally safe as well as nutritionally effective. (N Engl J Med 289:391-395, 1973)

TOTAL parenteral nutrition for patients who cannot be nourished through the gastrointestinal tract is being employed in hospitals with increasing frequency, particularly for surgical and pediatric patients,[1-3] including premature infants.[3,4] Protein digests, prepared either by enzymatic hydrolysis of casein or acid hydrolysis of fibrin, provide a relatively inexpensive source of amino acids for this form of therapy. There are striking differences, however, in the free amino acid composition of hydrolysate preparations in current use. For example, Stegink and Baker reported[5] that a casein enzymatic digest (Amigen), contained over five times as much glutamic acid (1960 μmoles per 100 ml) as a fibrin hydrolysate (Aminosol) (358 μmoles per 100 ml). On the other hand, the fibrin hydrolysate contained 112 μmoles per 100 ml of another acidic amino acid, cysteic acid, whereas the enzymatic digest contained none. The content of a third acidic amino acid, aspartic acid, is more nearly equal in the two preparations (660 μmoles per 100 ml in the fibrin hydrolysate

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and 500 μmoles per 100 ml in the casein enzymatic digest). For purposes of the present study it is noteworthy that the combined concentration of acidic amino acids in the casein enzymatic digest (2460 μmoles per 100 ml) is more than double that in the fibrin hydrolysate (1130 μmoles per 100 ml).

The present study focuses specifically on the acidic amino acids contained in protein hydrolysates because of recent findings that certain acidic amino acids, including glutamic, aspartic and cysteic acids, have unique brain-damaging properties.[6-9] The most extensively studied compound in this group, glutamic acid, which is usually given as the sodium salt, monosodium glutamate, has recently been shown by numerous investigators to induce hypothalamic damage when given to immature animals.[6-22] Susceptibility to hypothalamic damage induced by monosodium glutamate has been demonstrated in several species, including rhesus monkeys,[13-18] after either oral,[7,10-11] or subcutaneous[6,10,11,15,16] doses in the range of 0.5 to 1 mg per gram of body weight. Rodents treated with monosodium glutamate in the first 10 days of life become obese,[6,17,23,24] with neuroendocrine disturbances[6-23] and possible learning deficits[25] in later life. It has also been shown that subcutaneously administered glutamate gains entry to and is selectively accumulated by the arcuate nucleus of newborn-mouse hypothalamus, a process that parallels in time course the destruction of neurons in that nucleus.[20] The molecular specificity of brain damage induced by monosodium glutamate is such that it is reproduced by subcutaneous administration of a select group of other acidic amino acids (aspartic, cysteic, cysteine sulfinic and homocysteic acids and certain of their substituted synthetic analogues) that share with glutamate an ability to excite neuronal firing when introduced by microelectrophoresis into the mammalian brain.[7-9,16] An additive neurotoxic effect is produced by subcutaneous administration of any of the "neuroexcitatory" amino acids in combination with one another to immature mice.[7,9]

Since protein hydrolysates currently in use contain substantial concentrations of free acidic amino acids we postulated that the parenteral administration of these preparations to infants might entail risk of brain damage. Furthermore, if the total acidic amino acid content of casein hydrolysates substantially exceeds that of fibrin preparations, the former might produce more severe brain damage than the latter at any given exposure level. The present study was undertaken to test these hypotheses. Protein hydrolysates currently being used for parenteral alimentation of human infants were the preparations tested, but for obvious reasons, laboratory animals rather than human infants were chosen as test subjects.

Methods and Materials

A total of 120 Webster Swiss albino mice (National Animal Laboratories, St. Louis, Missouri), 10 days old, were studied. Ninety animals were given single subcutaneous injections of 1 of 3 protein hydrolysate preparations: casein enzymatic digest I (Travamin), Travenol Laboratories, Incorporated (previously called Amigen); casein enzymatic digest II (CPH), Cutter Laboratories, Incorporated; and fibrin hydrolyzed by acid (Aminosol), Abbott Laboratories. Each solution was given undiluted as commercially prepared (5 per cent hydrolysate to 5 per cent dextrose) to 6 animals at 1 of 5 doses (20, 40, 60, 80 or 100 μl per gram of body weight), 30 animals in all being treated with each solution.

Thirty additional mice, 6 being treated at each of the above doses, were given a control solution constituted as follows: L-lysine 800, L-tryptophan 100, L-phenylalanine 200, L-methionine 200, L-threonine 400, L-leucine 1200, L-isoleucine 400, L-valine 320, L-arginine 580, L-histidine 200, glycine 400, L-tyrosine 200, potassium metabisulfite 60, potassium chloride 51, and dextrose 5000 mg per 100 ml. The rationale for the control was that it excludes the acidic and sulfur amino acids, which have been shown to have brain-damaging properties while otherwise simulating the makeup of experimental solutions. Although the peptide content of the experimental solutions (protein hydrolysates are nearly 50 per cent peptides) could not readily be duplicated, we attempted to compensate for this deficiency by increasing the concentrations of amino acids used in the control solution. Instead of being 5 per cent hydrolysate (amino acids plus peptides), the control solution was 5 per cent amino acids. The control solution was adjusted to pH 7.0 with hydrochloric acid to bring it into the pH range of experimental solutions. All solutions were administered by injection under the skin of the back (Hamilton microliter syringe and 30-gauge needle), and the mice were housed in individual open containers under a lamp providing a constant ambient temperature of 31°C during the interval between treatment and post-mortem examination.

Five hours after injection both control and experimental mice were anesthetized with chloral hydrate and killed by perfusion fixation as described elsewhere.[7-14] Processing of brain tissue for alternative examination by either light or electron microscopy was performed by methods previously described.[13,14] For purposes of evaluating severity of lesions the portion of the hypothalamus containing arcuate nucleus (nucleus periventricularis arcuatus hypothalami)[26] was serially sectioned, and necrotic neurons were counted in a representative section (1 μm thick) cutting across the nucleus at its level of maximal damage. As indicated elsewhere,[7,8,13,14,16] the process of neuronal necrosis induced by monosodium glutamate is characterized by coarse clumping and condensation of nuclear chromatin accompanied by massive perikaryal swelling. Neurons thus affected are readily distinguished by light microscopy from healthy neurons, provided brains are prepared by appropriate histologic technics and examined in the acute post-treatment interval, 3 to 6 hours after treatment being optimal.

Results

The number of mice affected and the severity of hypothalamic damage at each dose of the administered solutions are shown in Table 1. None of the animals injected with the control solution at any dose sustained hypothalamic damage. None of those injected with the fibrin hydrolysate, at doses of 20, 40, or 60 μl per gram sustained lesions; of those treated at doses of 80 and

Table 1. Frequency* and Severity† of Hydrolysate-Induced Brain Damage.

Solution	Dose (μl/G)				
	20	40	60	80	100
Control	0	0	0	0	0
Fibrin hydrolysate	0	0	0	2(+)	3(+)
Casein enzymatic digest II	0	4(+)	6(++)	6(+++)	6(+++++)
Casein enzymatic digest I	1(+)	4(+)	6(++)	6(+++)	6(++++)

*No. of animals (out of 6 treated with each solution at each dose) sustaining hypothalamic damage.

†Necrotic neurons/representative section/per animal averaged for affected animals of each group. + = 1-10; ++ = 11-20; +++ = 21-30; ++++ = 31-40; & +++++ = 41-50.

100 μl per gram, however, ⅓ and ½ respectively did have lesions that, although small, were clearly of the type characteristically seen in animals treated with monosodium glutamate. The casein hydrolysates were essentially equal in neutotoxic potency, both being substantially more potent than the fibrin preparation. At the lowest dose tested (20 μl per gram) only one animal was affected by the casein hydrolysates, but ⅔ of those treated by either casein preparation were affected by the next dose (40 μl per gram) and all the animals treated by either preparation at 60, 80 or 100 μl per gram were affected. A linear dose-response relation was observed for each hydrolysate, with lesion severity increasing steadily with each dose increment. Figure 1 shows the normal appearance of the arcuate region of the hypothalamus in an unaffected control brain for comparison with a small lesion in the arcuate region of an animal given a high dose of fibrin hydrolysate (Fig. 2) and a large lesion in the arcuate region of one given a high dose of casein hydrolysate (Fig. 3).

Neither the animals receiving hydrolysate solutions nor those given the control preparation manifested acute symptoms of distress other than the periodic restlessness and helpless maneuvering about that is characteristic of rodent young isolated from the maternal nest. Laboratory animals did not differ from controls in these nonspecific behaviors.

Discussion

Stegink and Baker[3] recently measured amino acid blood levels in six human infants receiving parenteral alimentation therapy and reported no marked elevation of blood glutamate or aspartate levels while infusions of either fibrin or casein hydrolysates were being given. The authors concluded, "It is difficult to imagine glutamate-induced neuronal damage without substantial elevation of plasma glutamate levels. . . ." It may be questioned, however, whether the amino acid load being delivered to the brain of an infant receiving parenteral therapy would be reflected in measurements performed on peripheral venous blood. A venous-blood sample would be expected to reflect the amino acid makeup of the portion of the circulating blood that had passed through the body tissues and was returning to the infusion site (superior vena cava). Thus, it could provide a base line for glutamate levels just before the introduction of infusate into the system but does not reveal the extent to which the introduction of infusate altered glutamate concentrations in the portion of the blood leaving the heart to recirculate through the tissues. The arcuate nucleus of the hypothalamus lies at the base of the brain, where it is surrounded by the circle of Willis. Its rich arterial supply, not far removed in the circulatory system from the infusion site, might very well provide it with higher concentrations of acidic amino acids than venous-blood values would lead one to suspect. The striking tendency of arcuate nucleus to accumulate glutamic[20] and possibly other acidic amino acids, even against a steep tissue-to-blood concentration gradient, might cause these compounds to be siphoned off and accumu-

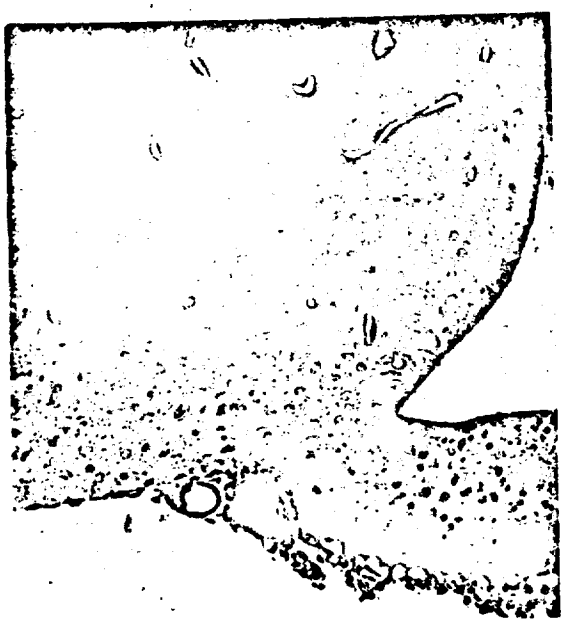

Figure 1. Normal-Appearing Arcuate Nucleus of Hypothalamus from a Newborn Mouse Given a High Dose (100 μl per Gram) of the Amino Acid Control Solution Five Hours Previously (X130).

Figure 2. Arcuate Nucleus of a Mouse Given a High Dose (100 μl per Gram) of Fibrin Hydrolysate (X130).

An inconspicuous (+) lesion affecting approximately 10 neurons at the angle of the third ventricle (⟶ ⟵) is evident upon close inspection. Degenerating neurons have clear, dilated cell bodies surrounding a shrunken, black nucleus. Lesions of this size and appearance were characteristically seen in animals treated either with a high dose of fibrin hydrolysate or with low doses of the casein preparations I and II.

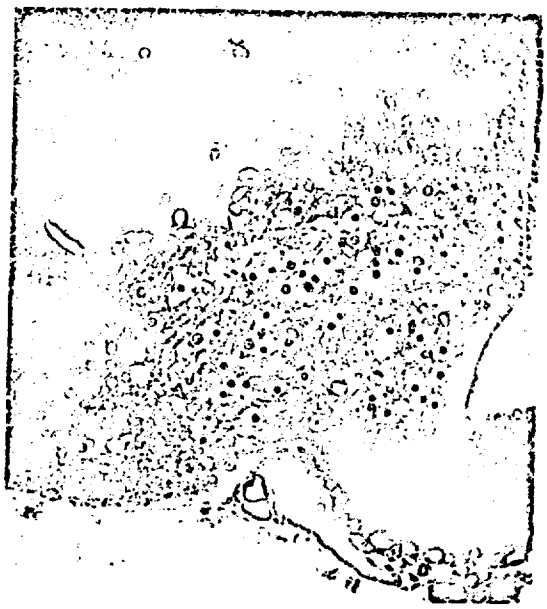

Figure 3. Arcuate Nucleus of a Mouse Given a High Dose (100 μl per Gram) of Casein Hydrolysate (X130).

A large (+ + + + +) lesion involving 40 to 50 arcuate neurons in various stages of degeneration is evident. Lesions of this size were seen in animals treated with high doses of casein hydrolysate I or II but not in those treated with fibrin hydrolysates.

lated in that hypothalamic region if even moderately elevated levels are being continuously circulated through it.

Absence of overt signs of acute toxicity in human infants receiving protein hydrolysate infusions has been advanced as evidence that human infants are invulnerable to glutamate-induced brain damage.[27] However, evidence that immature monkeys[15,18] as well as rodents undergo brain damage from doses of glutamate well below those required to produce acute symptoms and the present demonstration that neonatal mice sustain hydrolysate-induced brain damage without overt signs of acute central-nervous-system disturbance tend to invalidate this argument.

Vomiting, a well recognized side effect of protein hydrolysate infusions, is generally attributed to the glutamic (and aspartic) acid content of these preparations.[28-30] If the relation between glutamate-induced vomiting and brain damage were such that the former always preceded the latter, the occurrence of vomiting would be a useful warning signal. Unfortunately, experiments with rhesus monkeys[15] indicate that rhesus infants, particularly premature neonates, have a higher threshold for glutamate-induced vomiting than adults whereas the converse is true for glutamate-induced brain damage. Thus, vomiting occurs in newborn monkeys only at doses of glutamate exceeding those required to produce brain damage. If human beings parallel monkeys in this respect, vomiting by a human infant from an overload of glutamate would not be a warning signal that brain damage is about to occur; it would be a reminder that silent damage to the infant hypothalamus may already have occurred. For purposes of evaluating risk, the subcutaneous dose of casein hydrolysate required to destroy nerve cells in the hypothalamus of the immature mouse (20 to 40 ml per kilogram) and the rate at which a human infant might receive such a preparation intravenously (120 ml per kilogram per day)[5] cannot be directly compared since different routes of administration are involved. The general conclusion, however, that parenteral alimentation, particularly with casein hydrolysates, affords the human infant less than a wide margin of safety against hypothalamic damage seems warranted.

Although total parenteral therapy for infants with serious and sometimes life-threatening disorders is of unquestionable value, it is important to give continuing attention to possible means of improving the composition of the parenteral solutions used in this form of therapy. The amino acids that destroy hypothalamic neurons are not essential amino acids. In the absence of any nutritional requirement that they be introduced in high concentrations into the bloodstream, limiting their concentrations in parenteral alimentation preparations may be advisable. A step in this direction would be achieved by the simple choice of using fibrin instead of casein hydrolysates. Selectively removing the acidic amino acids from hydrolysates by absorbing them on ion-exchange resins, as suggested by Elwyn and Greenstein,[31] is worthy of consideration. Amino acid solutions constituted from crystalline amino acids are currently being developed and may, in the future, be the products of choice since the concentration of one amino acid in relation to others can readily be manipulated in such synthetic preparations. According to Heird et al.[32] either glutamic and aspartic acids or other anions that consume hydrogen ion such as acetate or lactate must be included in parenteral alimentation formulas for human infants to prevent metabolic acidosis from developing. Our findings suggest that the combined total concentration of acidic amino acids should be kept at a relatively low level to provide an adequate margin of safety against their brain-damaging potential. Maintaining the concentration of glutamic and aspartic acids at relatively low levels while relying upon other hydrogen-using anions for prevention of acid-base imbalance may be a feasible compromise formula.

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Effects of Chronic Excess Salt Feeding: Demonstration of Normal Thyroid Function in Salt-Fed Dogs with Hypercholesterolemia[1] (35056)

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Some rats and dogs chronically fed excess amounts of salt develop a significant increase in plasma cholesterol concentration (1–4). Salt-induced hypercholesterolemia could be important in contributing to long-term perturbations in lipid metabolism and to the development and progression of atherosclerosis (4–8). Observations in mice (9–11) and dogs (12) indicate that under certain circumstances a high NaCl intake can interfere with thyroid function. Therefore, it is possible that salt-induced hypercholesterolemia may be secondary to hypothyroidism. We report here studies of thyroid function in a colony of dogs fed excess salt for more than 5 years. Our data suggest that it is unlikely that the salt-induced hypercholesterolemia is mediated through a depression of thyroid function.

Materials and Methods. 17 pure-bred beagles, 16 females and 1 male castrated at age 9 months, were studied. Each dog consumed a daily average of 200 g of kibble,[4] 100 g of canned horse meat, and 1 teaspoonful of a sodium-free mineral–vitamin supplement. The 12 "salt-fed" test animals had received excess salt diets (water containing 1% NaCl or kibble containing 6–10% NaCl) for more than 5 years, starting at 10–18 months of age (4), consuming 12–21 g of NaCl/day. The 5 *control* dogs had an estimated daily NaCl intake of 2.5 g.

^{131}I-thyroidal uptake studies. Each animal received 0.25 μCi of carrier-free ^{131}I intravenously as the sodium salt in 4–6 ml of 0.9% NaCl solution. Thyroid uptake was measured at both 24 and 48 hr after injection of isotope, using a thallium-activated 2 × 2-in. NaI crystal enclosed in a cylindrical lead shield 2 in. thick, placed below a shielded opening in the platform upon which the dogs were positioned with the thyroid directly over the crystal for optimal counting. Each dog was anesthetized with a slow intravenous infusion of sodium pentobarbital (13) before counting.

Other procedures. Blood was obtained by venipuncture in heparinized tubes for determination of plasma cholesterol (14), of urea nitrogen (15), and of protein-bound iodine (PBI) (16). For histological examination, biopsies of thyroids of 6 representative dogs were obtained under pentobarbital anesthesia, fixed immediately in neutral 10% formalin, and stained with hematoxylin and eosin. Statistical analyses were calculated using Student's *t* test (17).

Results. On the basis of diet and plasma cholesterol levels, there were four groups to be compared: (i) control group of 5 dogs on low-salt diet with normal cholesterol; (ii) "salt-fed" group of 12 dogs, some with normal and some with elevated cholesterol; (iii) "salt-fed" subgroup of 6 dogs with normal

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[4] Kibble = dry dog food. Made by Warner's Dog Food Company, Brooklyn, N.Y. Ingredients: Wheat flour, meat meal, bone meal, wheat middlings, wheat germ meal, dried tomato pomace, irradiated dried yeast, dried corn fermentation solubles, calcium phosphate, manganese sulfate, potassium iodide, iron sulfate, copper carbonate, salt, Vitamin A feeding oil, dried skim milk, Brewers dried yeast, Turmeric, charcoal. Analysis: Min. crude protein, 20%; min. crude fat, 3.5%; max. crude fibre, 3%; calcium, calcium, 1%; phosphorus, 0.7%; sodium, 0.35%; potassium, 0.4%; vitamin A (USP units/lb), 1000; vitamin D_2 (USP units/lb), 2000; vitamin E (IU/lb), 2.25; riboflavin, 1000μ/lb; thiamine, 300 μ/lb; niacin, 500 μ/lb; and pantothenic acid, 1800 μ/lb.

cholesterol values (within 3 standard deviations of the mean of the control group); and (iv) salt-fed subgroup of 6 dogs with elevated cholesterol values (at least 5 standard deviations greater than the control group mean value). All dogs had normal renal function (BUN 10 ± 1 mg/100 ml) and comparable body weights (9.7 ± 0.4 kg). There was no significant difference in ($p > 0.05$) systolic blood pressure (4) for the 5 control dogs (± 160.2, SE ± 2.65, range 152–168 mm Hg) and for the 12 salt-fed dogs (± 153.3, SE ± 7.56, range 98–200 mm Hg). The mean plasma cholesterol concentration in the group of 12 salt-fed animals (242 mg/100 ml) was significantly increased over that of the control group (179 mg/100 ml) ($p < 0.01$). This difference was due to an elevation in the 6 salt-fed animals classified in the subgroup "high-salt, elevated cholesterol" (293 mg/100 ml), since the mean concentration in the remaining 6 "high-salt, normal cholesterol" animals (190 mg/100 ml) was not significantly different from controls ($p>0.05$).

The mean thyroidal ^{131}I uptake of the 5 control dogs was higher at 48 hr ($p < 0.05$) than that of the 12 dogs in the high-salt group, but there was no difference at either 24 or 48 hr between the mean ^{131}I uptakes of the 6 salt-fed dogs classified as normocholesterolemic and the 6 salt-fed dogs classified as hypercholesterolemic ($p > 0.05$). Because of the relatively high ^{131}I uptakes of dog No. 84, the data were reanalyzed with No. 84 excluded, but with similar conclusions.

All of the PBI values were within the normal range for dogs for this laboratory (Table I). There was no significant difference between the mean PBI values of the different groups and subgroups ($p > 0.05$), and there was no reciprocal relationship between PBI and cholesterol concentration.

Thyroid biopsies were obtained from dogs No. 11 and 93 (control group), 48 and 92 (high-salt, normal cholesterol), and 52 and 56 (high-salt, elevated cholesterol). All sections were reviewed by pathologists experienced in thyroid abnormalities, including those of the dog (18); no significant histological differences and no abnormalities could be recognized in a double-blind study.

Discussion. Among salt-fed dogs neither thyroidal ^{131}I uptake nor plasma PBI concentrations were significantly different between the group with and the group without hypercholesterolemia. Furthermore, histological review of thyroid biopsies indicated no differences. Thus, there was no evidence to support the suggestion that the hypercholesterolemia observed in salt-fed dogs and rats (1–4) is due to hypofunction of the thyroid. It is conceivable that the hypercholesterolemia was produced during a prior period of thyroidal hypofunction, with restoration of thyroid function to normal before correction of the hypercholesterolemia. However, the mean plasma cholesterol values of our hypercholesterolemic beagles were not significantly different at age 4.5 and 6 years. Similarly, in our salt-fed rats, repeated observations up to 22 months indicate that a spontaneous decline in plasma cholesteral seldom occurs, unless salt-feeding is stopped (unpublished data).

The somewhat lower ^{131}I uptakes observed in the salt-fed animals (group 2) as compared with the controls may be due to enhanced urinary excretion of iodide and a corresponding decrease in thyroid uptake, as observed in salt-fed mice (10). In the reports that iodine deficiency goiters could be produced by salt-feeding (9–11), special low-iodine diets were necessary. By neutron activation analysis, the iodine content of the dog kibble as found to be 3.9 mg/kg (±10%), so from this source alone each dog would received 750–800 μg of iodide/day, an amount in excess of normal requirements.

Certain other observations suggest that a high NaCl intake alone does not produce hypothyroidism. Daily ingestion of large amounts of NaCl increased the metabolic rate of young male Japanese (19), and salt-fed rats and rabbits had higher QO_2 values in liver and muscle slices *in vitro* (20). Chronic excess salt consumption had produced hypertension in several species [see (21)], while development of experimental hypertension can be blocked by inhibition of thyroid

TABLE I. ^{131}I-Thyroidal Uptake and PBI in Dogs According to Salt Intake and Plasma Cholesterol.

12 dogs on high-salt diets					5 dogs on low-salt control diet				
Dog no.	Total cholesterol (mg/100 ml)	^{131}I uptake (%) 24 hr	^{131}I uptake (%) 48 hr	PBI (μg/100 ml)	Dog no.	Total cholesterol (mg/100 ml)	^{131}I uptake (%) 24 hr	^{131}I uptake (%) 48 hr	PBI (μg/100 ml)
Subgroup with high cholesterol									
51	250	6.8	6.1	4.4	11	160	7.4	7.2	5.0
52	360	6.9	6.8	4.5	83	190	13.3	12.3	5.2
53	280	7.6	9.3	5.2	85	175	12.1	11.6	6.8
54	290	9.4	8.5	6.1	93	190	4.9	8.2	4.7
56	340	6.4	6.6	7.3	324	180	8.4	11.9	4.7
81	238	8.1	6.8	6.9					
Mean (±SE)	293 (±20)	7.5 (±0.4)	7.4 (±0.5)	5.7 (±0.6)		179 (±5.4)	9.0 (±1.4)	10.2 (±1.1)	5.3 (±0.4)
Subgroup with normal cholesterol									
46	198	6.9	8.5	4.4					
48	160	5.6	7.1	3.8					
55	218	6.9	6.5	4.1					
84	200	19.3	10.1	6.1					
92	183	7.6	8.3	4.6					
95	183	7.3	3.9	4.3					
Mean (±SE)	193 (±8.1)	8.9 (±2.1)	7.4 (±0.9)	4.6 (±0.3)					
Group mean	242 (±18)	8.2 (±1.0)	7.4 (±0.5)	5.1 (±0.3)					

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function surgically or chemically (22–24).

We have, therefore, concluded that the hypercholesterolemia which may be associated with chronic excess salt feeding is not the result of significant depression of thyroid function. The mechanism by which hypercholesterolemia may occur, as well as its significance to the development of atherosclerosis, remains unexplained. We attribute the observation that only half of the dogs on high-salt diets became hypercholesterolemic to a genetically-determined susceptibility to the effects of chronic excess salt ingestion, as has been demonstrated by inbreeding of rats to obtain strains resistant and susceptible respectively to salt-induced hypertension (25, 26). Since dogs are resistant to salt-induced hypertension, we are presently exploring in rats the question of whether these animals susceptible to salt-induced hypertension also are susceptible to salt-induced hypercholesterolemia and *vice versa*. Preliminary data suggest that this is indeed the case.

Summary. The hypercholesterolemia associated with chronic excess salt feeding is not due to hypothyroidism. As shown by thyroidal ^{131}I uptake, by plasma protein-bound iodine concentrations, and by histological studies of thyroid biopsy specimens, there were no significant differences in thyroid function between dogs with and dogs without hypercholesterolemia chronically consuming a high-salt diet.

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RENIN ACTIVITY, SODIUM AND POTASSIUM IN HYPERTENSIVE PATIENTS*

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INTRODUCTION

HUMAN peripheral plasma renin activity levels are altered in various pathological conditions, particularly those manifested by hypertension [1,2]. Likewise, hypertension may be associated with alterations in serum electrolyte levels [3,4], abnormalities of other chemical factors [5], and with disturbances of cardiac and renal function [6, 7]. However, most of the available studies deal only with a few of these isolated variables in any given patient population. Studies regarding the interrelationships of renin values and various physiologic and pathologic conditions and biochemical parameters are essentially lacking in large patient groups. Furthermore, except for the thiazide diuretics, there is little information regarding the possible effects that various drugs may have on plasma renin. Since the physiological and biochemical status of hypertensive patients represent complex interrelationships, which may be further complicated by drug therapy, it was of interest to determine how these interactions were expressed in a large hypertensive population.

The purposes of this retrospective study were: (a) to characterize our population of hypertensive patients in terms of age, sex and weight; (b) to survey peripheral plasma renin activity in these hypertensive patients so as to determine any relationships which may be present; and (c) to investigate the interrelationships of renin to biochemical, physiological and drug programs in the hypertensive population.

MATERIALS AND METHODS

The medical records of 200 carefully studied hypertensive patients were examined. Malignant hypertensives were not included in the population. A persistent diastolic pressure of 95 mm Hg or greater was chosen for the designation of hypertension. All subjects were patients at either the University of Iowa Hospitals or the Iowa City Veterans Administration Hospital. They were selected from a group of patients who had peripheral plasma renin activity assays performed.

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Normal subjects

The normal subject population was composed of ambulant men (N=20) and women (N=10) whose ages ranged from 19 to 68 yr. All of them had blood pressures below 130/80 mm Hg, with no evidence of renal or cardiovascular disease.

Patients

White male (N=105) and female (N=95) patients ranging in age from 16 to 73 yr were studied. Evaluation began with a complete history and physical examination. The blood samples for the determination of plasma renin activity were drawn and blood pressure measurements recorded in the sitting position. Some of the patients were hospitalized at the time the renin activity samples were taken, others were outpatients. All of the various laboratory and special diagnostic data to be subsequently described were obtained within 48 hr of the time that the renin samples were taken. The routine laboratory chemistries were drawn not more than 24 hr prior, or subsequent, to the drawing of the renin samples. Laboratory data as indicated were available for 137 essential hypertension patients and 50 renovascular hypertension patients. Special care was taken to exclude patients who, according to their hospital records, had undergone fluctuations in their clinical state during the time of evaluation.

History and physical examination

The data obtained from the medical history and physical examination included age, sex and weight. The parameters taken from the recorded physical examination were blood pressure, weight, height and general physical condition. It was noted if women were taking birth control pills.

Laboratory data

The routine laboratory studies which were evaluated included measurements of serum cholesterol [8], fasting blood glucose [9], serum uric acid [10], and serum chloride [11]. Serum sodium and potassium levels were measured with an IL Flame Photometer (Instrumentation Laboratory, Inc., Lexington, Massachusetts). Serum carbon dioxide content was determined by a Natelson Microgasometer (Scientific Industries, Springfield, Massachusetts). Renal function was evaluated by serum urea nitrogen [12], serum creatinine [13], and creatinine clearance measurements [13, 14]. Electrocardiograms were also noted.

Renin assays

Peripheral venous blood samples were drawn from an antecubital vein while the patient was seated. The majority of the renin assays were performed using rat bioassay techniques as described by Pickens *et al* [15]; however, a few were done according to the method of Helmer and Judson [16]. Renin activity was expressed as nanograms (ng) of angiotensin amide per milliliter of plasma.

Special diagnostic procedures

Intravenous pyelography, radioactive renography, aortography and renal arteriography observations were recorded. The description and interpretation of these techniques can be found in several recent articles [17–19].

Drugs

Drugs were recorded as present if the patient was taking drugs at the time the renin sample was taken, or within two days prior to the time the samples were drawn. An exception to this occurred with guanethidine, the thiazides and spironolactone, where a minimum of three drug-free weeks was used to classify the patients as 'untreated'. The term 'untreated' was also used to indicate that the patients had never been treated.

Diagnosis

Although no single classification of hypertensive disease is universally accepted, those used in this study are primary hypertension (essential), which denotes elevated blood pressure of unknown cause with or without clinically demonstrable symptoms, and secondary hypertension, which refers to elevated blood pressure of known etiology. A major subheading of secondary hypertension is 'renovascular hypertension', which we use to mean renal artery stenosis in conjunction with elevated blood pressure. In patients designated as renovascular hypertensives, the diagnosis of functionally significant renal artery stenosis was made by means of differential renal vein renins and/or split renal function tests [20, 21]. Cause and number of deaths within the population were also examined.

Data processing

Information characterizing each patient was coded and recorded on case history forms. The data were transferred to punch cards and verified by the University of Iowa Computer Center key punching staff. All computations were done on an I.B.M. 360 Model 65 computer by modifying the University of Iowa's Statistical Package for the Social Sciences. The statistics [22] that were used in the analysis of this study are: (a) standard deviation; (b) Pearson's Product Moment Correlation Coefficient (r); (c) Student's *t*-test; and (d) the modified *t*-test in conjunction with Pearson's Coefficient.

RESULTS

Personal and socioeconomic attributes of the hypertensive population

The population studied was composed of 200 patients; 105 (53.0 per cent) were males and 94 (47.0 per cent) were females. All of the patients were white. Their ages ranged from 17 to 73 yr, with the average age being 46 ± 1.0 for the males and 41 ± 1.3 for the females. The average height and weight for the male population was 5 ft 8 in. and 176 lb. The females averaged 5 ft 3 in. in height and 144 lb in weight. About one-half of the male (49.6 per cent) and female (47.2 per cent) hypertensive population was overweight as judged according to data compiled in an actuarial study of average body builds and blood dressure [23]. There were nearly twice as many males who had a normal body build as there were males who were classified as being underweight. The frequency of overweight females was nearly equal to the frequency of females with normal body builds. Nearly 75 per cent of the men were laborers or 'blue collar' workers, while most women (70.8 per cent) were housewives.

There were more essential hypertensive patients (68.5 per cent) in the population than all others combined. The other major diagnostic category was composed of renovascular hypertensive patients (25.0 per cent). Only 6.5 per cent of the hypertensive population was diagnosed as having pyelonephritis. Arteriosclerosis was the

most frequent secondary diagnosis which occurred concurrently with a major diagnosis. In the essential hypertensive population, 9.7 per cent of the patients had arteriosclerosis as coded on hospital charts, while only 5.2 per cent of the renovascular hypertensive patients had arteriosclerosis. Diabetes was the second most frequent disease occurring as a secondary diagnosis in the essential hypertensive (4.9 per cent) and renovascular hypertensive (0.9 per cent) population. Thirty-six per cent of the patients were untreated. Diuretics were the most frequently used (36.4 per cent) major class of drugs. Drugs which interfere with catecholamine metabolism, such as reserpine, guanethidine and methyldopa, comprised the second most frequent (27.4 per cent) drug group. This drug class was followed in descending order of frequency by sedatives, tranquilizers and hydralazine.

TABLE 1. SUMMARY OF ESSENTIAL AND RENOVASCULAR HYPERTENSIVE PATIENTS

	Essential hypertensive patients* N=137		Renovascular hypertensive patients N=50	
	No drug $\bar{x}\pm$S.D. (*n*)	Diuretics $\bar{x}\pm$S.D. (*n*)	No drug $\bar{x}\pm$S.D. (*n*)	Diuretics $\bar{x}\pm$S.D. (*n*)
Systolic pressure (mm Hg)	159.3±3.6 (66)	155.3±4.1 (49)	166.1±6.6 (18)	168.0±6.0 (23)
Diastolic pressure (mm Hg)	98.5±2.2 (66)	98.1±3.1 (49)	102.1±3.2 (18)	102.5±2.9 (23)
Age (yr)	41.8±1.6 (70)	47.2±1.6 (50)	47.2±3.7 (18)	46.9±2.5 (23)
Weight (lb)	160.6±5.0 (68)	176.8±5.0 (50)	153.5±6.8 (17)	140.0±6.4 (22)
Chemistry				
BUN (mg %)	16.1±0.6 (64)	19.3±1.1 (46)	17.9±1.6 (17)	20.0±1.9 (22)
Fasting glucose (mg %)	105.8±5.1 (43)	103.0±5.0 (40)	99.3±4.2 (8)	108.6±8.7 (15)
Cholesterol (mg %)	239.5±7.3 (45)	240.0±7.0 (37)	227.0±10.0 (14)	261.1±16.3 (21)
Creatinine (mg %)	1.1±0.1 (65)	1.2±0.1 (50)	1.6±0.4 (18)	1.5±0.3 (23)
Potassium (mEq/l)	4.1±0.1 (62)	4.0±0.1 (50)	4.0±0.1 (18)	3.8±0.2 (22)
Sodium (mEq/l)	140.3±0.6 (54)	140.2±0.6 (31)	138.4±0.9 (18)	139.1±0.9 (23)
Uric acid (mg %)	6.6±0.6 (31)	7.2±0.4 (35)	7.1±1.3 (5)	6.1±0.5 (12)
Carbon dioxide (mM/l)	24.9±0.5 (59)	25.1±0.5 (48)	25.9±0.9 (17)	25.2±0.8 (22)
Chloride (mEq/l)	100.7±0.9 (45)	101.9±1.4 (24)	98.0±1.4 (16)	98.1±1.5 (20)
Creatinine clear (ml/min)	88.9±5.4 (24)	86.8±8.4 (14)	75.0±14.0 (9)	64.8±5.9 (16)
Renin activity (ng/ml)	3.2±0.4 (70)	5.1±0.9 (50)	5.8±1.2 (18)[a]	12.2±2.5 (23)[b]

* Tests of significance between groups are designated [a], [b]; Essential hypertensives are denoted as EH, Renovascular hypertensives denoted as RH.

[a] RH no drugs significantly ($P<0.01>$) higher than EH no drugs.

[b] RH taking diuretics significantly ($P<0.1>$) higher than RH no drugs, also significantly higher than EH no drugs and EH diuretics.

Essential hypertensive population

Blood pressure of the essential hypertensive population (*Table* 1). The essential hypertensive population was composed of 80 males and 57 females. The male and female systolic and diastolic blood pressures were similar, with a mean value of 155/99. A very wide range of blood pressures was found in both the male and female patients.

Special diagnostic procedures. Since this group of patients was diagnosed as essential hypertensives, it would be expected that there would be very few positive results from the special diagnostic procedures. Nearly the same frequency of men (70 per cent) and women (71 per cent) were subjected to electrocardiographic procedures. About three times as many males (29) as females (11) had electrocardiographic evidence of left ventricular hypertrophy. However, the same frequency (54 per cent) of non-specific cardiac abnormalities, such as *st-t* wave changes, was observed in both the male and female essential hypertensive population.

Essential hypertensive patients not receiving drugs (*Table* 1). The blood pressures of these patients were moderately elevated, while all of the chemistry parameters were within the normal range, with the exception of an increased uric acid (mean 6.6 mg per cent, normal 2–6 mg per cent), and fasting glucose (mean 105.8 mg per cent, normal 65–100 mg per cent) values.

Essential hypertensive patients receiving diuretics (*Table* 1). Male and female patients receiving diuretics had nearly the same mean systolic and diastolic pressures as those patients who were not receiving therapy. The various chemical parameters were not different between the two groups. The mean renin activity level of the patients who were taking diuretics was considerably higher (5.1 ± 0.9 ng/ml) than the level of activity in the patients who were not taking drugs (3.2 ± 0.4 ng/ml (Table 2). Mean uric acid levels were slightly, but insignificantly, higher in this group (7.2 mg per cent in this group, 6.6 mg per cent in those patients not receiving drugs).

Essential hypertensive patients receiving anticatecholamine drugs in combination with diuretics and other drugs. There were no significant blood chemistry differences between this group of patients and the two groups just discussed. The mean renin activity level for this group was 4.7 ± 0.8 ng/ml, which was higher than the mean value obtained for the 'no-drug' group (Table 2). The mean uric acid concentration was 7.9 mg per cent, the highest of any group.

Renovascular hypertensive population

Blood pressure of the renovascular hypertensive population (*Table* 1). The renovascular hypertensive population was composed of 19 males and 31 females. The male and female systolic and diastolic blood pressures were similar, with a mean value of 167/102.

Special diagnostic procedures. All of the special diagnostic procedures revealed more positive results in the renovascular hypertensive population than were obtained in the essential hypertensive population. It was noted that left ventricular hypertrophy

TABLE 2. MEANS AND STANDARD ERROR AND NO. OF SUBJECTS

	ng of angiotensin/ml of plasma*		
	Male	Female	Both
Normal subjects	2.9±0.5 (20)	3.2±0.8 (10)	3.0±0.5 (30)
Essential hypertension (No drugs)	2.8±0.3† (39)	3.9±0.7† (31)	3.2±0.4 (70)
Essential hypertension (Anticatecholamines)	5.2±1.0† (28)	3.6±0.7† (11)	4.7±0.8 (39)
Essential hypertension (Diuretics)	5.1±0.9 (34)	5.0±2.0 (16)	5.1±0.9 (50)
Renovascular hypertension (No drugs)	4.6±0.9 (34)	7.0±2.2 (9)	5.8±1.2 (18)
Renovascular hypertension (Anticatecholamines)	8.4±3.3 (9)	16.4±3.7 (15)	13.4±4.4 (24)
Renovascular hypertension (Diuretics)	12.3±2.5† (23)	14.0±3.4† (15)	12.2±2.5 (38)
Contraceptive status of essential hypertensive women	Age	Blood pressure	Renin value
No birth control pills	33.9±2.5 (18)	157/99.4±6.9/5.0 (18)	3.8±0.6 (18)
Birth control pills	33.5±2.4 (16)	160/101±8.4/5.9 (16)	4.1±0.8 (16)

*Statistical evaluation of significance between means was done only within drug group, and only when N > 10 for both males and females.

†$P = < 0.05$ between males and females within a drug group.

was nearly twice as common in the male patients. Exactly 70 per cent of the renovascular hypertensive patients had either a non-specific cardiac abnormality or left ventricular hypertrophy. Ninety-two per cent of the renovascular hypertensive patients were evaluated by electrocardiographic techniques.

Renovascular hypertensive patients not taking drugs (*Table* 1). Only 36 per cent of the renovascular hypertensive patients were not receiving drugs, compared to 51 per cent of the essential hypertensive population. The untreated renovascular patients were not significantly different from the remainder of the population with regard to either physical or chemical parameters. The mean renin activity level of the renovascular hypertensive patients was 5.8±1.2 ng/ml, which was significantly higher ($P < 0.01$) than the same mean value of the essential hypertensive group who were not taking drugs.

Renovascular hypertensive patients taking diuretics (*Table* 1). There were no significant physical differences in the renovascular hypertensive group who were taking diuretics and the remainder of the renovascular population. A slight increase in blood urea nitrogen level (20.0±1.9 mg per cent) was noted. A decreased mean creatinine clearance rate of 64.8±5.9 ml/min was also observed in this population. The difference between the mean renin value of this group of renovascular hypertensive patients (12.2±2.5 ng/ml) when compared to the mean renin value of the renovascular hypertensive patients who were receiving no drugs was statistically significant ($P < 0.01$).

Renovascular hypertensive patients taking anticatecholamine drugs. Nearly one-half of the renovascular hypertensive patients were receiving anticatecholamine drugs. The mean blood urea nitrogen value (24.4 mg per cent) was significantly elevated ($P<0.01$) above the blood urea nitrogen levels of the other renovascular hypertensive drug groups. The mean creatinine clearance value was below normal limits (60.8 ± 5.4 ml/min); however, the difference was not statistically significant at the $P=0.05$ level when compared to the values of the other patients with renovascular hypertension. This group had an elevated serum uric acid level (mean 7.2 mg per cent) and an increased mean renin activity level of 13.4 ± 2.7 ng of angiotensin/ml of plasma. All of the other chemistry parameters were within normal limits.

Correlation studies. Because the blood urea nitrogen, serum sodium, serum potassium, creatinine clearance and renin activity values were borderline or abnormal in many of the population groups which were studied, it was necessary to determine what associations may be present. Pearson's Product Moment Correlation Coefficient (r) test was used to test the strength of associations. No correlation was considered to exist unless the correlation coefficient (r) had a positive or negative value of 0.5 or greater and a t-test significance of $P<0.05$.

The essential hypertensive and renovascular hypertensive patients were studied as distinct populations. Following this, they were subgrouped according to drug usage. For each group, blood urea nitrogen values, serum sodium values, serum potassium levels, and renin activity values were tested against systolic and diastolic blood pressures and several chemistry parameters. The parameters that were used in the correlations with the blood urea nitrogen, serum potassium and renin activity levels were as follows: (a) systolic blood pressure, (b) diastolic blood pressure, (c) cholesterol, (d) serum sodium, (e) uric acid, and (f) creatinine clearance.

No correlations regarding the above cited parameters were found in the essential hypertension population. However, there were several statistically significant correlations present in the renovascular hypertension population. In the 'no-drug' group, the highest correlation coefficient was found to occur for serum potassium levels and renin activity where $r=-0.7032$ at $P<0.001$ (Table 3). A high correlation was also present in renovascular patients taking diuretics ($r=0.5191$, $P<0.01$). The correlation coefficient for serum sodium and renin activity for the 'no-drug' group was only -0.4777 at $P<0.02$, while the coefficient for renin and sodium in patients taking diuretics was even lower ($r=0.1528$, $P<0.200$). Only renovascular hypertension patients taking diuretics showed a correlation between serum potassium levels and systolic and diastolic blood pressures of $r=-0.5529$, $P<0.01$, and $r=-0.6121$, $P<0.001$ respectively. All of the renovascular patients showed correlations between serum potassium levels and creatinine clearance rates as well as between BUN and renin activity (Table 3).

Renin activity in women taking birth control pills (*Table* 2). Since 14 per cent of the essential hypertensive women in the study were taking birth control pills, it was decided to determine differences in blood pressure or renin levels that might exist in these women and women who were not taking birth control pills. The control group was obtained by selecting 18 essential hypertensive women of approximately the same age range who were not taking birth control pills. Data obtained from the two

TABLE 3. CORRELATION COEFFICIENTS

Variables	Essential hypertensive patients		Renovascular hypertensive patients	
	r—Corr. coeff.	Prob.	r—Corr. coeff.	Prob.
No drug				
Serum potassium vs renin activity	−0.3202	0.006	−0.7032	0.001
Serum sodium vs renin activity	−0.0841	0.200	−0.4777	0.020
Serum potassium vs creatinine clearance	+0.2814	0.005	+0.5158	0.001
BUN vs renin activity	+0.2141	0.002	+0.5001	0.001
Diuretics				
Serum potassium vs renin activity	−0.1298	0.200	−0.5191	0.010
Serum sodium vs renin activity	+0.0884	0.300	−0.1528	0.200
Serum potassium vs creatinine clearance	+0.2643	0.005	+0.5283	0.001
BUN vs renin activity	+0.2551	0.002	+0.4503	0.001

groups are given in Table 2. As is evident from the table, there was no difference noted between groups by Students' *t*-test.

Deaths in the hypertensive population (*Table* 4). Data regarding the hypertensive individuals who died in the course of study are given in Table 4. The number of patients who died represents 6.0 per cent of the population. Of the total population, 7.5 per cent of the males and 5.5 per cent of the females died. By comparing these data with the overall population, it is noted that the mean systolic blood pressure was significantly higher in patients who died. The mean renin activity level as well as age was much higher in this group of individuals.

TABLE 4. CAUSE OF DEATH IN THE HYPERTENSIVE POPULATION

Patient	Sex	Renin	Blood pressure	Age at death	Autopsy report
G.C.	M	2.4	160/80	52	Adenocarcinoma
J.C.	M	2.3	140/70	51	Basilar artery thrombosis
E.F.	M	25.8	180/112	61	Cerebral vascular accident
C.G.	M	39.0	186/138	27	Renal failure
C.H.	M	11.8	120/80	37	Cardiorespiratory insufficiency; end stage kidney disease; renal failure
A.P.	M	3.6	190/95	70	Unknown*
E.R.	M	2.2	160/120	44	Cerebral infarction; pneumonia
H.S.	M	1.8	180/120	51	Cerebral vascular and coronary occlusion
C.S.	M	3.4	180/85	62	Myocardial infarction
J.B.	F	5.1	160/80	72	Adenocarcinoma
M.C.	F	2.1	230/120	57	Dissecting thoracic aneurism
A.P.	F	4.4	130/80	49	Unknown*
M.T.	F	1.7	210/140	52	Brain hemorrhage (Clinical diagnosis)
B.W.	F	1.9	220/94	75	Retroperitoneal hemorrhage
A.W.	F	1.9	170/110	35	Unknown*
		$\bar{x}$=7.2 S.E.±2.8	$\bar{x}$=172/102 S.E.±8.7/5.8	$\bar{x}$=53.0 S.E.±3.6	

*Autopsy report unavailable—died away from hospital.

DISCUSSION

It was reported in the Tecumseh, Michigan study [24] that the highest prevalence of hypertensive disease for males occurred at 45 yr of age and for females at about 50 yr of age. Perera [25] has commented that essential hypertension is always present by middle age. In the present study, the mean age of the male patients was 46 yr, and the mean age of the females was 41 yr.

It is popular to consider that hypertension and obesity go together; however, obesity is a very common finding among virtually all strata of the adult population of the United States. Stamler [26] reported that among both middle-aged white men and women, about 53 per cent of the population is overweight. The present study revealed that about 50 per cent of the male and female patients were overweight. However, since obesity is so commonplace among the middle-aged, it is difficult to judge the significance of correlations regarding hypertension and obesity. On the other hand, it was noted that about one-half of the hypertensive population was of average weight, or underweight. A study by Bechgaard [27] reported that only 3 per cent of a group of hypertensive patients were underweight. The present data showed that about 15 per cent of the males and females were underweight. It is also generally considered that there is a strong correlation between relative weight and systolic blood pressure [26]. The present study and the study by Kannel *et al* [28] both showed a very low correlation ($r=0.3$) between relative weight and systolic blood pressure.

A recent study by Weinberger *et al* [29] reported that plasma renin activity and blood pressure were higher in some hypertensive patients during oral contraceptive administration. Many hypertensive women who stopped taking contraceptive pills for several months experienced a significant decrease in blood pressure. Using age-matched control women, we could not demonstrate any significant differences in blood pressure and renin activity between women taking birth control pills, and those who were not taking contraceptive pills.

Of the 12 patients in the hypertensive population who died of known lesions during the course of the study, the cause of death was either congestive heart failure, coronary heart disease or cerebrovascular accident in all but three cases. A study by Smith *et al* [30] of 376 patients with hypertension found three main causes of death. These major categories were congestive heart failure and coronary heart disease, cerebrovascular accidents and uremia. Smirk and Hodge [31] reported that coronary artery disease was the chief cause of death (41 per cent) in a hypertensive population of 518 persons under treatment.

In the currently studied population, it was apparent that the chief causes of death were due to cardiovascular and cerebrovascular lesions. It is well known that unchecked hypertension leads to major complications which involve key target organs, in particular the heart, brain and kidney. In untreated essential hypertension, a prospective prognostic study by Sokolow and Perloff [6] has clearly shown that the higher the casual arterial pressures of the patients, as a group, the greater the mortality over a 5-yr period. It was also shown in this study that the increase in mortality was directly correlated with the degree of abnormality of the fundi, heart, brain and kidney. Since the advent of medical treatment for hypertension, congestive heart failure and uremia as a cause of death in hypertension have decreased in frequency [32, 33]. In contrast, there is little suggestion that treatment of hypertension has reduced morbidity and mortality due to coronary artery disease [31, 34]; our data support this concept.

In addition, many of our patients had moderate increases in serum cholesterol, a possible factor in the development of arteriosclerotic vascular disease.

Although the incidence of renovascular hypertension is unknown, it has been estimated to occur in about 5–15 per cent of the hypertensive population [21]. In the present study 25 per cent of the hypertensive population was composed of patients with renovascular hypertension. The higher frequency of renovascular hypertension in our group may be due to the fact that the University of Iowa Medical Center receives a relatively large number of patients with severe hypertension; many patients are referred here specifically for evaluation of renal artery disease. Another factor may be related to increased ability to detect renal involvement by use of intravenous pyelograms, radioactive renograms, renal arteriograms and split renal vein renins.

Hyperuricemia in essential hypertensive patients has been cited in several reports [5, 35] with the conclusion that the hyperuricemia resulted from diminished renal excretion of urate. Normal uric acid levels for our laboratory are 2–6 mg per cent. Uric acid levels in our untreated patients with essential hypertension and with renovascular hypertension are somewhat higher than this, although not significantly so. This tends to confirm the observations of Cannon *et al* [5]. The higher uric acids noted in those patients treated with thiazide diuretics also would be expected, since an increase in serum uric acid is a known response to these drugs [36].

The renin activity levels were within normal limits in the essential hypertensive population. Early observations [37, 39] have reported decreased or no renin activity in some patients with essential hypertension. Conn *et al* [40] proposed that many, if not all, of these patients had 'normokalemic primary aldosteronism'. Some workers have suggested that low renin levels in some patients with essential hypertension occur as an inherited or racial characteristic. However, this phenomenon would not be apparent in our study because our population was composed entirely of white individuals. Our normal subjects were selected to cover a wide age range, and to include both men and women. In view of this, and since our untreated essential hypertensives fall well within the normal range, we must conclude that few, if any, of these patients have hidden aldosterone producing adrenal adenomas.

Chemistry values in essential hypertensive patients receiving diuretics or anti-catecholamine drugs were not different as compared to essential hypertensive patients not taking drugs. However, there was a significant increase in renin activity in patients who were taking diuretics. This is most likely related to the volume and/or sodium depletion which is known to occur with this type of drug [43, 44]. It indirectly suggests that in many patients the effects on volume and sodium persist as long as the drug is given. As an alternative, it is possible that these patients had more severe hypertension, and that their disease in some manner increased the renin levels. The lack of correlation between blood pressure and renin activity in all of the groups suggest that this is not the determining factor, however.

The renovascular hypertensive patients showed many more abnormalities. There was a significantly higher mean systolic blood pressure in the renovascular hypertensive patients. Serum potassium, serum sodium and creatinine clearance rates were somewhat low. In contrast, the mean blood urea nitrogen and renin activity levels were significantly elevated. Exactly 70 per cent of the renovascular hypertensive patients had some cardiac abnormality such as left ventricular hypertrophy or abnormal electrocardiographic results. These abnormalities suggest generalized involvement of

vessels, particularly cardiac, cerebral and renal vessels, with arteriosclerosis in many of the patients with renovascular hypertension, even though this diagnosis was not coded on their hospital charts.

Renovascular hypertensive patients taking diuretics had an abnormally high blood urea nitrogen level, as well as an increased plasma renin activity, as compared to renovascular hypertensives not taking drugs. Winer *et al* [45] have demonstrated that the use of thiazides and other diuretics will increase plasma vasoconstrictor activity (renin activity). This effect may be particularly pronounced in the presence of a pre-existing significant renal artery lesion.

Since many of the chemistry parameters were abnormal in the renovascular hypertensive population, correlation studies were performed. Renin values, although elevated, did not show a strong correlation with blood pressure, serum sodium or creatinine clearance. A strong inverse relationship was shown between renin activity and serum potassium in these patients, however. These data support the finding of Fitz and Armstrong [46]. The negative relationship between increased renin activity and potassium may be due to the effect of the renin–angiotensin system on aldosterone secretion, as proposed by many investigators. Perhaps decreased potassium levels stimulate the renin–angiotensin system, as has been suggested from human [47, 50] and animal studies [48, 51–53]. Clinical evidence for this relationship is demonstrated by normotensive patients with Bartter's syndrome, where high levels of plasma renin activity are accompanied by increased levels of circulating angiotensin, hypokalemia, and increased secretion and excretion of aldosterone [54]. Furthermore, Muller *et al* [55] have experimental evidence that potassium administration reduces elevated renin levels. Further study is needed to clarify the role of potassium in renin release.

In the present study, both systolic and diastolic pressures were negatively correlated with potassium levels in the same drug groups as were found for the renin activity correlations. However, no significant correlation was found between blood pressure and renin activity.

Although serum sodium levels were abnormally low in many of the renovascular hypertensive patients, there was no significant correlation with either blood pressure or renin activity levels. This finding is in contrast to the reports of Brown *et al* [3], who proposed that elevated peripheral renin activity in patients with renovascular hypertension is correlated with hyponatremia, and who coined the phrase 'hyponatremic hypertensive syndrome'. This syndrome was not apparent in the presently studied population.

A moderate correlation between renin activity levels and blood urea nitrogen was found in the renovascular hypertensive population. A significant positive correlation was found between serum potassium levels and creatinine clearance rates. Cannon *et al* [5] observed an increase in uric acid with increased cholesterol levels, but they did not perform a statistical correlation analysis. No significant correlation was found in the present study, although both uric acid and cholesterol levels were elevated. No strong correlations were noted for renin activity in patients with essential hypertension.

Renin activity measurements have been criticized on many accounts. It is true that plasma renin activity is an indirect measurement and cannot be equated directly to angiotensin concentration. Also, inhibitors to the action of renin may not allow the reaction to proceed, or angiotensin may be more rapidly destroyed in one situation

than in another. Despite the legitimate criticism of the renin activity assay, in this study it consistently showed higher values in the renovascular hypertensive patients. The demonstration of significantly higher peripheral vein renin levels is of interest in view of current discussion concerning the role of the renin–angiotensin system in human renovascular hypertension. Although when judging individual renin values this difference may be difficult to detect, the trend seems to be clear-cut in our patients, and supports the concept that the renin–angiotensin system may be important in the etiology of renal vascular hypertension. Statistical, biochemical and clinical investigations regarding the biological levels could contribute much toward the understanding of the complex interactions in these patients.

SUMMARY

A retrospective study of 200 hypertensive patients was performed in order to investigate various clinical and biochemical relationships. The physical parameters which were studied included serum cholesterol, fasting blood glucose, serum sodium, potassium, uric acid, carbon dioxide, chloride and plasma renin activity. Renal function was evaluated by blood urea nitrogen, serum creatinine and creatinine clearance tests. Special diagnostic data such as intravenous pyelography, radioactive renography, aortography and renal arteriography were also considered. Electrocardiographic data were also recorded. Various drug regimens were taken into account along with specific diagnosis. The above information for each patient was transferred to punch cards and various statistical computations were done with a computer.

The population was composed of two distinct populations; patients with essential hypertension and those with renovascular hypertension. With the exception of increased levels of uric acid and fasting glucose, all of the chemistry values, including plasma renin activity, were within the normal range. Nearly 30 per cent of the essential hypertensive population had evidence of left ventricular hypertrophy. The blood pressures of the renovascular hypertensive (RVH) population were generally much higher than those of the essential hypertensive (EH) population. Exactly 70 per cent of the RVH population had either a non-specific cardiac abnormality or left ventricular hypertrophy. Plasma renin activity was significantly elevated in the RVH population only. RVH patients who were taking diuretics had an increased blood urea nitrogen level and a decreased creatinine clearance rate. These patients also had a significantly higher plasma renin activity level. Correlation studies revealed a significant negative correlation between serum potassium levels and renin activity, and between serum potassium levels and systolic and diastolic blood pressures in the RVH population. This population also showed a strong positive correlation between serum potassium levels and creatinine clearance rates as well as between BUN and renin activity. No other statistically significant correlations, including serum sodium levels, were demonstrated in either the RVH or EH population.

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SALT AND WATER ABSORPTION BY THE INTESTINAL TRACT

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1 General principles
2 Exchanges of salt and water across the intestinal mucosa
3 Evidence for active Na transport
  a Evidence for transcellular transport
  b Evidence for membrane function
4 Cl absorption
5 Water absorption
6 Source of energy for fluid and salt transport
7 K transport
8 Other consequences of Na absorption
References

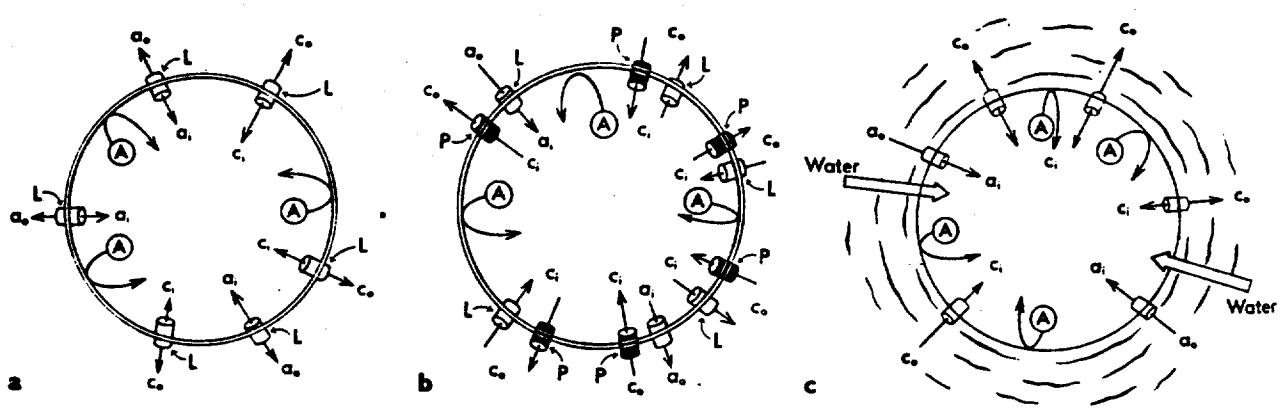
The absorption of salt by the intestinal tract resembles in many ways the processes of transcellular Na transport widely distributed in nature and to be found in other epithelial cells such as those of the kidney tubules, gall-bladder, urinary bladder of toads, and the skin of frogs and toads (Ussing, 1960; Leaf, 1965). These systems of transcellular absorption appear to depend upon the operation of processes of transport consisting of "pumps" and passive "leaks" located in the limiting membranes of the cells. Recent evidence also suggests that the mechanisms of salt transfer by the intestine are closely coupled with those for the transfer of other molecules; however, it is possible that a coupling between the transport of amino acids and of ions at the cell membrane may be wide spread among other tissues (Christensen, 1962).

1. General Principles

The processes of salt transport across the limiting membranes of animal cells appear to have evolved as a means of stabilizing the cell volume. For any cell capable of exchanging fluid and electrolytes with its environment and thus provided with membranes permeable to electrolytes and water, the presence in the cell contents of molecules and molecular aggregates which function as polyvalent electrolytes, but to which the cell membrane is impermeable (e.g., soluble enzymes, intermediates of metabolism, or organelles carrying a charge on their surfaces), introduces additional osmotic stresses by virtue of Gibbs–Donnan effects (see fig. 1a, b & c).

In many cells the stabilization of the volume appears to depend upon the regulation of the concentration of the diffusible ions inside the cell by the operation in the limiting membranes of active transport systems (pumps) for some of these ions (usually Na and K), the membranes being also leaky. In the dynamic steady state there is a constant exchange of the ions between the cell interior and the environment, the rate of ionic movement in one direction through the leaks being equal to that through the pumps in the opposite direction, while the concentrations (strictly, the electrochemical potentials) remain steady inside the cell, and the volume stable. In most cells, the functioning of the ionic pumps results in the electrochemical potential of Na inside the cell being less than that outside, and that of K greater. The operation of the pumps naturally requires a continual supply of free energy, and the interruption of this supply, by placing the tissue in a metabolically unfavourable environment (e.g., by cooling

FIG. 1. Stabilization of volume of symmetrical cell by ionic pumps in membrane



a: cell membrane enclosing impermeant anions A carrying charge z but leaky (through L) to cations and anions c_i, c_o, a_i and a_o. From Gibbs–Donnan equilibrium

$$\pi_i = A + \sqrt{\pi_o^2 + z^2A^2}$$

where π_i = internal osmotic activity = $A + [c]_i + [a_i]$, π_o = external osmotic activity = $[c_o] + [a_o]$.

If membrane is permeable to water, cell will swell as in (c)

b: if cell membrane is furnished with pumps (P) operating in conjunction with leaks (L) on c_i and c_o, the concentration of diffusible ions inside the cell can be regulated, π_i would be equal to π_o and the cell volume is stable

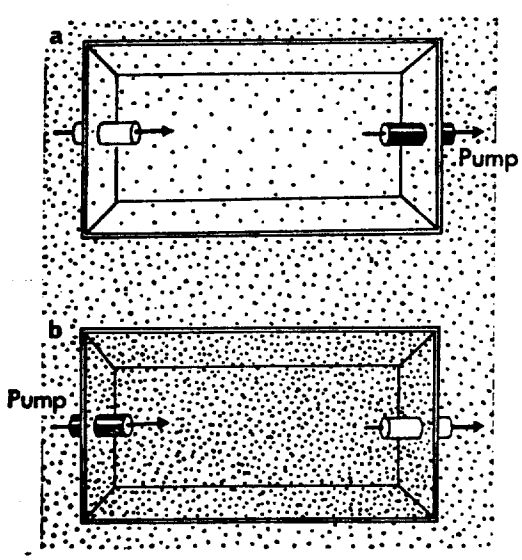
c: with cell in metabolically unfavourable environment, ionic pumping ceases, small ions are redistributed across membrane through leak in accordance with (a), and the cell swells

or by interrupting the blood supply), leads to a cessation of the pumping, a loss of cell K and a rise in the intracellular Na and Cl and water (tissue swelling) (see Leaf, 1956; Heckmann & Parsons, 1959; Robinson, 1960).

For a non-epithelial cell, e.g. an erythrocyte, the salt-transporting processes will be distributed symmetrically between all the faces of the cell which are in contact with its environment (fig. 1b). For a salt-absorbing epithelial cell, on the other hand, the transport systems will be distributed asymmetrically between the two faces bordering the two fluid compartments between which transport is occurring. Thus Na absorption may be regarded as one example of an important general principle that movement across an epithelial layer, that is to say, transcellular transport (translocation), depends upon the ability of the individual epithelial cell to sustain a concentration difference or electrochemical potential difference between its interior and the environment.

The system for the Na transport across the intestinal cells is in contrast to the systems which exist for the transport of sugars and amino acids across the cells (fig. 2). Absorption of sugars and amino acids also depends upon the existence of pumping systems situated in the limiting membranes of the cells, but the translocation depends upon the ability of the mucosal cells to accumulate the substances so that the intracellular concentration is greater than that in the environment (see papers by Newey and by Fisher, pp. 236 and 241 respectively of this Bulletin). The translocation is then achieved by the substance being pumped in at one face of the cell and leaking out at the other. For Na, on the other hand, the evidence is that the translocation depends upon the ability of these cells to maintain their internal Na at a lower electrochemical potential than is found in the environment. In this case the translocation results from a movement down a leak into the cell at one face and then outwards from the cell through a pump at the opposite face.[1]

FIG. 2. Two sorts of asymmetrical cell for transcellular transport



a: transported substance (e.g. Na) is pumped out of the cell at one pole and diffuses into the cell at the other pole down its electrochemical gradient

b: transported substance (e.g. glucose) is pumped into the cell at one pole and diffuses out at the other pole down its concentration gradient

In epithelial tissues the unit cells are usually packed in polygonal array, with some part of the lateral membranes in close contact with those of the neighbours. It has been generally assumed that the two cell membranes in contact at tight junctions present such a marked barrier to diffusion that no exchanges are possible between adjacent cells. However, recent experiments by Loewenstein (1966) indicate that the junctional membranes in many epithelia are structures which are highly permeable to ions, and possibly to larger molecules such as intermediates in metabolism. It therefore now seems likely that individual epithelial cells are linked to form a syncytium which functions as a concerted system for transport, but direct evidence for this in the specific instance of intestinal cells is lacking at the present moment.

2. Exchanges of Salt and Water across the Intestinal Mucosa

From what has been said, it is not surprising to find that active Na transport accounts for most of the net ionic traffic observed across many epithelia. Thus, across frog skin and the toad bladder, the net transcellular ionic traffic consists almost entirely of Na accompanied by Cl; more moles of Na than of any other ion are reabsorbed by the renal tubules of mammals; the salivary, biliary and pancreatic secretions contain Na as the predominant ion. In man, the gastro-intestinal secretions present to the intestine a daily load of water and salt which considerably exceeds the dietary intake. It has been estimated that the total load of salt presented daily for net absorption by the adult human intestine amounts to about 750 m-moles in some 8.5 l. and that, of this, only one-fifth is derived from the diet (Carter, Coxon, Parsons & Thompson, 1959). In dog and man, the fact has now been established that isotopically labelled Na and Cl and water rapidly exchange in both directions between the blood and the lumen contents.

It seems to be a feature of the duodenum and jejunum that, when hypotonic solutions are introduced into the lumen, NaCl moves from the blood until the intestinal contents become isotonic, when net absorption of the isotonic solution occurs. In the lower small intestine, on the other hand, a net absorption of NaCl occurs from hypotonic solutions. These differences in intestinal activity, which are an example of the different absorptive characteristics of different regions of the intestine, have been emphasized by Visscher, Varco, Carr, Dean & Erickson (1944), by McHardy & Parsons (1957) and by Code (1965). In the absence of a knowledge of the transmucosal potential difference in these various experiments, it is not clear whether it is the Na or the Cl which is being moved against its electrochemical gradient and therefore fulfils an operational definition of being subjected to active transport. What is clear from these experiments is that at least one of the ions must be subjected to active transport by the ileal mucosa.

[1] The functioning of these systems for transcellular transport raises the question of the mixing of the cell contents and the movement of the absorbed substances within the cell. Is this movement achieved entirely by diffusion through the cytoplasm, which lies in the interstices between the organelles, or do cytoplasmic movements play an additional part?

3. Evidence for Active Na Transport

a. Evidence for Transcellular Transport

The evidence that Na is subjected to active transport from the lumen across the ileal intestinal mucosa into the blood is now substantial and stems from the observation that in vivo, and also with identical Ringer solutions bathing both sides of segments of intestine isolated in vitro, under favourable metabolic conditions, there is a net transport of Na inwards across the cells from the lumen (see, e.g., Curran, 1965). There is also, incidentally, a substantial water absorption. This inward transport of Na continues although a potential difference exists across the whole wall, the fluid bathing the serosal suface being positive—*ca* 10 mv for rat intestine—to that in the lumen (see paper by Barry, p. 266 of this Bulletin). Under these circumstances the net inward movement of Na from the lumen constitutes a movement against an electrochemical gradient and therefore falls within the operational definition of active transport. The gradient of electrochemical potential across the mucosal cells can be reduced to zero by exposing the mucosal and serosal surfaces of segments surviving in vitro to Ringer solutions of identical composition and by clamping the transcellular potential difference to 0 v. Any single ionic species subject to independent active transport will then move more rapidly across the tissue in one direction than in the other and will contribute to the current which has to be passed through the voltage-clamp circuit to maintain the zero potential difference across the epithelium. In certain circumstances the short-circuit current across the intestinal wall is found to equal exactly the Na current (e.g. in rabbit ileum) (Schultz & Zalusky, 1964), but in many cases the short-circuit current does not match the net movement of Na (see, e.g., Barry, Smyth & Wright, 1965). However, a net inward movement of Na is invariably found in all these sorts of experiments. The occurrence of net inward movement of Na under zero electrochemical potential gradient proves the occurrence of active transport of the ion.

b. Evidence for Membrane Function

We must now consider the evidence for the dependence of transcellular Na transport upon the existence of active transport systems in the limiting membranes of intestinal mucosal cells.

i. There is an analogy with other tissues. Both in squid axon and in a transporting tissue, frog skin, the electrical phenomena associated with transport are generated in the limiting membranes. For squid axon the electrical potentials of resting and active nerve are found to originate in the limiting membranes and, for example, are independent of the position of the internal electrode relative to the membrane (Hodgkin, 1964). In the case of frog skin, the potential profile across the tissue exhibits quantal jumps (Ussing & Windhager, 1964). Transcellular transport is differently affected by the environment to which each face of the tissue is exposed. The permeabilities of the two faces of frog skin and of toad bladder to various electrolytes are different, so that there appear to be intrinsic differences between the properties of the two faces in these tissues (MacRobbie & Ussing, 1961). The electrical activity of the squid axon and ion transport between the interior of erythrocytes and the environment can be maintained with the cellular contents homogeneous and replaced by artificial media (see Hodgkin, 1964; Whittam, 1964). Many tissues possess the capacity to accumulate amino acids and also K ions (Christensen, 1962). The amino acids appear to be free inside the cells, and the transport seems to depend on membrane function. The majority of the accumulated K ions appear to be free and are not bound. Insoluble enzyme systems associated with membranes have been isolated from a wide variety of tissues; the activity of these systems of enzymes appears to be directly related to that of the active transport of Na and K. The systems hydrolyse adenosine triphosphate (ATP) and are affected by the presence of Na and K and the cardiac glucosides in a manner similar to systems for cation transport in the intact cell (Skou, 1965).

ii. There is some reasonably direct evidence for the existence of transport systems in the limiting membranes of intestinal mucosal cells. Thus there is histochemical evidence for such a localization of adenosine triphosphatase (ATPase) systems (Hollands & Smith, 1964). Cellular fractionation procedures which yield preparations of uncontaminated sheets of microvilli have permitted the identification of ATPases, and incidentally of disaccharidases and peptidases in the brush-border region of the cell (Miller & Crane, 1961; Berg & Chapman, 1965). For Na, the intracellular concentration appears to be less than that outside the intestinal mucosal cell (Schultz, Fuisz & Curran, 1966). Since there is also evidence that the interior of the cell is electrically negative to the exterior, it follows that the electrochemical potential of Na is lower inside the cell than outside. For sugars and amino acids there is strong evidence that the transport systems are located in the cell membranes, notably in the brush-border region (Kinter & Wilson, 1965; and see paper by Newey, p. 236 of this Bulletin).

iii. Systems for transcellular transport based upon the functioning of transport systems in the limiting membranes of cells are feasible. Examination of the properties of model systems permits specification of the conditions for transcellular transport by cells furnished with pumps and leaks in their limiting membranes (Parsons & Prichard, 1966).

4. Cl Absorption

In theory, provided that a movement of Na through the cell is induced and the cell membranes have a finite conductance for Cl, Cl transport could follow Na translocation through the cell in an entirely passive fashion. However, there is evidence that, in the small intestine and colon of some species, the Cl absorption is not related in a simple fashion to that of Na. It is found, for example, that the rates of Cl and Na absorption differ and that this difference depends upon the site in the intestinal tract where the absorption has been observed. Thus, in rat intestine, Na is absorbed faster than Cl in the jejunum but, in the ileum and colon, Cl is absorbed at least as fast as Na (Table I).

The data in Table I are derived from experiments in which isotonic salt solutions containing 25 mM-HCO_3 are initially present in the intestinal lumen. Since it can be calculated from the pH changes of the mucosal fluid that the movements of CO_2 largely occur as HCO_3 ions and it is also found that, in the contents of the ileum, the HCO_3 ion concentration during absorption may exceed that of the plasma, it appears that the movements of this ion can occur against an electrochemical gradient, the lumen of the intestine being negative to that in the plasma. From the operational point of view, the HCO_3 ion movements across rat ileum in vivo thus appear to be an example of active transport. It has been pointed out that the

TABLE I. Absorption rates for rat intestine in vivo (from Parsons (1956))

(The values given are the means of six experiments, with the S.E. of the mean. The rates are expressed in m-mole/g. dry wt./4 hr. Negative values indicate net entry into the lumen of the intestine.)

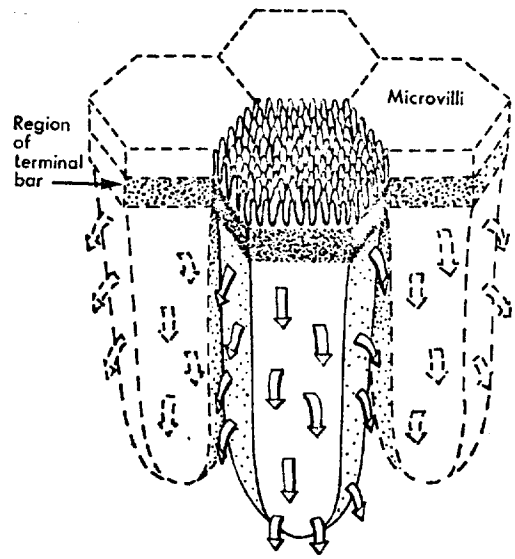
Segments	Amounts absorbed		
	Na	Cl	Total CO_2
Jejunum	10.2±1.1	6.0±0.8	3.8±0.3
Ileum	5.6±0.6	6.5±0.3	−1.1±0.3
Colon	7.0±0.4	7.3±0.4	−0.6±0.2

fact that, in rat jejunum, CO_2 is absorbed from HCO_3-containing solutions but is secreted in the ileum would be explicable if the Na were absorbed, at least in part, in exchange for H ions, and the Cl by an independent process involving an exchange with HCO_3 ions, the former process being relatively more active in the jejunum than in the ileum (Parsons, 1956). Processes such as this require that the short-circuit current across the tissue would not be equal to the net Na influx; unlike rabbit ileum, this appears to be the case for the combined jejunum and ileum of the rat (Barry *et al.* 1965).

5. Water Absorption

The water absorption observed in the small intestine and on appears to be closely coupled to solute absorption. ater movement has never been observed from isotonic solutions without concomitant solute absorption (Curran, 1965; Parsons, 1967). There is also evidence that water movement may occur from the lumen of the small intestine and colon into a solution bathing the serosal surfaces of the absorbing cells and against a gradient of water activity. This finding has been unequivocally demonstrated in vitro (Table II) and there is evidence for the phenomenon in vivo. Just as is found for Na absorption, the water absorption depends upon the cellular metabolism (see section 6). However, although this sort of transport falls within the operational definition of an active transport, the movement of water observed may be

FIG. 3. Relationship of adjacent epithelial cells



Cells of intestinal mucosa are laterally adherent at the terminal-bar region immediately below the microvilli. The remaining cell membrane below the terminal bar may be regarded as the "base" of the cell. The lateral membranes in the region of the cells are closely adjacent, but during fluid absorption become separated to form intercellular channels

explained without the necessity for postulating the existence of a system of specific water pumps. The experimental evidence now available indicates that water absorption of the sort described can be produced by the accumulation of solutes in a multicompartmental system in which the several compartments are arranged in a serial fashion and separated by membranes possessing differing permeability properties (Curran & Macintosh, 1962). It must be understood that, under in-vitro conditions, a much greater accumulation of the transported substrate will occur in the intestinal wall than when the region immediately subjacent to the mucosal cells is perfused with blood in vivo.

The morphological evidence shows that the absorbing cells are attached laterally to each other via the tight junction referred to in section 1. The tight junction extends for but a short distance immediately below the brush border. The remainder of the cell surface on the other side of the junction away from the brush border must therefore be regarded as the base of the absorbing cell through which water and solutes leave. A potential intercellular space exists between the interdigitating basal membranes of adjacent cells. (fig. 3). In epithelial tissues such as gall-bladder and intestine, which are absorbing fluid, this space may become visible (Diamond & Tormey, 1966). The flow of the fluid through these relatively narrow channels towards the blood capillaries would also give rise to electrokinetic phenomena if the surface of the channels carries a charge.

TABLE II. Effects of tonicity and glucose on water absorption by rat intestine in vitro (based on Parsons & Wingate, 1961)

(The experiments lasted 60 minutes, and the saline medium was Krebs bicarbonate–Ringer. The tonicity of the luminal fluid was changed by alteration in the NaCl concentration. The values given are the means of the number of experiments (in parentheses), together with the S.E. of the mean. Positive values indicate movement from the lumen; negative values, movement into the lumen.)

Initial concentration difference between luminal and serosal fluids (m-osmole/kg. H_2O)	Water absorption (µl./mg. dry wt.)			
	Glucose present		Glucose absent	
	Jejunum	Ileum	Jejunum	Ileum
−43	18.8±1.7 (8)	12.0±1.0 (6)	6.7±0.1 (3)	5.7±0.7 (3)
0	14.9±1.0 (8)	8.1±0.6 (6)	0.4±1.2 (3)	0.6±1.2 (3)
43	9.3±0.5 (6)	3.2±0.4 (6)	−2.4±0.5 (3)	−1.2±0.3 (3)
86	7.3±0.7 (4)	2.0±0.9 (4)	−4.5±0.1 (3)	−4.2±0.8 (3)
130	4.0±0.2 (4)	−0.5±0.3 (4)	−4.6±0.4 (3)	−5.9±0.3 (3)

Electrokinetic phenomena associated with fluid movements are known to occur in the intestine (Smyth & Wright, 1964; and see paper by Barry, p. 266 of this Bulletin).

6. Source of Energy for Fluid and Salt Transport

The active transport of Na appears to be coupled energetically to the cellular metabolism in a fashion similar to that found in other tissues; for the small intestine of many adult animals, Na transport depends upon cellular aerobic metabolism. The case of the rat intestine is of some interest; fluid and electrolyte transport in rat jejunum in vitro appear to be closely related to the occurrence of aerobic glycolysis, which occurs at a high rate in this tissue. If glucose or mannose (Lifson & Parsons, 1957; Duerdoth, Newey, Sanford & Smyth, 1965) is present at a sufficiently high concentration in the fluid bathing the serosal surface of rat jejunum in vitro, fluid transport from the lumen is stimulated. Surprisingly, acetate, pyruvate and the lower fatty acids are not able to sustain Na and water absorption in this tissue. In the ileum and colon of the rat, on the other hand, fluid absorption is always stimulated under aerobic conditions by the presence of substrates such as acetate, pyruvate and the lower fatty acids as well as glucose (see Table III).

7. K Transport

Very little is known about K absorption by the intestine; however, the information available on K metabolism of the intestine does present features of interest. There is evidence that, like those of the other tissues, the intestinal mucosal cells possess high intracellular concentrations of K ions. Any system for the absorption of K must thus be presumed to operate on the principle of intracellular accumulation derived from the activity of inwardly directed pumps, at least at the mucosal pole, with passive outward leaks, at least at the basal pole. It appears that the maintenance of the high cellular K in rat jejunal mucosal cells depends upon a cellular metabolism requiring glucose as substrate. Provided that glucose is present, the tissue K can be sustained under anaerobic conditions. A striking feature is that the presence in the intestinal lumen of certain sugars which are not metabolized and therefore not capable of acting as substrates leads to a loss of K ions by the mucosa. The absorption of these sugars by rat jejunal mucosa therefore appears to be associated with loss of the tissue K (Table IV). There is also evidence that the K and Na transport mechanisms in the intestinal mucosal cellular membranes may be linked; replacing the lumen Na by Li leads to a fall in the tissue K.

8. Other Consequences of Na Absorption

There is now evidence that the intracellular accumulation and transport of amino acids and some sugars by the intestine are very closely linked to the transport of Na. An early explanation of the stimulating effects of the Na on sugar transport was that the presence of Na in the intestinal lumen is necessary to maintain an intracellular concentration of this ion sufficient to activate the necessary intracellular enzyme systems. In the absence of internal Na, it was argued, the enzyme systems were inactive or possessed decreased activity. The more recent evidence indicates that a direct coupling exists between the inward movement of Na at the brush border of the absorbing cells and the process of accumulation and hence of transport of the sugars and amino acids. Thus, the transport of sugars and amino acids is stimulated when Na absorption is proceeding and the transport of Na is stimulated when the absorption of sugars and of amino acids is proceeding. It would then follow that the energy for sugar and amino-acid transport would be derived directly from the movements of ions such as Na or K down an electrochemical gradient and that this electrochemical gradient is maintained by cellular metabolism via processes involving the hydrolysis of energy-rich substances such as ATP. The replacement of the lumen

TABLE III. Effect of different substrates and inhibitors on water absorption by rat intestine in vitro

(Values are the means ± S.E. of the mean relative to glucose = 100. Unless otherwise indicated in parentheses, each value is based on the mean of 4–25 observations. Substrate concentrates in range 10–28 mM. Data recalculated from Smyth & Taylor (1957), Gilman & Koelle (1960), Parsons & Paterson (1965), and Detheridge, Matthews & Smyth (1966).)

Substrate	Water absorption		
	Jejunum	Ileum	Colon
Glucose	100 ± 5.8	100 ± 6.4	100 ± 5.8
Mannose	—	—	90.9 ± 6.6
Fructose	37.5 (2)	—	71.1 ± 14.9
Pyruvate	2.8 (3)	60.0 ± 4.9	71.9 ± 13.2
Acetate	0.0 (2)	—	90.1 ± 6.6
Butyrate	1.7 (2)	—	91.7 ± 9.1
Succinate	—	—	52.1 ± 7.4
Citrate	19.8 ± 2.3	75.0 ± 9.6	39.7 ± 7.4
Anoxia + glucose	33.6 ± 3.9	1.8 ± 1.8	4.1 ± 2.5
Fluoroacetate + glucose	86.5 ± 12.3	53.2 ± 12.8	26.4 ± 5.0
None	27.1 ± 2.6	68.8 ± 5.5	50.4 ± 5.8

TABLE IV. K content of mucosal layers of rat jejunum after 30 minutes' absorption in vitro with various substrates (based on Brown & Parsons, 1962)

(The values are the means of the number of experiments (in parentheses), together with the S.E. of the mean.)

Tissue K (m-mole/kg. dry wt.)			
Aerobic conditions		Anaerobic conditions	
No substrate	330 ± 9 (9)	No substrate	231 ± 6 (5)
5.5 mM-glucose	442 ± 12 (9)	5.5 mM-glucose	371 ± 7 (3)
27.5 mM-glucose	500 ± 12 (6)	27.5 mM-glucose	410 ± 11 (6)
5.5 mM-galactose	249 ± 13 (6)		
27.5 mM-galactose	220 ± 5 (9)		
27.5 mM-3-O-methyl-glucose	269 ± 8 (3)		
Before incubation (fresh tissue)	447 ± 4 (70)		
Effects of Na, glucose present (Li replacing Na in mucosal fluid)	309 ± 14 (6)		

Na by K will abolish the ionic gradient across the mucosal border of the cell and hence abolish the transport of sugars and amino acids (Curran, 1965; Schultz *et al.* 1966; Parsons, 1967). The maintenance of the ionic battery inside the cell by metabolism therefore appears to promote not only salt absorption but also, by virtue of the coupling effects, translocation across the mucosal cells of other solutes such as amino acids and sugars and of water. It is possible that, at least to a limited extent, Cl and K transport are also coupled to that of Na.

Membrane ATPases apparently implicated in Na and K transport have been described in the microvillous region of the cells as well as in other parts of the bordering membranes (section 3*b*). There may therefore be, at least in the jejunum, an outwardly orientated Na pump in the brush-border region. The existence of such a pump would explain the rapid entry of Na into hypotonic solutions in the jejunal lumen (section 2). Further, if a coupling exists between net Na and sugar absorption on a 1:1 molar basis, then a human subject consuming carbohydrate at the rate of, say, 2 moles (360 g.) of hexose units/day will require 2 moles of Na for the absorption. Since the daily exocrine digestive secretions appear to contain less than 1 mole Na (section 2), an important function of any outwardly directed Na pump in the brush-border region could be to ensure that, in the intestinal lumen, adequate quantities of Na are available to drive the absorption of sugars and amino acids.

It is to be noted that, in rat jejunum in which a non-metabolized sugar such as 3-*O*-methylglucose is being absorbed, the accompanying Na which enters the cells is presumably returned to the lumen; when glucose is being absorbed, the Na accompanied by Cl and water leaves the base of the cells. In rat jejunum it is possible that a brush-border Na pump may depend upon endogenous energy sources, while that at the base of the cell may depend upon energy derived as discussed in section 6.

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THE RELATIONSHIP OF SODIUM CHLORIDE TO HYPERTENSION [1]

9670

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Restriction of the sodium chloride intake as a therapeutic measure in hypertensive vascular disease was first advocated many years ago (1, 2, 3). Although subsequent investigators (4, 5, 6, 7) claimed that the addition or removal of salt from the diet failed to influence the blood pressure level significantly, interest in the subject has recently been renewed.

Selye and his coworkers (8) noted a striking hypertensive effect when sodium chloride was administered to experimental animals receiving injections of desoxycorticosterone acetate. Grollman and his associates (9) observed that drastic reduction in sodium intake resulted in a decline in blood pressure in some hypertensive patients. They believed it probable that the beneficial effects of the diet proposed by Kempner (10) might be due to the restriction of salt. Recently evidence has been presented (11) that sodium chloride potentiates the pressor activity of desoxycorticosterone acetate when injected into rats rendered nephritic with nephrotoxic serum.

The present study was undertaken to reexamine the relationship of sodium chloride to hypertensive vascular disease in man.

METHODS

Men and women with uncomplicated hypertensive vascular disease were studied on the wards of the Presbyterian Hospital and the Research Service, First Division, of the Goldwater Memorial Hospital. Subjects were included only if the antecedent blood pressure consistently exceeded 140/90 mm. of mercury and in the absence of cardiac pain or insufficiency, renal or cerebral complications or fever. All patients were free of albuminuria, showed normal phenolsulfonephthalein excretion and urine concentration tests, and in all instances the venous pressure was within normal limits.

Blood pressures were measured each morning in the same arm by the same observer, with the subject quiet and relaxed in bed, the head of which was raised to a 30° angle. At least 5 and usually 7 or 8 readings were taken at half-minute intervals and the lowest systolic and diastolic value recorded. This value was designated the *"resting"* blood pressure as compared to *"casual"* readings taken under various conditions of activity at other times during the day. In order to secure an adequate baseline, it was found obligatory to carry out preliminary observations for at least 3 weeks.

All subjects were kept in bed until after blood studies and blood pressure measurements were made, and ambulatory activity was standardized at a constant level the rest of each day. The patients were weighed daily before breakfast on the same scales. The daily fluid intake and urine output were recorded, distilled water being supplied for drinking purposes.

Throughout the study the patients were given a constant diet and fluid intake. Identical salt-poor daily menus were prepared. Repeated direct analyses of aliquots taken from an entire day's cooked diet gave values of between 0.25 to 0.35 grams of sodium or considerably less than 1 gram of sodium chloride per day. These salt-poor diets yielded 1,700 to 2,200 calories and 70 to 80 grams of protein, and did not include special ingredients such as dialyzed milk. Sodium chloride was administered by mouth using weighed salt shakers, additional supplements being given in some instances in the form of enteric-coated tablets. When indicated, desoxycorticosterone acetate [2] (DCA) was injected subcutaneously in doses of 5 mgm. twice daily.

The urine chloride was determined daily from the 24-hour collection, daily aliquots being measured at approximately weekly intervals for their sodium content. Fasting blood samples were obtained at intervals for CO_2 content, chloride, sodium, potassium, protein and urea nitrogen determinations. The serum volume was measured with the blue dye T. 1824. Weekly electrocardiograms were taken and in no instance showed evidence of significant myocardial damage or serial changes.

A sample chart (Figure 1) illustrates the general form

[1] This study was made possible through the generosity of the Albert and Mary Lasker Foundation. We are indebted to Miss Ann D. Barrows, dietitian, Miss Margaret G. Hawthorne, head nurse, Miss Katherine Vislocky, technician, John T. Sharp and Dominic G. Iezzoni for their invaluable assistance.

[2] Desoxycorticosterone acetate (Doca) was supplied through the courtesy of Dr. L. A. Pirk of Roche-Organon, Inc., Nutley, New Jersey.

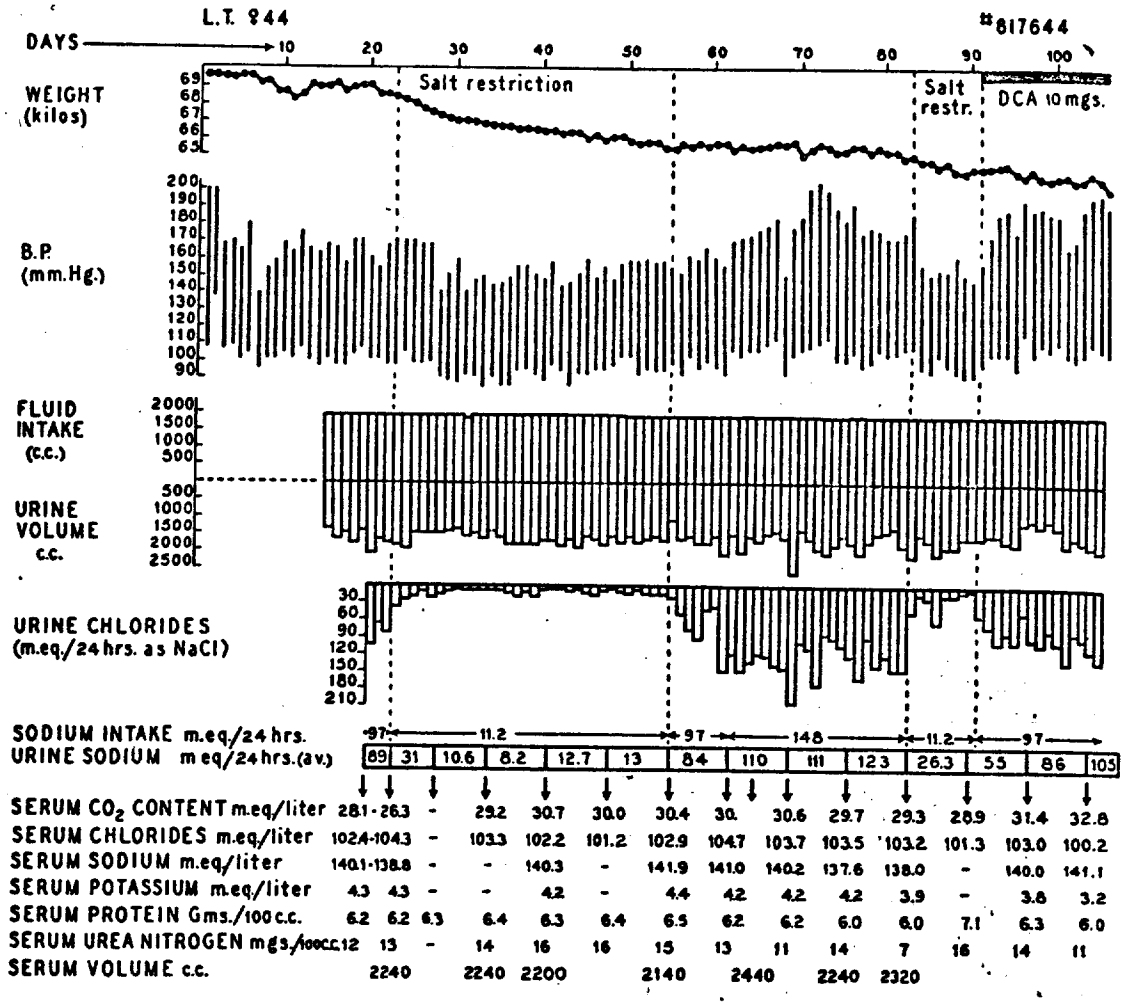

FIG. 1. SAMPLE CHART ILLUSTRATING GENERAL FORM OF INVESTIGATIONS ON EACH HYPERTENSIVE SUBJECT

in which subsequent investigations were conducted and recorded on each patient.

RESULTS

I. *Effect of rigid sodium chloride restriction on blood pressure.* Six hypertensive patients were studied following a preliminary baseline period and constant regimen which included 4 grams of sodium chloride added to the salt-poor diet. A decrease in *"resting"* blood pressure was observed in all patients in from 3 to 5 days after the withdrawal of the sodium chloride from the diet, with a return to previous levels only after sodium chloride administration was resumed (Figure 2, I). The majority of blood pressure readings, during the period of observation, remained above 140/90 mm. of mercury. *"Casual"* readings were apparently uninfluenced by sodium chloride restriction, and each patient exhibited the same variation, the same response to such stimuli as activity, apprehension or the cold pressor test as was demonstrated during the fore-period.

The withdrawal of sodium chloride from the diet for a period of 2 weeks was tolerated in these subjects without discomfort. Slight weight loss and small increases in urine output were generally observed. The decreased intake was sharply

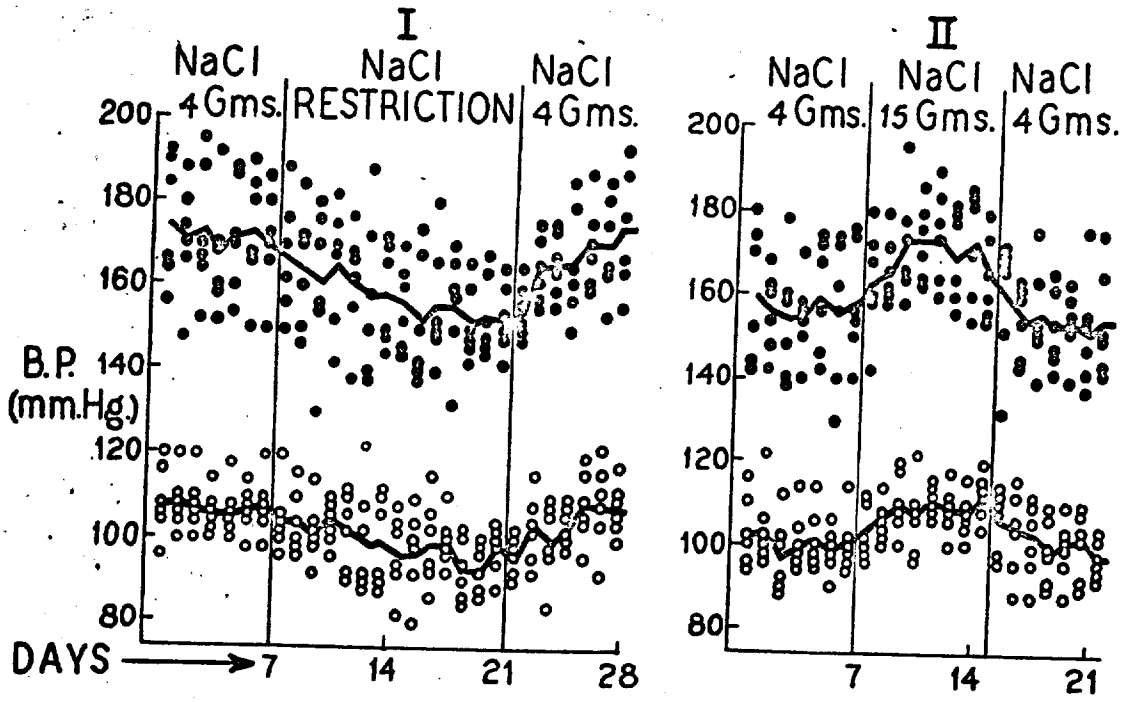

FIG. 2. EFFECT OF RIGID SODIUM CHLORIDE RESTRICTION AND OF INCREASED SODIUM CHLORIDE INTAKE ON 6 PATIENTS WITH HYPERTENSION

reflected in urine sodium and chloride levels, but serum CO_2, chloride, sodium, potassium, protein, urea nitrogen and volume measurements were not materially influenced (Table I). Ballistocardiographic tracings were unchanged on repeated records taken before and after sodium chloride restriction in 2 of these patients, both of whom exhibited a significant drop in blood pressure on salt restriction.

II. *Effect of increased sodium chloride intake on blood pressure.* Six hypertensive patients, 4 of whom had been included in the previous study, were investigated following a preliminary baseline period and constant regimen which included 4 grams of sodium chloride added to the salt-poor diet. The intake of sodium chloride was then increased to 15 grams daily for an 8-day period. These subjects were able to tolerate this dosage without gastric intolerance or diarrhea.

A rise in *"resting"* blood pressure was observed in 5 patients within 1 to 4 days after the addition of sodium chloride to the diet, with a return to previous levels after the initial dosage was resumed (Figure 2, II). (In 2 patients a week's trial with placebo pills was without effect on the blood pressure.) Again *"casual"* readings were uninfluenced and each patient exhibited the same variation, the same response to such stimuli as activity, apprehension or the cold pressor test as was demonstrated before the increased sodium chloride intake.

The further addition of sodium chloride for a period of 8 days was followed by an increase in urine sodium and chloride excretion, and slight weight gain and decreases in urine output were generally observed. No significant change was recorded in serum CO_2, chlorides, sodium, potassium, protein, urea nitrogen, or volume measurements (Table II).

III. *Effect of rigid sodium chloride restriction*

TABLE I

Clinical and laboratory data of 6 hypertensive patients before and after rigid sodium chloride restriction

Case	Mean "resting" B.P.		Mean "casual" B.P.		B.P. response to cold pressor test		Before and after 2 weeks of rigid NaCl restriction								
							Urine*		Serum						
	week before	*2nd week of*	*week before*	*2nd week of*	*before*	*after*	Sodium	Chloride	CO_2 content	Chloride	Sodium	Potassium	Proteins	Urea nitrogen	Volume
	NaCl restriction						*m.eq. per 24 hrs.*	*m.eq. per 24 hrs. as NaCl*	*m.eq. per liter*	*m.eq. per liter*	*m.eq. per liter*	*m.eq. per liter*	*grams per 100 cc.*	*mgm. per 100 cc.*	*cc.*
1	164/106	148/92	182/114	180/112	196/116	200/120	86– 9	88–11	26.4–29.6	104.1–103.1	139.1–140.5	4.3–4.2	6.4–6.6	13–16	2460–2420
2	168/110	152/96	178/116	180/116	194/122	190/120	82–13	94–14	29.0–28.6	102.4–100.3	138.4–139.3	4.2–4.0	6.1–7.0	8–14	2710–2680
3	182/104	160/92	—	—	—	—	68–13	90–16	27.7–24.2	104.6–104.1	142.5–135.7	4.4–5.4	6.7–6.9	7– 9	
4	160/98	140/90	174/104	170/100	182/110	188/112	69– 8	64–20	33.5–30.1	97.5– 99.0	140.1–139.8	4.4–4.8	6.7–6.8	12–14	2360–2280
5	162/110	146/100	176/120	172/118	—	—	68– 9	70–14	27.9–29.7	100.4– 98.1	138.5–138.5	4.5–4.5	7.3–7.2	14–18	
6	182/116	168/108	204/110	208/114	216/120	206/118	66–14	76–15	27.3–28.5	100.8– 99.2	137.5–137.7	4.4–4.4	7.2–7.4	11–10	2000–2000

* Mean values for each period.

on pressor action of DCA (12). Five hypertensive patients, 3 of whom had been investigated in the previous studies, were studied following a preliminary baseline period and constant regimen which included 4 grams of sodium chloride added to the salt-poor diet. DCA was then injected subcutaneously in doses of 5 mgm. twice daily, with added dietary sodium chloride increased in 3 subjects but maintained at 4 grams in 2. After 10 days, all added sodium chloride was withdrawn from the diet without other change in regimen and while the subjects continued to receive DCA.

In all instances an increase in *"resting"* blood pressure followed the injection of DCA, this rise not being maintained when sodium chloride was withdrawn (Figure 3). During the period of sodium chloride and DCA administration, the expected slight increases in weight, hemodilution and serum volume were generally noted, together with slight reduction in urine volume (12, 13). Significant increases in serum chloride and sodium were observed in 2 patients, while reductions in serum potassium of from 0.4 to 1.5 m.eq. took place in all subjects. When DCA injection was continued but with rigid sodium chloride restriction, slight reductions in weight and serum volume were seen, together with hemoconcentration, slight increases in urine volume, and a sharp decrease in urine sodium and chloride values. The serum chloride and sodium were significantly reduced by sodium chloride restriction in 2 instances, while the serum potassium levels showed

TABLE II

Clinical and laboratory data of 6 hypertensive patients before and after increased sodium chloride intake

Case	Mean "resting" B.P.		Mean "casual" B.P.		B.P. response to cold pressor test		Before and after 8 days of increased NaCl intake								
							Urine*		Serum						
	week before	*8 days of*	*week before*	*8 days of*	*before*	*after*	Sodium	Chloride	CO_2 content	Chloride	Sodium	Potassium	Proteins	Urea nitrogen	Volume
	Increased NaCl intake						*m.eq. per 24 hrs.*	*m.eq. per 24 hrs. as NaCl*	*m.eq. per liter*	*m.eq. per liter*	*m.eq. per liter*	*m.eq. per liter*	*grams per 100 cc.*	*mgm. per 100 cc.*	*cc.*
1	140/96	158/108	160/110	160/112	178/120	174/118	57–190	74–176	31.4–32.8	99.4– 99.0	140.0–140.8	4.4–4.2	6.4–6.4	13–11	2440–2500
2	166/100	180/112	210/120	206/120	220/120	220/120	72–204	80–200	27.4–28.0	100.4– 99.6	138.4–138.4	4.9–4.6	6.0–5.9	18–18	2620–2580
3	162/112	164/116	176/122	174/120	—	—	66–210	74–210	26.3–27.0	101.9–101.9	138.1–138.9	4.6–4.9	7.0–7.2	14–17	
4	146/92	160/102	162/104	162/100	180/118	182/118		90–216							2480–2520
5	162/102	178/110	186/114	188/114	196/118	200/120	86–204	68–178	29.9–30.4	104.4–103.3	140.9–138.0	4.9–4.9	6.2–6.0	14–16	2840–2920
6	172/102	184/110	196/116	194/118	—	—	65–186	74–230	24.4–26.1	106.3–108.0	140.7–140.0	5.2–4.7	6.1–6.0	9–13	

* Mean values for each period.

no further consistent change (Figure 4, Table III).

As the pressor action of prolonged DCA injection was on occasion found to be transitory, the order was reversed in 2 additional patients, *i.e.*, following the usual baseline observations, rigid sodium chloride restriction was instituted simultaneously with the start of DCA administration. These were patients who had not been subjected to previous studies. No rise in *"resting"* blood pressure ensued until the addition of sodium chloride (Figure 5).

COMMENT

The present study, utilizing measurements of the *"resting"* blood pressure, confirms the reports of some previous observers that the rigid restriction of sodium chloride in the diet may lower the blood pressure of patients with hypertensive vascular disease. Conversely, it has been shown under similar conditions that large amounts of dietary sodium chloride may exert a pressor effect in hypertensive subjects. Lastly, the observations of Knowlton and her associates (11), that sodium chloride participates in the potentiation of the pressor activity of DCA, have been supported by studies in patients with an elevated blood pressure. It has been previously shown that this pressor response to DCA is not due to salt and water retention alone (12).

Because of the great variability of blood pressure readings in association with any of a large

number of environmental changes, the need has been stressed for an adequate baseline and constant regimen as well as for the differentiation between *"resting"* and *"casual"* values. It must be emphasized that the magnitude of blood pressure fluctuation observed in this study was often small, although consistent, and that standard conditions were required.

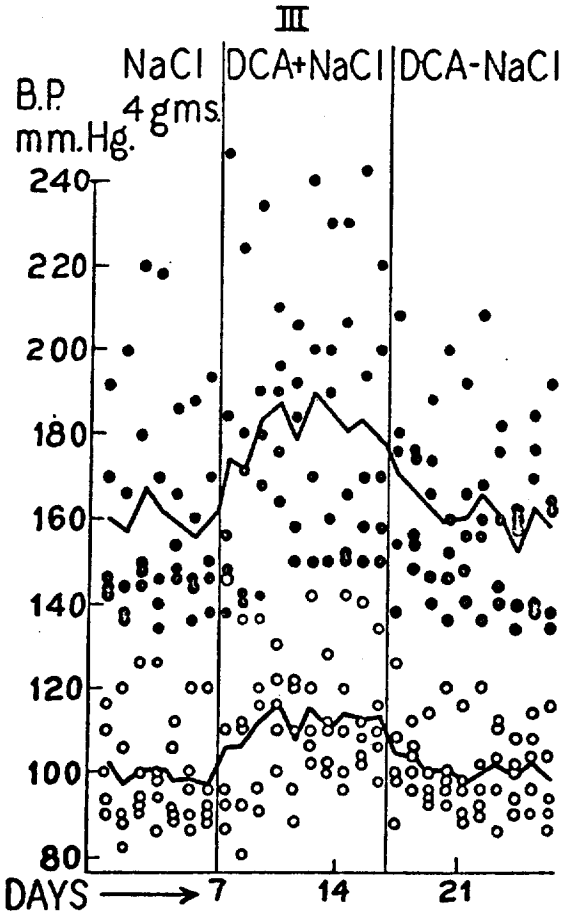

FIG. 3. EFFECT OF RIGID SODIUM CHLORIDE RESTRICTION ON PRESSOR ACTION OF DCA IN 5 HYPERTENSIVE SUBJECTS

The effect of rigid sodium chloride restriction is apparently unrelated to changes in circulatory volume or cardiac output, hence must be concerned with alterations in peripheral resistance. It must be noted that a normal blood pressure is not routinely achieved by salt restriction and that the patient's ability to respond to autonomic or neurogenic stimuli is unaffected. The demonstration in this study that there is an absence of correlation between *"resting"* and *"casual"* blood pressure readings, suggests that the factors (at least in part neurogenic) influencing the "casual" readings may be independent of those influenced by sodium chloride or DCA. This may explain the results of sympathicolytic drugs and of surgical procedures which modify vasoconstriction of sympathetic origin yet do not invariably relieve the hypertensive state. Previous uncertainties concerning the effect of many therapeutic agents may have been due to the failure to dissociate between extrinsic (neurogenic) and direct actions on peripheral resistance, both of which seem to be involved—in variable proportions—in the clinical picture of hypertension.

There is nothing to suggest that the small changes in *"resting"* blood pressure associated with rigid sodium chloride withdrawal are of therapeutic significance or that prolonged restriction will exert any influence on the natural history of this disorder.

The modification of *"resting"* blood pressure levels by sodium chloride and the relationship of sodium chloride to the pressor action of DCA are consistent with the view that some product of the adrenal cortex may play a part in the mechanism of hypertensive vascular disease (12, 14, 15).

CONCLUSIONS

1. Observations have been made on patients with uncomplicated hypertensive vascular disease under controlled conditions of diet and activity.

2. Despite an otherwise constant regimen, rigid sodium chloride restriction resulted in a slight decrease in *"resting"* blood pressure in 6 subjects, whereas large amounts of dietary sodium chloride produced a slight rise in *"resting"* blood pressure in 5 of 6 subjects.

3. The pressor action of desoxycorticosterone acetate was observed in 7 subjects, but only when sodium chloride was included in the diet; the rigid restriction of sodium chloride obliterated this pressor response.

4. It is suggested that vasoconstrictive factors (at least in part neurogenic) in hypertensive patients are independent of those alterations in peripheral resistance influenced by sodium chloride or desoxycorticosterone acetate.

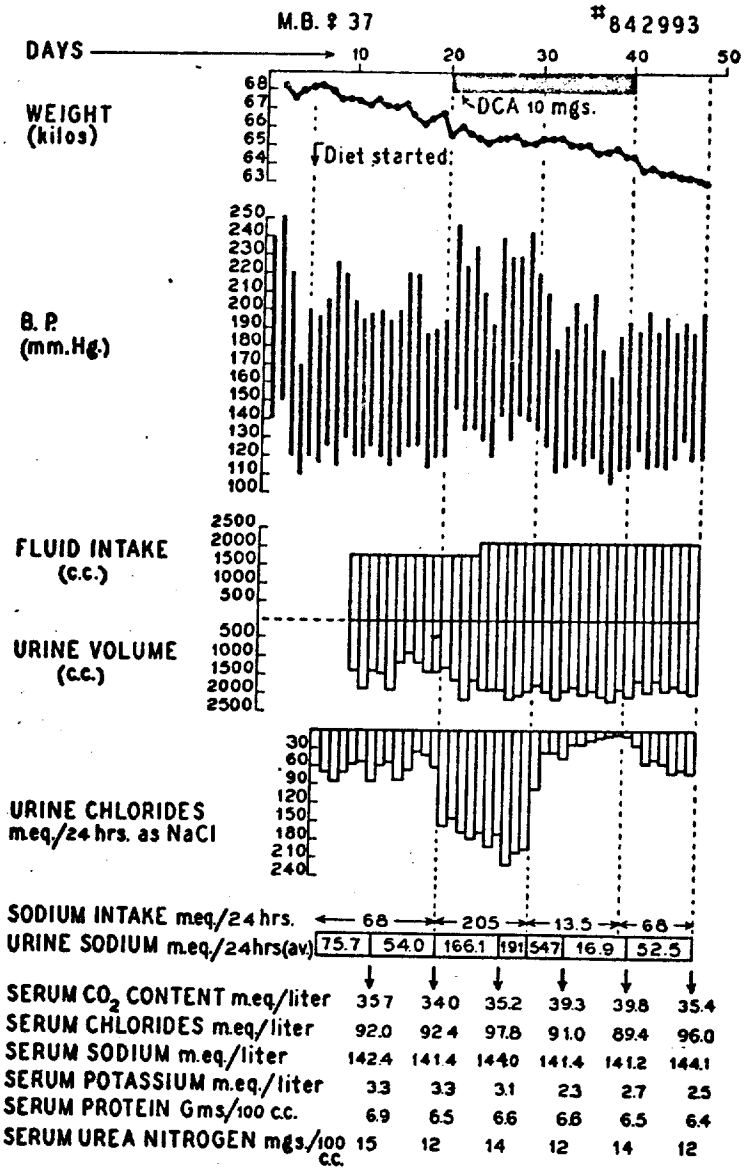

FIG. 4. CLINICAL AND LABORATORY DATA ILLUSTRATING EFFECT OF RIGID SODIUM CHLORIDE RESTRICTION ON PRESSOR ACTION OF DCA.

TABLE III

Laboratory data of 5 hypertensive patients—effect of rigid sodium chloride restriction on pressor action of DCA

Case	Mean "resting" B.P.			Urine†						Serum values at end of each period																				
				Sodium			Chloride			CO_2 content			Chloride			Sodium			Potassium			Proteins			Urea nitrogen			Volume		
	I*	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
				m. eq./24 hrs.			*m. eq./24 hrs. as NaCl*			*m. eq./liter*			*m. eq./liter*			*m. eq./liter*			*m. eq./liter*			*grams/100 cc.*			*mgm./100 cc.*			*cc.*		
1	200/118	226/120	198/110	57	133	29	62	182	32	27.6	30.6	32.8	101.5	101.3	97.2	138.0	140.0	139.0	4.7	3.2	4.0	7.2	5.8	6.8	12	9	14	2940	3000	—
2	166/120	200/136	174/118	54	166	22	58	179	25	34.0	35.2	39.8	92.4	97.8	89.4	141.4	144	141.2	3.3	2.6	2.7	6.5	6.6	6.5	12	13	14		—	
3	146/98	172/110	156/104	72	58	12	82	70	18	23.7	30.0	31.7	104.6	102.8	98.0	135.8	139.2	140.4	4.3	3.4	3.5	7.0	6.7	7.3	13	11	12	2000	2290	1705
4	142/94	160/100	146/92	85	78	18	92	84	28		—		103.8	107.1	104.2	137.2	137.4	136.9	4.8	4.4	4.4	6.4	6.0	6.2		—		2640	2860	2720
5	144/90	152/100	140/90	65	210	15	78	246	27	25.6	30.0	28.4	104.1	98.6	101.2	136.0	139.8	135.4	4.9	3.9	3.6	6.6	6.4	6.8	10	8	12	2400	2580	2420

* I. Preliminary week—NaCl 4 grams added to diet.
II. Ten-day period—DCA 10 mgm. daily, NaCl 4 to 15 grams added to diet.
III. Ten-day period—DCA 10 mgm. daily, rigid NaCl restriction.
† Mean values for each period.

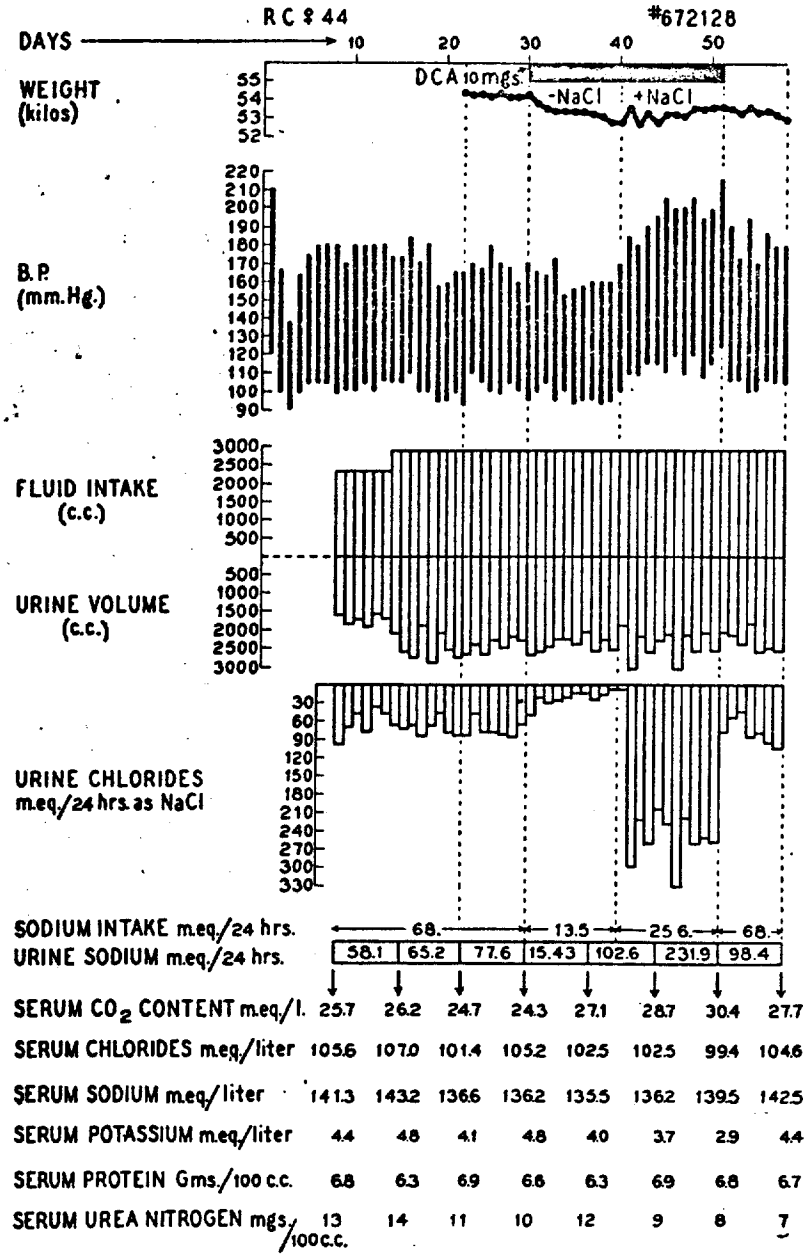

FIG. 5. CLINICAL AND LABORATORY DATA ILLUSTRATING EFFECT OF RIGID SODIUM CHLORIDE RESTRICTION ON PRESSOR ACTION OF DCA

5. The relationship of sodium chloride and desoxycorticosterone acetate to hypertensive vascular disease is consistent with the view that some product of the adrenal cortex may play a part in the mechanism of this disorder.

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Response of Thymus to Increase of Intake of Sodium Chloride

It is a well-known fact that the metabolism of the body fluids and electrolytes is closely connected with all metabolic processes in tissues. I have examined the significance of the changes in sodium balance for the activity of the lymphatic tissue of the thymus.

Adrenalectomized rats were used (Wistar, males, average weight of each group 190–222 gm.): (1) treated with deoxycorticosterone acetate (DCA), 2 mgm. intramuscularly per rat daily, and supplied with tap water; (2) treated with the same doses of DCA and supplied with isotonic saline solution. Treatment with DCA started immediately after adrenalectomy and later after 24 and 48 hr. respectively. 72 hr. after adrenalectomy the rats were killed by decapitation. The thymus was weighed and the lymphocytes of thymus were counted. At the same time a part of muscle (m. quadriceps femoris) was removed for electrolyte analysis. The specimens of the muscle were dried, defatted, and the potassium and sodium content was determined by flame photometry and expressed in milliequivalents of ions per 100 gm. of fat-free dried material.

The analysis of the electrolyte concentration of the muscle has shown that the animals of the group treated with DCA and supplied with tap water maintain a relative balance in electrolytic proportions (intact control rats: potassium, 46·0 m.equiv./100 gm.; sodium, 10·5 m.equiv./100 gm.). Animals treated with DCA and saline have shown an increased retention of sodium which is demonstrated by a lower concentration of potassium and a corresponding increase of sodium in muscle. It is a well-known fact that sodium chloride stimulates the excretion of potassium in animals treated with DCA (ref. 1).

Table 1 shows that the animals treated with DCA and saline have an enlarged thymus. An increase in the average weight of the thymus is conditioned by an increase in the number of cells. The mean weight of the thymus of animals treated only with DCA does not differ from the mean weight of the thymus of intact control animals of the same body-weight (n-13, mean thymus weight: 180 mgm.).

Table 1

Treatment	Mean body-weight (gm.)	Mean thymus weight (mgm.)*	No. of cells × 10⁶*	Muscle electrolytes (m.equiv./100 gm.) Na	K	Na/K*
DCA	192 (10)†	177	576	12·1	44·9	0·26
DCA + NaCl	199 (21)	241	872	13·3	41·0	0·31

* Statistical significant differences between both groups ($P < 0·01$).
† In parentheses, number of rats used.

The experiments have shown that the thymus of adrenalectomized animals reacts to stimuli produced by an imbalance in the electrolytes of the internal environment (increase of sodium intake) by intensified activity. The mechanism of this action is not clear. We may assume both direct and indirect influence. It is known that the maintenance of ion concentration gradients requires metabolic energy. The hyperfunction of the thymus tissue may perhaps be an expression of increased metabolic efforts to maintain the homeostasis of the internal environment. My results indicate that the electrolyte metabolism has

an important role in the regulation of the growth of thymolymphatic tissue.

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Determination of Physiological Rhythms of Unrestrained Animals by Radio Telemetry

THE results of 24-hr. rhythm measurements are frequently influenced by perturbations due to the presence of an observer and the measuring equipment. These perturbations can be minimized if the desired physiological data are obtained by radio telemetry[1,2]. The transmitter carried by the animal should be characterized by small size, long battery life, and sufficient range for unrestrained movements. Several transmitters with these design goals were constructed successfully and used to relay temperature and heart-rate information to a distant observer.

The transmitters contain a basic oscillator which is frequency-modulated by a voltage proportional to the phenomenon measured. For a description of the design, see ref. 3. The frequency of each temperature transmitter is controlled in part by the intrinsic dependence on temperature of the transistor in the oscillator and also by a voltage derived from a thermistor half-bridge circuit. The transmitter is built around the antenna, which is a ferrite core. The life of the 75 MAH mercury cell in this circuit exceeds 200 days. The frequency of the E.K.G. transmitter is controlled by amplified potentials derived from the heart. Additional circuitry is needed for amplification; this increases power consumption and reduces battery life to about forty days. Units with more batteries have a longer life. Both the temperature and the E.K.G. units are suitable for surgical implantation provided a non-toxic covering is used. In these experiments temperature transmitters were hermetically sealed in cases machined from nylon rod. The temperature-sensitive elements were covered with the tip from a finger of a latex surgical glove, although they could be designed to protrude into a portion of a nylon shell. Seals between the latex finger tip and the nylon case were made successfully by wrapping and tying with nylon suture. No toxic reactions were noted when a unit prepared in this manner was implanted in a dog. Ref. 3 describes the pretreatment required before implantation.

In a series of short experiments 24-hr. rhythms (circadian rhythms) of heart-rates for a dog, a cat and a rabbit were obtained. In each case the animal was trained to wear a harness on which the transmitter was mounted. The input leads to the transmitter were connected to tantalum electrodes which were implanted surgically weeks in advance. The electrodes were put in subdermally over the right scapula and on the dorsal mid-line near the first lumbar vertebra, a position favouring the detection of heart-potentials. Fourier analysis of the data was made in order that the 24-hr. periodicity could be expressed quantitatively in the form:

$$F(t) = C_0 [1 + C_1 \sin(15t + \theta_1) + C_2 \sin(30t + \theta_2) + \ldots + C_n \sin(15t + \theta_n)]$$

One may think of this Fourier series as a curve of best fit for the given frequency components. The Fourier series approximation, which is uniquely

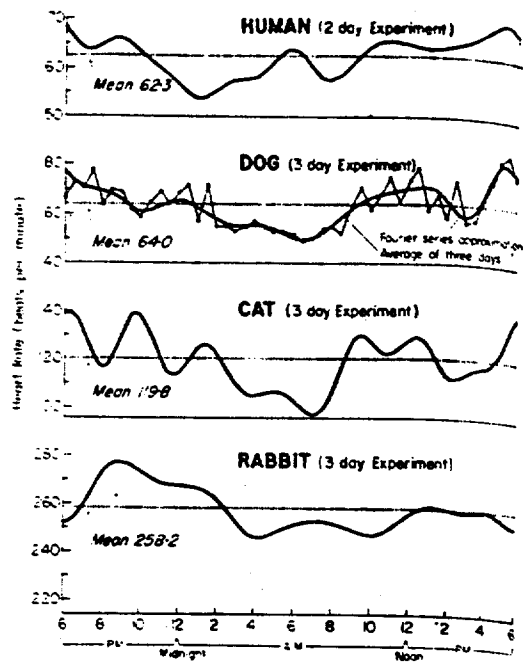

Fig. 1. 24-hr. periodicity in heart-rates of man and three laboratory animals. A Fourier series approximation was used for curve smoothing. Original data for the dog are included

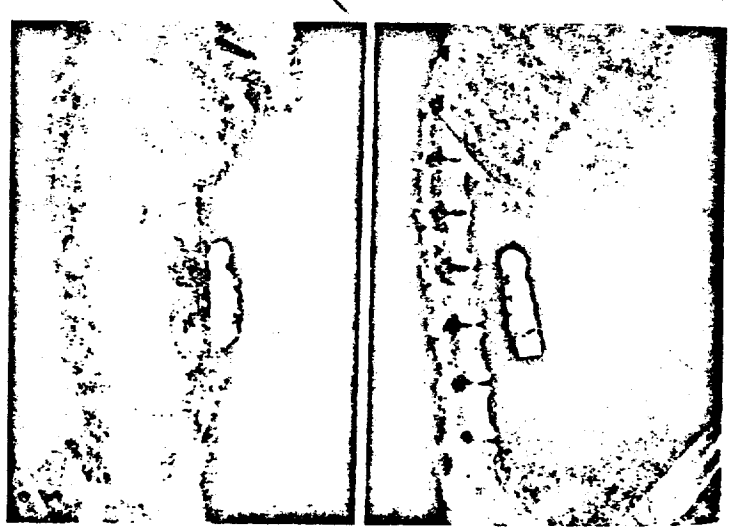

Fig. 2. Dorsal-ventral (left) and right to left lateral (right) roentgenographs showing position of the temperature transmitter in the dog

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SODIUM INTAKE AND BLOOD PRESSURE IN TWO POLYNESIAN POPULATIONS*

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Abstract The populations of the Polynesian islands Rarotonga and Pukapuka are ethnically similar but observe different economic, social and dietary habits. In Rarotonga mean blood pressure increases with age, particularly in women. In Pukapuka rise of blood pressure with age is slight and only seen in women. Blood-pressure differences between the populations are greater than are accounted for by differences in height and weight. Dietary surveys and 48-hour urine collections from subsamples indicate that sodium intake in Rarotonga is about 50 mEq a day higher than in Pukapuka. No within-population correlation between blood pressure and 24-hour urinary sodium output is demonstrable. Many differences between the two groups may explain the differences in blood pressure, but the findings are compatible with Dahl's hypothesis that higher salt intake and higher blood pressures are related.

IT is generally accepted that the level of blood pressure is determined by many factors, both constitutional and environmental. Dahl[1] has proposed that in some cases, habitual salt intake may be one of these factors, and epidemiologic observations that support the hypothesis have been published.[2-4] Other studies have indicated no evidence of difference of salt intake between subjects with different levels of blood pressure[5] or have noted similar blood pressures in groups assumed to have differences in salt intake.[6] Here, we report a study of blood pressure and salt intake in two South Pacific Polynesian populations.

POPULATION SAMPLES

The populations studied were of the same broad ethnic origin. One consisted of the entire adult population of the isolated coral atoll of Pukapuka in the Northern Cook Islands. These people live under a subsistence-level economy and a diet composed chiefly of coconut, taro and fish. The other group was 98 per cent of a sample of adults who had lived under town conditions in Avarua on Rarotonga in the Southern Cook Islands for at least 10 years. This group enjoys a cash economy, with ready access to a varied diet including canned goods. Further details of the populations and their diets have been given elsewhere.[7]

METHODS

Dietary information on total food consumption and use of salt in cooking and at table was collected by the 24-hour recall method, and by household dietary surveys.[7] The same nutritionist (F.D.) surveyed the two populations. Blood pressure was measured to the nearest even number of millimeters of mercury with the use of an ordinary sphygmomanometer and 14-cm cuffs. The pressures reported here were measured with the subject seated, at a fourth-phase diastolic level. Measurements were made by one observer (I.A.M.P.) in Rarotonga and by the same observer and one other (H.P.B.H.) in Pukapuka. Between-observer comparisons revealed no bias. Weights were measured to the nearest pound, with subjects in a minimum of clothing for which an allowance of two pounds was made. Temperature and relative humidity were measured daily in the examination centers at 2 p.m.

Casual samples of urine were collected from all participants, and sodium concentration determined in the field, where possible, with the use of an EEL photometer with a Titrasol standard. In addition, subsamples of the participants, 51 in Rarotonga and 60 in Pukapuka, made 48-hour collections of urine. From these specimens mean 24-hour outputs of sodium were estimated, and, in most of them, creatinine excretion was also calculated by Jaffe's method in an Auto-Analyzer. For logistic reasons creatinine estimations had to be performed on urine specimens that had been deep frozen for a year. To estimate the effect of this delay, a subsample of 25 specimens were examined again after a lapse of a further year. It was found that in this period creatinine concentration had diminished by a mean of 15 mg per 100 ml (17 per cent), but there was a high correlation (r equal to 0.96) between first and second estimations. The choice of participants for the 48-hour collections of urine was largely determined by their willingness and ability to co-operate, but was made without knowledge of their blood pressure, dietary habits and biochemical or physical findings.

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Results

Temperature and Humidity

During the surveys the mean temperature in Rarotonga was 77.8°F (range, 74 to 80°F), with a mean humidity of 73.4 per cent (range, 53 to 93 per cent), and in Pukapuka the mean temperature was 83.3°F (range, 79 to 86°F), with a mean humidity of 76.3 per cent (range, 65 to 87 per cent).

Dietary Sodium

In Rarotonga the mean daily dietary intake of sodium was estimated as 120 to 140 mEq, and that in Pukapuka as 50 to 70 mEq. The Rarotongans used a considerable amount of salty tinned corned beef, tinned fish and salted coconut sauce. The Pukapukans had only occasional tinned corned beef, with less frequent coconut sauce, and did not add salt to foods in cooking.

Blood Pressure

Age and sex distributions of the subjects, together with mean blood pressures in 10-year age groupings, are shown in Table 1. The social organization of the islands is such that inaccuracy of reported ages was probably little greater than in European groups. Pregnant women were excluded from analysis. Frequency distributions of blood pressure in broader age groups are shown in Tables 2 and 3. The Rarotongans showed a rise of systolic blood pressure with age that was steeper in females. The mean diastolic pressure reached a peak of 97.8 mm in males in the fifth decade, and 105.1 mm in females during the sixth decade. In the Pukapukan males there was little change of pressure with age; the females showed a rise, reaching a systolic peak of 130, and a diastolic peak of 80 to 82 mm, in the fifth and sixth decades. In subjects 40 years of age and over, 70 per cent of men and 74 per cent of women in Rarotonga had pressures above 139 systolic or 89 diastolic, as compared with 8 per cent of men and 21 per cent of women in Pukapuka. In the same age groups 28 per cent of men and 47 per cent of women in Rarotonga had pressures above 159 systolic or 94 diastolic, as compared with 3 per cent of men and 7 per cent of women in Pukapuka. The Rarotongans tended to be taller and heavier than the Pukapukans,[7] and in both populations blood pressure was positively correlated with body weight. To examine the extent to which the differences in blood pressure could be attributed to differences in stature, mean blood pressures were adjusted for regression on body weight within age groups. Regression coefficients were similar in the two populations, so that a pooled within-population regression coefficient for blood pressure on weight was derived by analysis of covariance technics[8] for each age-sex group, and used to adjust blood pressures to the mean body weight of that group. Table 4 presents the regression coefficients, together with the adjusted mean blood pressures and their standard errors. A comparison of Tables 1 and 4 shows that, on the basis of the statistical model used, little of the difference in blood pressure between the two populations was attributable to differences in body weight. Because the correlation between blood pressure and height at constant weight was negative, further adjustment of blood-pressure means for height was not done.

Casual Urinary Sodium Concentrations

A casual urinary sodium concentration was measured for 87 per cent of the participants in Rarotonga and in 98 per cent of those in Pukapuka. Mean values are indicated in Table 5. In both populations and sexes the concentrations were lower in subjects in the sixth and seventh decades than in younger persons. The Rarotonga females had lower mean concentration than the males at all ages, but no consistent difference between the sexes was found in Pukapuka. The Rarotongans show significantly

Table 1. *Mean Blood Pressures According to Sex and Age Group.**

Age	Blood Pressures in Rarotonga					Blood Pressures in Pukapuka				
	No. of Subjects	Systolic		Diastolic		No. of Subjects	Systolic		Diastolic	
yr		*mean*	*S.D.*	*mean*	*S.D.*		*mean*	*S.D.*	*mean*	*S.D.*
Males:										
20-29	83	131.5	13.5	84.1	11.1	43	116.9	8.6	76.4	9.1
30-39	45	131.3	13.4	86.7	10.5	47	118.2	11.1	77.8	10.0
40-49	40	145.6	24.2	97.8	16.2	33	114.2	9.9	79.9	9.6
50-59	40	141.7	22.1	93.3	12.5	32	119.9	14.6	76.8	8.6
60-69	18	156.5	32.5	94.6	14.6	20	120.6	11.2	76.5	7.4
≥70	17	154.8	33.0	91.5	16.3	13	114.2	20.5	71.2	11.8
Females:										
20-29	75	116.8	12.9	77.6	10.0	48	107.7	9.6	71.4	8.0
30-39	31	132.2	18.4	86.7	12.0	42	112.8	11.4	77.4	9.8
40-49	40	155.6	28.8	97.9	17.5	27	118.2	18.2	76.6	13.1
50-59	33	172.3	40.6	105.1	20.1	31	130.4	22.5	81.7	15.3
60-69	20	173.7	38.2	102.3	19.9	21	130.8	18.7	80.3	14.4
≥70	13	170.1	38.5	93.4	18.4	10	128.6	29.6	72.8	17.4

*S.D. = standard deviation.

TABLE 2. *Distribution of Systolic Pressure According to Age and Sex.*

Age	No. of Subjects with Systolic Pressure Indicated											
	90-99	100-109	110-119	120-129	130-139	140-149	150-159	160-169	170-179	180-189	190-199	≥200
yr	*mm Hg*											
Males:												
20-39:												
Pukapuka	3	10	39	28	8	2	–	–	–	–	–	–
Rarotonga	–	3	16	42	31	21	10	4	1	–	–	–
40-59:												
Pukapuka	2	16	22	16	5	2	2	–	–	–	–	–
Rarotonga	–	2	8	14	12	14	9	13	3	2	1	2
≥60:												
Pukapuka	2	8	11	3	7	1	1	–	–	–	–	–
Rarotonga	–	2	1	4	5	5	4	3	2	2	1	6
Females:												
20-39:												
Pukapuka	11	33	32	10	3	1	–	–	–	–	–	–
Rarotonga	3	24	25	21	17	8	4	4	–	–	–	–
40-59:												
Pukapuka	3	10	15	12	8	2	4	1	–	2	1	–
Rarotonga	–	1	3	5	12	11	10	3	7	8	2	11
≥60:												
Pukapuka	2	3	6	4	7	4	1	2	1	1	–	–
Rarotonga	1	–	–	3	3	5	3	–	3	1	2	12

higher mean concentrations than the Pukapukans in all age groups of both sexes. In both populations there was a low positive correlation between casual urinary sodium concentration and body weight. Adjustment for body weight, with the use of linear regression coefficients within age groups, had little effect on the differences between populations in sodium concentration; the overall Rarotongan mean concentrations of 126 mEq per liter in males, and 116 mEq in females, were lowered to 121 and 110 mEq respectively, and in Pukapuka the mean of 65 mEq in males was raised to 66 mEq, and that of 68 mEq in females became 74 mEq per liter.

48-Hour Urine Collections

Table 6 lists mean ages, 24-hour urinary volumes and sodium outputs for the subsamples. There was no significant difference in sodium output between the sexes within populations, but the difference between populations was highly significant (p less than 0.001). There was no consistent age trend. Pukapukan women produced smaller volumes than the men (p less than 0.05), but there was no difference between the sexes in Rarotonga. Volumes were significantly lower (p less than 0.05) for both sexes in Rarotonga than in Pukapuka. This suggests that the "48-hour" collection in Rarotonga may have been incomplete and there is further evidence for this. Mean 24-hour creatinine output was estimated from the urine samples for 26 of the men and 11 of the women in the Rarotongan subsample, and 36 of the men and 21 of the women in Pukapuka. Mean values were similar in the two groups, 1106 mg per 24 hours (S.D., 438) for men, and 790 mg (S.D., 355) for women in Rarotonga as compared with 1215 mg (S.D., 494) and 783 mg (S.D., 228) respectively in the Pukapukans. Because of the evidence given above under "Methods" that there had probably been a decline in measurable creatinine concentration between collection and analysis of the specimen we cannot use estimated 24-hour creatinine output as an index of completeness of urine collection in the usual manner. However, in view of the high correlation (r equal to 0.96) between repeat determinations on the specimen after an interval of a year we can put the data to use in a different way. The correlation coefficient between sodium and creatinine outputs was 0.73 (p less than 0.001) in the Rarotongan samples (0.55 in women and 0.80 in men). In the Pukapukans the coefficient was 0.12 (not significant) – 0.06 for women and 0.17 for men.

TABLE 3. *Distribution of Diastolic Blood Pressure According to Age and Sex.*

Age	No. of Subjects with Diastolic Pressure Indicated							
	50-59	60-69	70-79	80-89	90-99	100-109	110-119	≥120
yr	*mm Hg*							
Males:								
20-39:								
Pukapuka	1	16	28	34	9	2	–	–
Rarotonga	–	7	24	50	31	11	5	–
40-59:								
Pukapuka	–	8	29	22	4	2	–	–
Rarotonga	–	1	5	16	30	12	10	6
≥60:								
Pukapuka	1	8	14	8	1	1	–	–
Rarotonga	–	1	5	6	13	3	4	3
Females:								
20-39:								
Pukapuka	1	25	39	19	6	–	–	–
Rarotonga	1	14	33	32	20	4	2	–
40-59:								
Pukapuka	2	11	17	18	3	6	–	1
Rarotonga	–	–	4	15	21	10	9	14
≥60:								
Pukapuka	2	7	9	6	3	3	1	–
Rarotonga	–	2	2	7	6	6	3	7

TABLE 4. *Regression Coefficients and Mean Blood Pressures Adjusted for Body Weight, with Standard Errors* According to Sex and Age Group.*

Age	Systolic Blood Pressure			Diastolic Blood Pressure		
	Regression Coefficient	Rarotonga	Pukapuka	Regression Coefficient	Rarotonga	Pukapuka
yr						
Males:						
20-29	0.133	131.0 (1.3)	117.8 (1.8)	0.090	83.8 (1.1)	78.0 (1.6)
30-39	0.106	130.7 (1.6)	118.8 (1.6)	0.133	85.9 (1.3)	78.6 (1.3)
40-49	0.179	143.3 (2.9)	116.9 (3.2)	0.162	95.8 (2.0)	82.3 (2.2)
50-59	0.171	141.4 (3.0)	120.2 (3.3)	0.117	93.1 (1.7)	77.0 (1.9)
60-69	0.114	155.8 (5.3)	127.6 (5.0)	0.080	94.1 (2.5)	77.0 (2.4)
≥70	−0.138	155.6 (6.7)	113.2 (7.7)	0.026	91.4 (3.4)	71.4 (3.9)
Females:						
20-29	0.153	116.0 (1.3)	109.0 (1.6)	0.140	76.9 (1.4)	72.6 (1.8)
30-39	0.182	130.0 (2.4)	109.3 (2.1)	0.174	84.6 (1.8)	78.9 (1.6)
40-49	0.248	151.6 (3.9)	124.0 (4.8)	0.185	94.9 (2.2)	81.0 (2.8)
50-59	0.233	168.0 (5.6)	134.9 (5.8)	0.192	101.6 (2.9)	85.4 (3.0)
60-69	0.112	171.1 (6.7)	133.4 (6.5)	0.075	100.5 (3.9)	82.1 (3.8)
≥70	0.195	167.4 (8.8)	132.1 (10.2)	0.117	91.8 (4.6)	74.9 (5.3)

*In parentheses.

The implication is clear that a significant proportion of the variance in sodium excretion in the Rarotongan subsample was due to inaccuracy of urine collection, but there is no evidence for such an inaccuracy in the Pukapukan subjects. (It is worth noting that the correlation coefficients would be unaffected by the possibility that subjects "made up" their specimens with fresh water.) This may reflect the closer supervision of participants that was possible in Pukapuka.

For all 60 of the Pukapukan samples and 38 of the Rarotongans (14 women and 24 men) casual urinary sodium concentration had been estimated several days before the 24-hour collections of urine had been made. Correlation coefficients between the casual concentration and the 24-hour sodium output in Pukapuka and Rarotonga respectively were 0.05 and 0.12 for males and 0.32 and 0.36 for females. None of these attains statistical significance.

TABLE 5. *Mean Casual Sodium Concentrations with Standard Errors* According to Sex and Age Group.*

Age	Rarotonga		Pukapuka	
	No. of Subjects	Mean	No. of Subjects	Mean
yr		*mEq/liter*		*mEq/liter*
Males:				
20-29	74	130 (6)	43	67 (6)
30-39	37	134 (8)	47	72 (6)
40-49	37	134 (6)	33	73 (8)
50-59	35	122 (6)	32	64 (7)
≥60	29	101 (9)	32	45 (6)
Females:				
20-29	65	126 (6)	46	71 (7)
30-39	29	128 (8)	41	78 (8)
40-49	34	113 (8)	24	69 (9)
50-59	27	107 (8)	31	64 (6)
≥60	31	93 (6)	31	56 (8)

*In parentheses.

Blood Pressure and Urinary Sodium within Populations

No within-population association between blood pressure and 24-hour urinary sodium was detectable. When subjects in the same sex and population group, but in different tertiles of sodium-output distribution, were compared, no gradient of mean systolic or diastolic pressures was found. Regarding casual urinary sodium concentration, first-order linear correlation coefficients between blood pressure and sodium concentration holding body weight constant were derived in each age-sex group, and then pooled across age groups. The pooled coefficients are shown in Table 7. None of the coefficients were significant, but there was a suggestion of a low correlation of casual urinary sodium concentration with systolic blood pressure at least in females. If the coefficients are further pooled between populations that for females (r equal to 0.13) attains conventional significance (p less than 0.05).

DISCUSSION

Our finding of considerable differences between the two populations in mean blood pressures confirms the work of other investigators.[9,10] These dif-

TABLE 6. *48-Hour Urine Volume and Sodium Outputs According to Mean Age of Participants.**

Location	No. of Subjects	Age	Urine Volume	Sodium
		yr	*ml/24 hr*	*mEq/24 hr*
Males:				
Rarotonga	35	48 (14.5)	1118 (480)	114 (59)
Pukapuka	38	51 (12.5)	1784 (197)	62 (37)
Females:				
Rarotonga	16	48 (9.5)	1063 (412)	102 (36)
Pukapuka	22	52 (8.5)	1401 (732)	63 (33)

*Standard deviation in parentheses.

TABLE 7. *Pooled First-Order Linear Correlation Coefficients between Casual Urinary Sodium Concentration and Blood Pressure, with Body Weight Constant.*

LOCATION	MALES			FEMALES		
	D.F.*	SYSTOLIC	DIASTOLIC	D.F.*	SYSTOLIC	DIASTOLIC
Rarotonga	197	0.06	−0.02	171	0.13	0.05
Pukapuka	172	0.13	0.02	158	0.13	0.08

*Degrees of freedom.

ferences are not attributable merely to the relation of blood pressure to body weight and height so far as this relation is revealed by regression analysis within the populations.

We have adduced three lines of evidence that habitual salt intake is higher in Rarotonga than in Pukapuka. The first, and most direct, evidence comes from dietary surveys, which estimated mean salt intake as 50 to 70 mEq a day in Pukapuka and 120 to 140 in Rarotonga. The second piece of evidence comes from the 48-hour urine collections carried out by subsamples of the populations. Estimates based on these gave a mean of 63 mEq per day in Pukapuka and 110 mEq a day in Rarotonga. Although participants in the 48-hour urine study were not chosen randomly in a strict sense, we have no reason to suppose that they were biased in any way that might affect comparison of sodium output between populations. The main criticisms of the evidence of sodium intake derived from timed urine specimens are based on the accuracy of urine collection, and differences between the populations in sodium loss through sweat. We have evidence that the Rarotongan specimens were inaccurate and, on the average, incomplete. Their mean volumes were lower than those of the Pukapukans — significantly so in males (p less than 0.05) — and there was a highly significant correlation between sodium and creatinine outputs. No significant correlation was found in the Pukapukan specimens, so that, on the evidence, we must assume that the overall effect of incomplete collection of urine was to underestimate the real difference in mean sodium output of the two subsamples. These considerations increase the already close correspondence between the dietary estimate of sodium intake and that based on 24-hour urine output. This correspondence is necessary to exclude the possibility that the difference in urinary output of sodium reflected differences in sweat loss between the two groups. Pukapuka was somewhat hotter and more humid than Rarotonga during the surveys, and we had no way of estimating loss of sodium in sweat.

Mean values of casual urinary sodium concentration, estimated on most participants in the survey, show highly significant differences between the populations. One factor in the variance is salt intake, but there are obviously others, particularly fluid balance. Mean 24-hour urine volumes were 50 per cent higher in Pukapuka (Table 6), and, although there is evidence that this difference is at least partly due to incomplete collection of specimens in Rarotonga, we cannot rule out the possibility of a lower fluid intake there; even if the difference in urine volumes were entirely due to fluid intake, this would not be sufficient to account for the 80 per cent difference in mean sodium concentrations. It is unlikely that diurnal variation in sodium output contributed significantly, for in both islands, casual specimens were collected throughout the working day. Since we do not know of any independent validation of casual urinary sodium concentration as an index of salt intake, we can only postulate that the population difference reflected, at least in part, differences in salt intake.

If it is accepted that we have demonstrated that Rarotongans have higher average salt intake and blood pressure than Pukapukans, how far can this be adduced as evidence for Dahl's[1] hypothesis that salt intake affects blood pressure? There are many other differences between the islands and their inhabitants, and our evidence could be regarded as anything more than compatible with Dahl's hypothesis only if we could demonstrate an association between blood pressure and salt intake *within* as well as *between* the populations. Ability to do this in small samples would depend on the proportion of subjects susceptible to the influence of salt, the validity of the method of assessing salt intake in the face of diurnal and seasonal variations in the individual and the group, and the variance of salt intake between individuals. If the last is small, as it may be in culturally close-knit groups such as the Pukapukans, it may not be possible to establish consistent individual differences in salt intake. Dietary questionnaires are recognized as being suitable only for detecting fairly gross differences between groups. Twenty-four-hour urine samples are potentially a reliable method of estimating salt intake if sufficient samples could be collected for each individual to smooth out day-to-day and seasonal variation. Unfortunately, we have data only on small numbers based on two consecutive daily collections that, in Rarotonga, were probably inaccurate. Thus, the failure to detect a within-population relation of sodium output to blood pressure is not evidence against Dahl's hypothesis. Our data suggest that casual urinary sodium concentration may show a low within-population correlation with systolic blood pressure, at least in females, that deserves further investigation, but we do not at present interpret this as reflecting an association between sodium *intake* and blood pressure. We were not able to demonstrate a significant correlation between casual urinary sodium concentration and a subsequent 24-hour urinary sodium output. Our conclusions are therefore restricted to the differences between the populations that, like the find-

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ings of others,[2-4] are compatible with Dahl's hypothesis.

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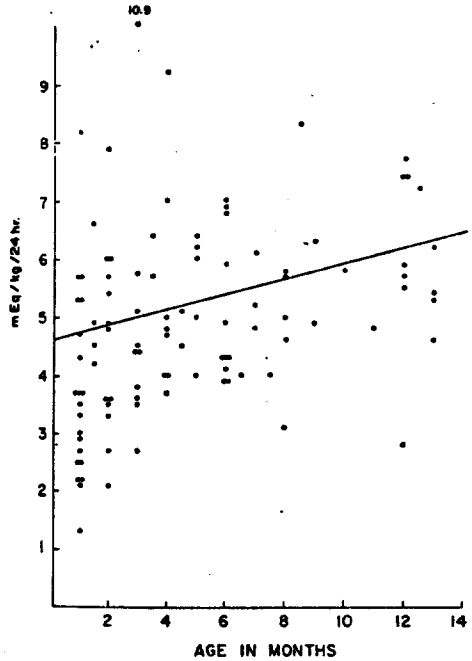

Fig 1.—Sodium intake per kilogram of body weight per 24 hours of 103 well infants in the first year of life.

the first year of life (Fig 2). The daily mean caloric intake is 125 calories/kg at one month,* falling to 95 calories/kg at one year.

In Fig 3 are shown the values of the ratio of sodium intake to total calories ingested. In order to relate the two variables, the total sodium intake was divided by the total calories ingested per 24 hours and multiplied by 1,000. The mean value of 33 mEq/1,000 calories at one month increases to 65 mEq/1,000 calories at 1 year of age. These data indicate that the sodium concentration in the diet is increased with age.

The changes in the composition of the diet in the first year of life are illustrated best by considering three separate groups of these infants. The total sodium intake for the infants below 2 months, from 5-7 months and from 11-13 months of age were calculated separately according to the dietary components and averaged. The increased intake of sodium is due to the large amounts ingested with canned vegetables, meat, and eggs at 1 year of age, as is shown in Fig 4. The total sodium intake from cereal is increased moderately in both older groups, while sodium derived from milk increases only during the middle of the first year.

It is of interest that the mean caloric intakes from milk in the three groups were 425 calories/24 hr below 2 months of age (87% of total intake); 610 calories/24 hr from 5-7 months (73% of total intake); and 435 calories/24 hr from 11-13 months (44% of total intake). Apparently milk ingestion increases in the early months of infancy, but falls as solids become an important source of calories toward the end of the first year of life.

Comment

The validity of data obtained by dietary interview may well be questioned, but infants fed by currently accepted methods would likely be the best possible subjects for obtaining reliable quantitative information by this technique. Their diets are quite stable from day to day, both as to composition and quantity. Chemical analysis of the food collected by aliquot supports the validity of the interview technique. In addition, the mean caloric intakes found in this study by interview agree with published studies of the caloric intake of normal infants in the first year of life.[3-5]

The study clearly indicates that modern feeding techniques have resulted in a moderately large sodium load being offered toward the end of the first year of life. The general availability of processed foods and precooked cereals has resulted in widespread

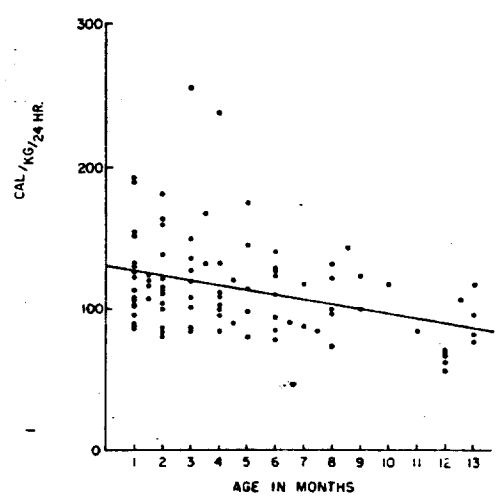

Fig 2.—Caloric intake per kilogram of body weight per 24 hours of 103 well infants in the first year of life.

*Infants of low birth weight were excluded.

Infant Feeding Practices, 1966

Salt Content of the Modern Diet

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OUR INTEREST in dietary sodium was greatly stimulated by the surprising observation that an infant we were studying was ingesting 50-60 mEq of sodium daily from a diet of homogenized milk and commercial baby food. The patient, aged 15 months, was in chronic cardiac failure due to coarctation of the aorta. After a review of the literature, it was apparent that there was little published data as to the "normal" sodium intake of infants being fed modern diets. There are no recommendations as to optimum dietary sodium for infants by the National Research Council of the United States [1] or in the Canadian Dietary Standards.[2]

The purposes of this report then are: (1) describe the results of a laboratory survey of the sodium contents of the diet of infants in the Well Baby Clinic at Vanderbilt University Hospital, and (2) to question the soundness of sodium levels of currently popular infant feeding practices.

Methods

A total of 103 infants, 1 to 14 months of age, were selected at random in the well baby clinic from July 1964 to February 1965 by taking every tenth patient registered. Before the infant's checkup, the mother of each infant was interviewed as to the current diet being fed the baby. She was asked to estimate as accurately as possible the total amounts of milk, cereal, strained fruit, meat, and vegetables that the infant received daily. Questions were asked so that the mother would give an estimate of what the infant would take on an average day in the past week, the day immediately preceeding, and the day of the interview. When the total intake was compiled, estimates of the caloric and sodium intakes were made from standard tables supplied by manufacturers of baby foods.

The accuracy of the method of interview was determined in eight additional infants by having the mothers aliquot an exact duplicate of a meal into a plastic container. The intake of three separate days was acquired and analyzed for sodium and potassium content. The interview technique was shown to be a very reliable method of estimating sodium and potassium intakes. The correlation coefficient of the measured and estimated sodium intake was 0.96, and of the potassium intake was 0.99.

The reliability of the tables supplied by the manufacturer was investigated by analyzing 20 commonly used foods of the same brands purchased from local suppliers. The sodium levels listed in the tables were 100% ± 25% of the values found by analysis.

The weights and heights of the infants were recorded at the time of interview, after which the mothers were usually given dietary instructions for the ensuing weeks and months.

Regression lines were calculated from the dietary estimates with caloric or sodium intake as ordinate and age in months as the abscissa. The mean values referred to in the text were read directly from the calculated regression lines.

Results

The mean total daily sodium intake rises rapidly in the first year of life, from 18 mEq/24 hr at one month to 61 mEq/24 hr at one year. An increase in sodium intake is expected in the first year of life due to growth and increased appetite. In Fig 1, the increase in intake is related to the factor of body size. At one month the mean sodium intake is 4.6 mEq/kg/24 hr with a rise to 6.3 mEq/kg/24 hr by one year.

Caloric intake increases rapidly in the first year of life, in keeping with the normal growth of a group of healthy infants. The mean value increases from 550 calories/24 hr at one month to 1,050 calories/24 hr at one year. However, the total caloric intake in proportion to body size is decreasing in

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introduction of these products to very young infants. Infants as young as 2-3 months are being fed pureed vegetables, meat, and eggs. Of the commercial canned foods, these are highest in sodium content, apparently the result of the addition of sodium chloride during preparation and processing.[6,7]

Beal,[8] in 1957, called attention to the fact that infants were being fed solids at an earlier age than in studies she had conducted in the previous ten years. She noted that occasionally this resulted in nonacceptance of the food later on, which contradicts the popular belief that early feeding promotes the change from milk. It is also a commonly accepted notion that infants "sleep all night" when fed solids as an evening meal. This appears to be a prominent reason in the feeding of cereal in the first month of life, though probably without foundation.[9] From the nutritional point of view, the major need for solids in the first year of life is related to iron requirements since milk will not supply the needs for growth unless artificially fortified.

A large sodium intake may prove to be very hazardous in patients in cardiac failure, especially when the diet is changed from one lower in sodium.[10] From the practical point of view, one can avoid excessive ingestion of sodium in such patient by not feeding precooked cereals and strained meats and vegetables. We have adopted the practice of keeping infants with congestive heart failure on milk with added iron, strained fruits, and cereals which are not precooked. Thus, it is usually possible to maintain sodium intake below 20 mEq/day for the entire first year of life.

Since pureed infant foods are occasionally prescribed for geriatric patients, consideration of the salt content of these foods is important because of the high incidence of cardiac disease in the elderly.

The higher sodium intakes found in this study are near the levels which produce hypertension in experimental animals. Meneely showed that rats fed diets high in sodium develop hypertension.[11,12] The occurrence of hypertension in the rats was proportional to the total level of sodium ingested. The lowest sodium concentrations of the rat feed mixture which caused significant hypertension was 2.8%. Since this mixture contained fat, protein, and carbohydrate with a total nutritive value of 480 calories/100 grams, there were 100 mEq sodium/1,000 calories. Further refinement of these experiments have indicated that dietary potassium has a protective effect when the Na:K ratio is 1.0.[13] The eight infants in this study whose diets were analyzed from aliquots were found to have a Na:K ratio from 0.7 to 1.5. They may be protected by the high potassium contents of these foods. Dahl, however, has reported experiments in which genetically susceptible rats developed hypertension while being fed commercially available baby food.[14]

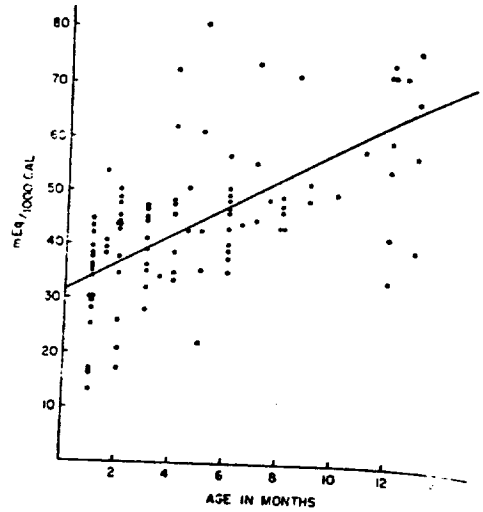

Fig 3.—Sodium intake per 1,000 calories of 103 well infants in the first year of life.

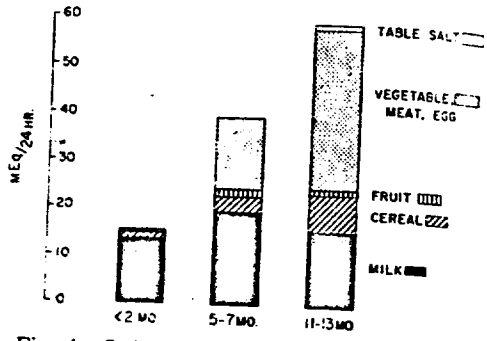

Fig 4.—Sodium intake from various dietary components of three groups of infants in the first year of life.

Dahl has completed several geographical surveys which relate the high rates of hypertension in certain areas of the world to large daily sodium loads.[11,15] Hypertension is very prevalent among the people of northern Japan where 20% of the adult

population is affected. The diet of these people contains as much as 500 mEq of sodium daily; thus, an 80 kg (176 lb) adult would have an intake of 6.2 mEq/kg/24 hr. This level is comparable to the average intake of the 1-year-old infant as found in this study. The average 1-year-old American infant apparently has a relatively higher sodium intake than his parents. In the United States the average daily intake of sodium of adults varies from 130 to 300 mEq/24 hr,[1] or 1.6 to 3.8 mEq/kg for an 80 kg (175 lb) individual.

In 1934, Thompson reported several diabetic children who developed hypertension while on extraordinary salt intakes of 60-80 gm daily. The blood pressure returned to normal when the salt intake was dropped to 4 gm daily or when high intakes of potassium were added.[16] There are no data available at the present time which would relate sodium intake during infancy to essential hypertension at any age. Idiopathic or essential hypertension is a very rare disease in childhood and is virtually unknown in infancy.[17]

There are wide margins to optimal sodium intake for infants who are well. Growth requirements have been estimated to be 76 mEq for each kilogram of body growth.[18] Thus, a rapidly growing infant, gaining at the rate of 30 gm (1 oz) daily would require 2.3 mEq every 24 hours. The maximum upper limits of sodium intake are much less well established; the level 8 mEq/kg has been suggested by Pratt.[19] Five of the infants in this study were taking this level and higher without apparent ill effect.

More study of this problem is indicated, giving consideration to possible subtle alterations in renal function and blood pressure. In addition, the observations should be extended beyond the first year, since many factors alter the diet during this period, including physiological tapering of appetite, slowing of growth rate, and a change to table food, more comparable to the usual diet of the adult members of the family.

Summary

The daily sodium intake of 103 well infants was estimated by dietary interviews and substantially confirmed by aliquot analysis in eight. Total sodium intake increases rapidly, and out of proportion to weight gain, during the first year of life. This increase results chiefly from the introduction of solid baby foods, which are high in sodium content, particularly strained meat and vegetables. For those infants where high sodium intake is not desirable, avoidance of these baby foods appears indicated. Possible long-term consequences of excessive sodium intake are discussed.

This study was supported by grants from the Middle Tennessee Heart Association, the Hartford Foundation, and the US Public Health Service, grants No. FR-95 and HE-08195.

The statistical analysis was carried out by Dr. C. Federspiel of the Biostatistic Department of Vanderbilt University.

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The University of Maryland Agricultural Experiment Station

Bulletin No. 340 — December, 1932

Salt Tolerance of Baby Chicks

By GEO. D. QUIGLEY and ROY H. WAITE

INTRODUCTION

Common salt (sodium chloride) has come to be generally recognized by poultrymen as an important ingredient of the poultry mash. There is, however, a general and persistant belief that salt has extremely toxic or poisonous properties when fed to chickens in anything more than comparatively small amounts. This belief has, for the most part, been based on tradition. The reported work of various investigators, such as Zurn (1) in 1882, Collier (2) 1892, Suffram (3) 1910, Pearl, Surface and Curtis (4) in 1915, and the work of Edwards (5) with pigeons in 1918, has, as a rule, been so interpreted by poultry raisers as to seem to further the idea that salt is likely to be extremely poisonous. In 1926 the problem with respect to partly grown chickens was studied at some length by Mitchell, Card and Carman (6). With the exception of Edwards' work with pigeons, the various investigations have been conducted with comparatively mature chickens, leaving some doubt as to the behavior of baby chicks when given heavy doses of salt.

A considerable portion of the time of teachers, investigators, and extension workers in poultry husbandry, as well as salesmen for poultry feeds and poultry equipment, is spent in attempting to determine the cause of trouble and mortality in flocks of chickens, particularly in immature birds during the spring months. Often the feed is blamed, although usually unjustly so, as has been demonstrated by numerous tests conducted at the Maryland Station during the last six years. A portion of these

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tests have been reported by Quigley and Waite (7) in 1931. Occasionally a sample of feed is received for examination which contains more salt than is usually considered safe. A lack of specific information concerning the effect of salt on baby chicks has been a handicap in efforts to check on samples of this kind. The Maryland Station has considered the problem of sufficient importance to warrant investigation and an experiment was started in April, 1932.

Previous Work With Poultry

As indicated in the foregoing discussion the bulk of the experimental work has been carried on with adult fowl. Only a very small amount of salt work has been done with baby chicks. Zurn (1) is reported as having found that 15 to 30 grams (approximately ½ to 1 ounce) of salt will kill a healthy hen in 8 to 12 hours. Calculated on this basis a bird weighing 4 pounds would not survive a minimum dose, amounting to 8.3 grams of salt per kilogram of body weight (salt to the amount of 0.83% of body weight). Collier (2) stated that no bad effects were observed until salt intake in food reached 0.063 ounces per bird per day. Since this figure represents, for a 4-pound bird, a daily dose of 0.98 grams of salt per kilogram of body weight, or 0.098% of body weight, it is obvious that in the work of Collier an amount of salt approximately one-ninth of the amount quoted as lethal by Zurn causes "bad effects". Collier mentions no mortality and indicates that the bad effect was a diarrhea.

Suffram (3), after experimentation, concluded "that a dose of 4 grams per kilogram of body weight is sufficient to produce death". It is interesting to note that Suffram called attention to the possibility of the development of tolerance to salt as a result of repeated injections of small doses, which possibility seems to have been overlooked by other investigators making a similar study. The work of Edwards (5) with pigeons is of interest because of another phase of the problem attacked. While he was able to show that 3.33 and 4.5 grams per kilogram would produce death in pigeons, his experimental birds had previously been used for smaller doses. His work reveals that the percentage of salt found in the crops of the dying pigeons was 0.76% and 0.73% respectively or close to physiological salt solution, and indicates the difficulty of diagnosis by means of chemical analysis.

The investigations of Mitchell, Card and Carman (6) disclosed that, contrary to general belief, "chickens could be raised from 9 to 21 weeks of age on rations containing as high as 8% of salt", or they could be allowed free access to rock salt, in both cases without apparent detrimental effects. The birds used in the various salt levels were later employed in tests to determine the individual dose that would produce death. Although the question of tolerance to previous feeding of salt would seem to be involved, their results substantially confirm those of Suffram, their findings being, "the minimum lethal single dose of salt for birds weighing from 3 to 5 pounds was found to be close to 4 grams per kilogram of body weight". In this work the conclusion was also reached that salt given in solution was more toxic than equal amounts consumed in feed, also that with repeated equal doses of salt, calculated to eventually produce mortality, there seemed to be little difference in survival period whether birds were fasting or had access to feed.

A paper by Smith (8) (1929) pertaining to chicks reared from day-old to eight weeks reveals best results in growth from those fed 1.5% of salt in an all-mash ration, and that mortality was measurably increased after the salt level exceeded 2% of the ration. In this experiment the percentage of salt ranged from 0% to 4.5% with intervals of 0.5%. An interesting observation by Smith was the bloated appearance of some of the chicks receiving more than 2% of salt, which was diagnosed as acidosis, and was reported to have responded favorably to a peritoneal injection of 2 cc. of an 8% solution of sodium carbonate. Examination of the chicks that died "showed the body cavity filled with fluid; a dropsic condition of the cardiac sac; hemorrhage at the top of the heart; a very pale condition of the liver, spleen and lungs, and a very irritated condition of the mucosa of the intestinal tract."

Purpose of the Experiment

The purpose of the experiment herein described was to determine: (1) the percentage of salt in feed which would be required to produce sudden and severe mortality; (2) the maximum percentage of salt in feed which would be tolerated by baby chicks; (3) the effect of various levels of salt upon growth and mortality, and (4) the minimum lethal dose.

Procedure

The experiment was divided into two parts, one investigation concerned with the reaction of the chicks to various levels of salt in the feed and another in which lethal dose determinations were made.

The Effect of Salt in Feed

A total of 98 hand-selected chicks, all apparently healthy, were used in this part of the experiment. Of these chicks, 84 S. C. W. Leghorns were selected and divided into six lots of 14 chicks each, and each lot was further subdivided into 2 pens of 7 chicks

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each and placed in separate compartments of a special brooding device on the order of a battery brooder (Fig. 1), and which was known by previous experience to produce excellent brooding conditions for such work. These pens were immediately self fed on a ration containing the following amounts of salt: Pen 1, 1% salt; pen 2, 3%; pen 3, 5%; pen 4, 8%; pen 5, 10%; and pen 6, 15% of salt. The basal ration used was the Wisconsin rachitic formula* which experience had shown to give good results under the conditions of the experiment:

59 lbs. ground yellow corn.
25 " pure wheat flour middlings.
12 " crude casein.
1 " salt (sodium chloride).
1 " precipitated calcium carbonate.
1 " precipitated calcium phosphate.
1 " dried yeast.
Tested Vitamine D potent cod liver oil, 1%.

To the above ration salt was added in proper amounts to bring the basal 1% up to the percentage desired. No other food was

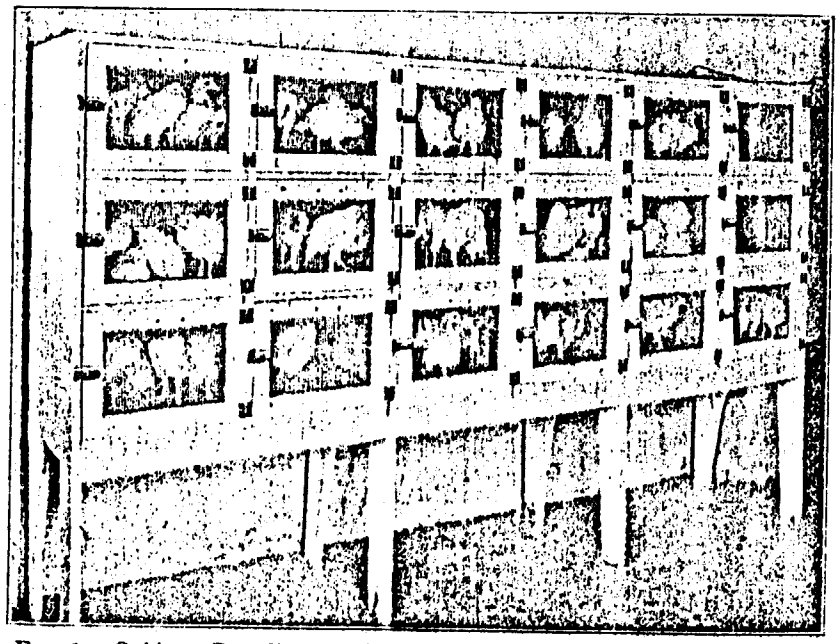

FIG. 1. *Cabinet Brooding Device in which chicks were brooded. This equipment was designed for use in testing commercial brands of cod liver oil and was made available for the salt feeding experiment through the courtesy of Mr. L. E. Bopst of the State Inspection and Regulatory Service.*

* Ration by Hart, Kline and Keenan, Science, 73 (1931), No. 1904, pages 710, 711.

given. Water was kept available to the birds at all times. Weight of chicks and amount of feed consumed was calculated daily during the first three weeks and weekly thereafter through the ninth week.

In order to learn something of the effect of sudden switching to a mash heavily loaded with salt the fourteen remaining chicks previously mentioned of the 98 were divided into two pens of seven chicks each, one (pen 7) with 7 S. C. W. Leghorns, and the other (pen 8) with 4 S. C. W. Leghorns and 3 Hamburgs. These were held in reserve on the 1% basal ration during the first week of the experiment, after which they were placed on a 15% salt ration. Pen 7 was switched to the 15% salt mash by simply emptying the 1% mash from the feeder and immediately replacing it with the 15% salt mash. Pen 8 was fasted for four hours before being placed on the 15% mash. Both pens were continued on this heavy salt mash (15%) for seven days, then both were returned to the 1% mash for 10 days, after which pen 7 was switched to a mash containing 22½% of salt and pen 8 was similarly transferred to a 30% salt mash, each for a period of ten days. The mortality was heavy under this treatment but the surviving chick in each of the pens (7 and 8) was returned to the 1% salt mash at the end of the ten days in an attempt to determine if there were any permanent deleterious effects from the treatment.

Adequate notes and records were made as to the appearance of the various pens, condition of the droppings, and gross autopsical appearances of birds dying during the course of the experiment.

The ability of chicks to survive excessive amounts of salt, which seemed to shatter certain traditions among poultrymen, resulted in a desire for verification of the amounts of salt used. Samples of all six rations used in the general feeding tests were submitted to the State Feed Control Laboratory* which reported a content of 1.32%, 3.30%, 5.44%, 8.41%, 10.89% and 16.17% of sodium chloride indicated by chemical analysis of the respective rations as fed to pens 1 to 6. These figures approximate the theoretical amount calculated in the mixing formula and as they run uniformly slightly in excess of the percentage expected they would seem to indicate that the intended amount of salt was administered.

RESULTS OF THE FEEDING TESTS

Data showing the body weight of pens 1-6 is given in Tables I and II, and is presented graphically in Fig. 2. While the weight of all pens was substantially the same at the start, differences began to appear at once, particularly in pen 5 (10%), where the bloated condition to be discussed later, gave them a decided ad-

* Mr. L. E. Bopst in charge.

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TABLE I.

BODY WEIGHT AND FEED CONSUMED PER CHICK PER DAY FOR LOTS FED 1%, 3%, AND 5% OF SALT IN ALL-MASH RATION

(See Table II for Higher Percentages)

	Pen 1 (1% Salt)				Pen 2 (3% Salt)				Pen 3 (5% Salt)			
Day	No. Chicks	Weight Per Chick: Grams	Weight Per Chick: Lbs.	Feed Per Chick, Lbs.	No. Chicks	Weight Per Chick: Grams	Weight Per Chick: Lbs.	Feed Per Chick, Lbs.	No. Chicks	Weight Per Chick: Grams	Weight Per Chick: Lbs.	Feed Per Chick, Lbs.
0	14	34.4	.0758	.....	14	34.6	.0763	.....	14	34.1	.0752	.....
1	14	34.3	.0756	.0071	14	36.6	.0807	.0043	14	38.5	.0849	.0043
2	14	44.2	.0974	.0100	14	45.4	.1001	.0043	14	46.4	.1023	.0100
3	14	46.8	.1032	.0143	14	47.2	.1041	.0157	14	49.3	.1087	.0100
4	14	51.1	.1127	.0143	13	51.5	.1135	.0138	14	51.9	.1144	.0143
5	14	53.5	.1179	.0200	13	53.9	.1188	.0138	14	55.0	.1213	.0143
6	14	57.2	.1261	.0185	13	56.0	.1235	.0169	14	58.1	.1281	.0143
7	14	59.8	.1318	.0185	13	58.4	.1287	.0123	14	62.5	.1378	.0143
8	14	64.1	.1413	.0228	13	61.2	.1349	.0184	14	66.7	.1470	.0200
9	13	70.5	.1554	.0292	13	66.5	.1466	.0246	14	70.6	.1556	.0228
10	13	75.3	.1660	.0276	13	69.6	.1534	.0246	14	72.9	.1607	.0214
11	13	78.5	.1731	.0215	13	73.6	.1623	.0169	14	76.9	.1695	.0171
12	13	83.2	.1834	.0276	13	75.8	.1671	.0215	14	80.7	.1779	.0214
13	13	91.3	.2013	.0292	13	79.7	.1757	.0292	14	80.8	.1781	.0242
14	13	94.5	.2083	.0292	13	81.2	.1790	.0215	14	81.8	.1803	.0228
15	13	102.3	.2255	.0292	13	92.9	.2048	.0276	14	92.9	.2048	.0257
16	13	104.9	.2313	.0230	13	92.1	.2030	.0292	14	93.6	.2064	.0242
17	13	112.0	.2469	.0338	13	94.2	.2077	.0215	14	88.1	.1942	.0200
18	13	122.5	.2701	.0384	13	106.4	.2346	.0276	14	105.4	.2324	.0314
19	13	128.2	.2826	.0384	13	120.7	.2661	.0261	14	106.2	.2341	.0257
20	13	136.2	.3003	.0415	13	120.5	.2657	.0400	14	114.4	.2522	.0371
21	13	139.9	.3084	.0430	13	126.7	.2793	.0400	14	121.4	.2676	.0285
*28	13	181.4	.3999	.0353	13	162.1	.3574	.0358	14	145.6	.3210	.0310
*35	13	230.6	.5084	.0538	13	207.4	.4572	.0483	14	183.2	.4039	.0348
*42	13	325.7	.7180	.0725	13	293.2	.6464	.0591	14	241.9	.5333	.0493
*49	13	386.3	.8516	.0841	13	326.9	.7207	.0553	14	282.7	.6232	.0485
*56	13	498.6	1.0992	.1013	13	395.5	.8719	.0725	14	366.8	.8086	.0651
*63	13	542.4	1.1958	.1029	13	464.7	1.0245	.0661	14	427.8	.9431	.0514

* In these weekly calculations feed consumption is averaged.

TABLE II.

BODY WEIGHT AND FEED CONSUMED PER CHICK PER DAY FOR LOTS FED 8%, 10% AND 15% OF SALT IN ALL-MASH RATION

(See Table I for Lower Percentages)

	Pen 4 (8% Salt)				Pen 5 (10% Salt)				Pen 6 (15% Salt)			
Day	No. Chicks	Weight Per Chick: Grams	Weight Per Chick: Lbs.	Feed Per Chick, Lbs.	No. Chicks	Weight Per Chick: Grams	Weight Per Chick: Lbs.	Feed Per Chick, Lbs.	No. Chicks	Weight Per Chick: Grams	Weight Per Chick: Lbs.	Feed Per Chick, Lbs.
0	14	33.8	.0745	.....	14	34.0	.0750	.....	14	34.5	.0761	.....
1	14	43.0	.0948	.0029	14	38.1	.0840	.0086	14	35.4	.0780	.0014
2	13	48.5	.1069	.0062	14	46.4	.1023	.0128	14	42.6	.0939	.0014
3	13	49.0	.1080	.0107	14	50.6	.1116	.0057	14	46.4	.1023	.0071
4	13	52.5	.1157	.0107	14	56.4	.1243	.0011	14	48.3	.1065	.0100
5	13	54.7	.1206	.0130	14	61.0	.1345	.0011	14	49.5	.1091	.0086
6	13	56.2	.1239	.0130	14	62.7	.1382	.0010	14	53.4	.1177	.0100
7	13	57.6	.1270	.0130	13	63.5	.1400	.0092	14	54.8	.1208	.0100
8	13	58.3	.1285	.0169	11	61.5	.1356	.0145	13	57.8	.1274	.0130
9	13	61.6	.1358	.0230	11	66.1	.1457	.0181	13	65.2	.1437	.0123
10	12	62.8	.1384	.0150	11	68.5	.1510	.0127	12	64.5	.1422	.0166
11	12	67.0	.1477	.0183	11	67.0	.1477	.0109	12	65.8	.1451	.0166
12	12	71.3	.1572	.0183	11	64.5	.1422	.0127	11	64.2	.1415	.0127
13	11	72.4	.1596	.0218	10	65.7	.1448	.0160	10	65.3	.1440	.0120
14	10	74.0	.1631	.0280	10	62.7	.1382	.0140	10	65.1	.1435	.0200
15	10	78.3	.1726	.0180	10	71.6	.1578	.0160	9	64.4	.1420	.0066
16	10	77.8	.1715	.0340	10	75.1	.1656	.0240	9	67.3	.1484	.0080
17	10	80.6	.1777	.0240	10	69.2	.1526	.0140	9	69.7	.1537	.0111
18	10	90.2	.1989	.0280	10	80.5	.1776	.0140	9	71.8	.1583	.0177
19	10	95.5	.2105	.0220	9	81.6	.1799	.0220	8	81.1	.1788	.0225
20	10	99.2	.2187	.0320	8	88.0	.1940	.0375	8	77.4	.1706	.0150
21	10	103.8	.2288	.0320	8	93.9	.2070	.0275	8	79.6	.1755	.0175
*28	9	132.0	.2910	.0488	8	110.4	.2434	.0225	5	95.0	.2094	.0177
*35	9	163.6	.3607	.0228	8	128.8	.2840	.0325	4	96.5	.2127	.0207
*42	9	196.6	.4334	.0422	7	162.7	.3587	.0420	4	108.0	.2381	.0200
*49	9	243.8	.5375	.0463	7	189.6	.4180	.0359	3	135.0	.2976	.0266
*56	9	276.2	.6089	.0431	7	239.1	.5271	.0416	3	137.0	.3020	.0266
*63	9	316.6	.6980	.0473	7	263.6	.5811	.0395	3	172.3	.3799	.0361

* In these weekly calculations feed consumption is averaged.

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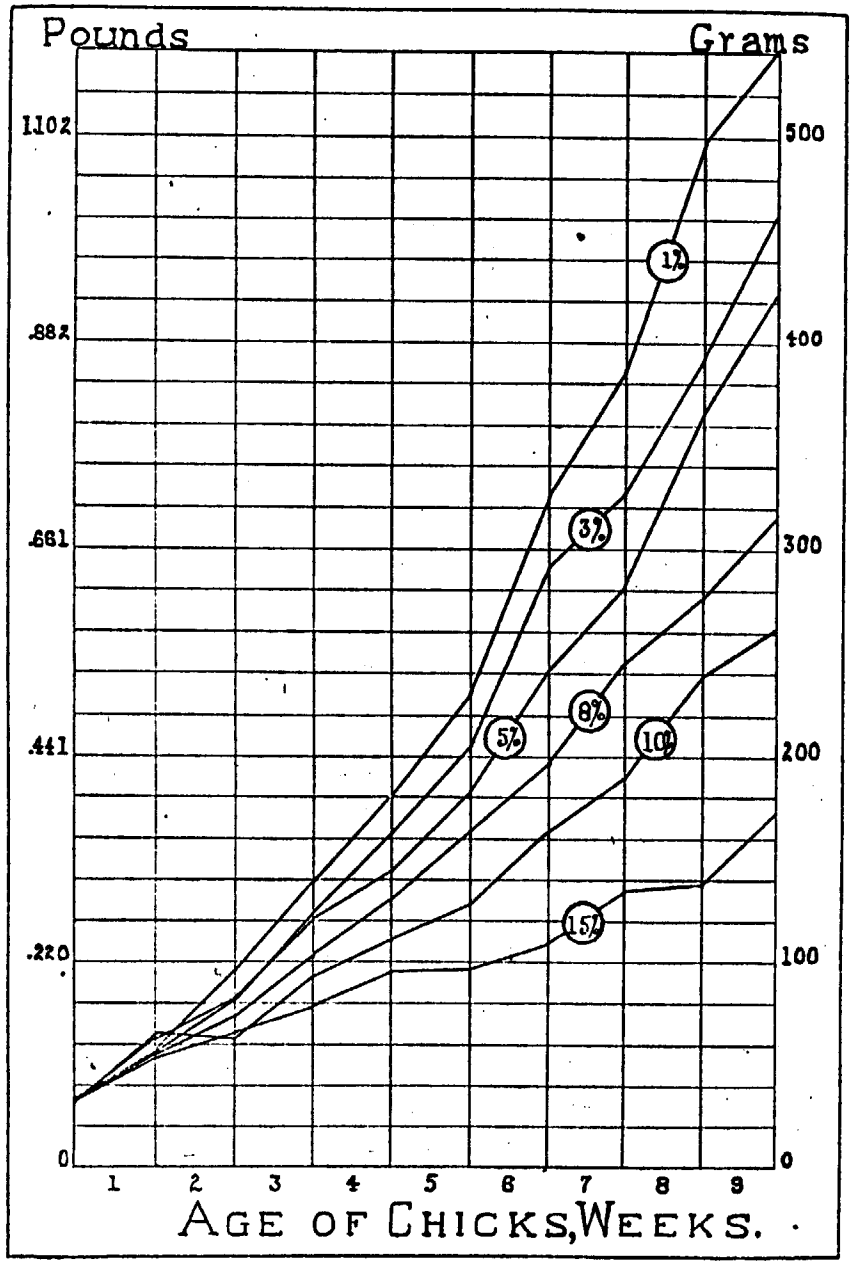

FIG. 2. *Growth Curves of S. C. W. Leghorn Chicks Fed 1%, 3%, 5%, 8%, 10%, 15% of Salt in Ration.*

vantage in gains in weight made during the first week. Commencing with the third week all pens maintained the same relative position with respect to weight until the completion of the experiment at the end of the ninth week.

Pen 1 (1% salt) made what may be considered a normal gain as judged by the standards available.* In comparison with pen 1, other pens made lesser gains which became pronounced after the salt exceeded 5% of the mash as in pens 4, 5, and 6. Figs. 3, 4, 5, 6, 7, and 8 give a fairly good illustration of the difference in size at the conclusion of the experiment.

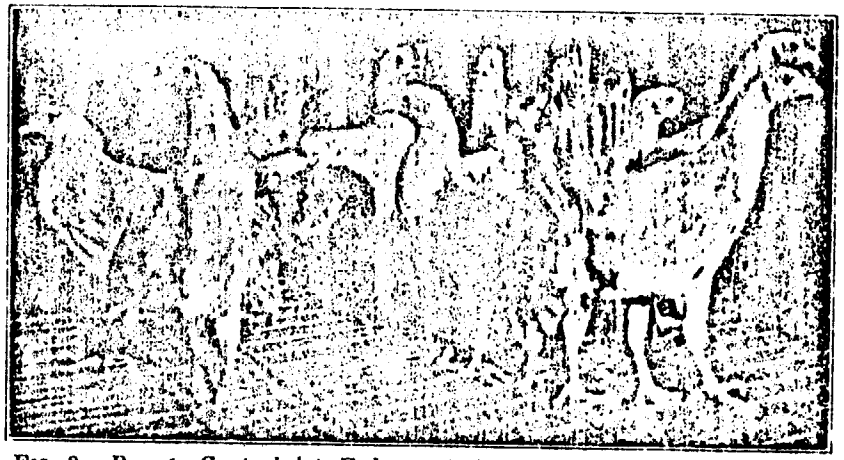

FIG. 3. *Pen 1, Control lot Fed a 1% Salt Ration. Photographed at Conclusion of Experiment, Age Nine Weeks.*

FIG. 4. *Pen 2, 3% Salt in Ration. Age 9 Weeks.*

* Growth figures show close agreement with those furnished by Card and Kirkpatrick, Storrs Bulletin 96, and by those of Hart, Klien and Keenan, for the same ration as used in this experiment, as reported in Science, 73 (1931), No. 1904, pages 710, 711.

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Mortality for the various pens is shown in Table III. The mortality in pens 1 and 2 (1 chick in each pen) occurred during the first two weeks and autopsy findings strongly indicate that these deaths were probably not due to salt poisoning. It is interesting to note that in all pens receiving more than 5% of salt, mortality was not excessive until the second week, and that in pen 6 (15% salt) no mortality occurred until the second week of the experiment. This is shown graphically in Fig. 9.

FIG. 5. *Pen 3, 5% Salt in Ration. Age 9 Weeks.*

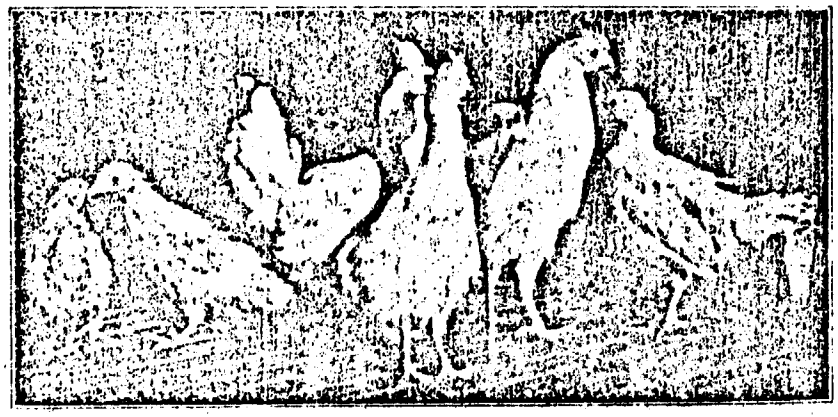

FIG. 6. *Pen 4, 8% Salt in Ration. Age 9 Weeks.*

TABLE III.

MORTALITY DURING NINE WEEKS IN THE SALT FED LOTS.

Pen No.	Per Cent Salt in Ration	Number Chicks	Number Died	Per Cent Loss
1	1	14	1	7.14
2	3	14	1	7.14
3	5	14	0	0.00
4	8	14	5	35.71
5	10	14	7	50.00
6	15	14	11	78.57

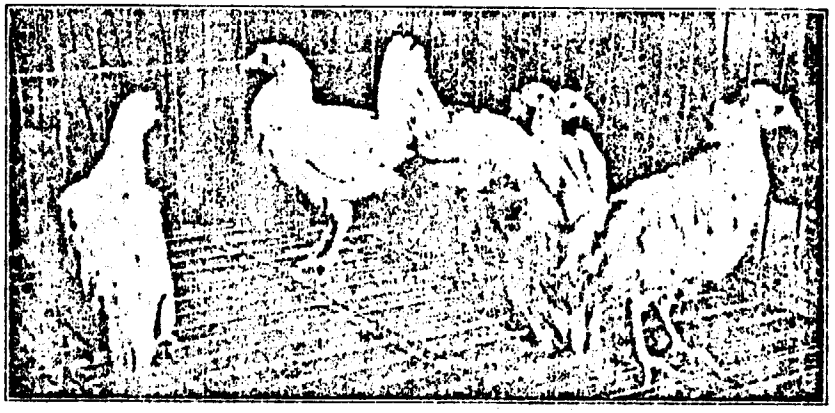

FIG. 7. *Pen 5, 10% Salt in Ration. Age 9 Weeks.*

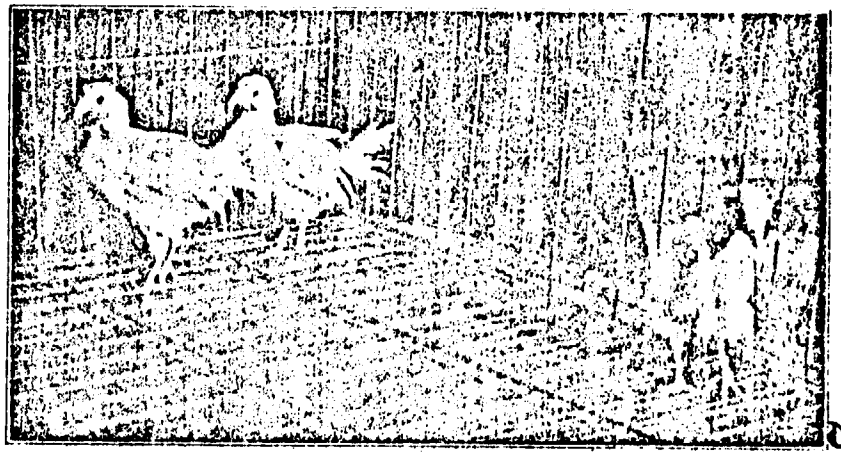

FIG. 8. *Pen 6, 15% Salt in Ration. Age 9 Weeks.*

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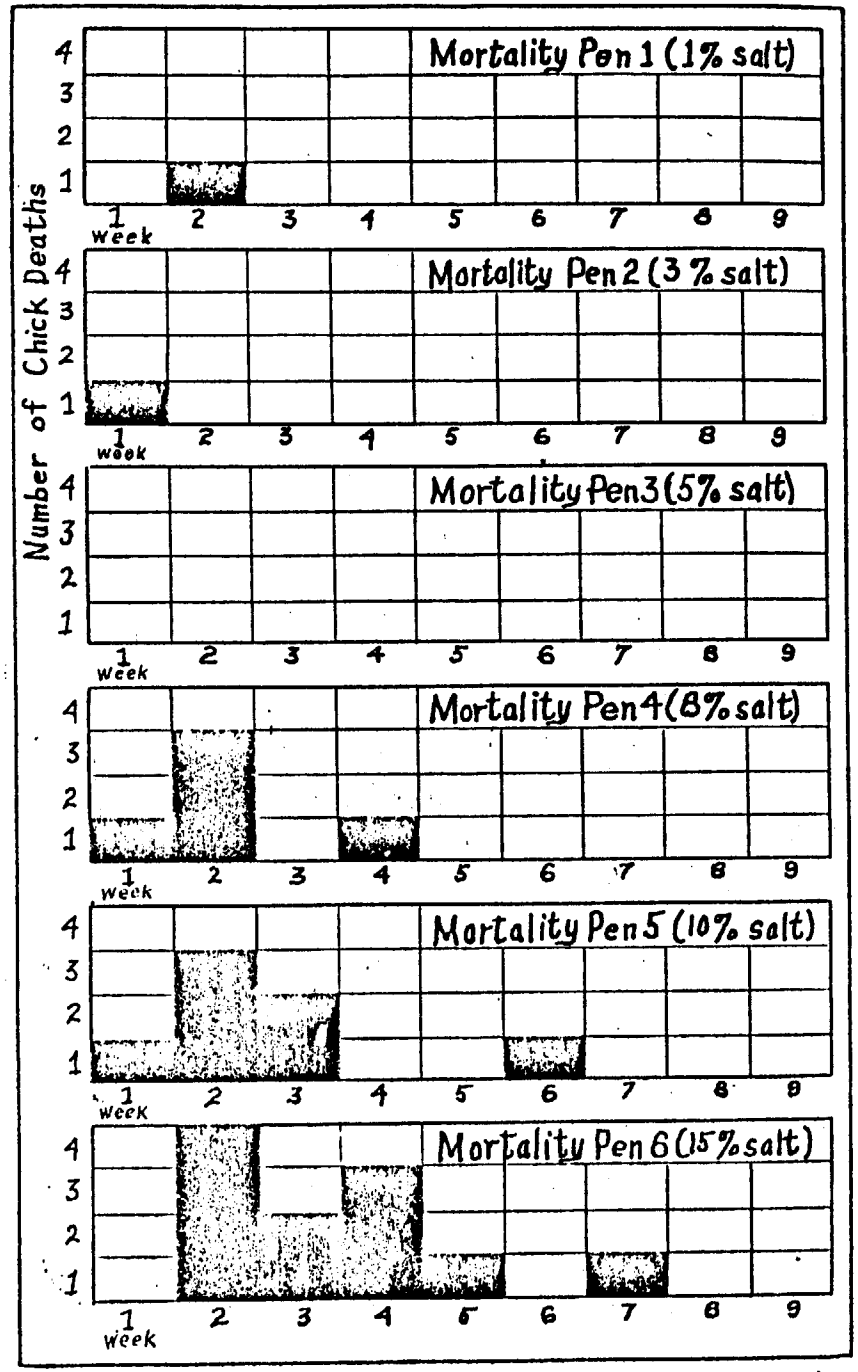

FIG. 9. *Distribution of Mortality by Weeks in Salt Fed Pens.*

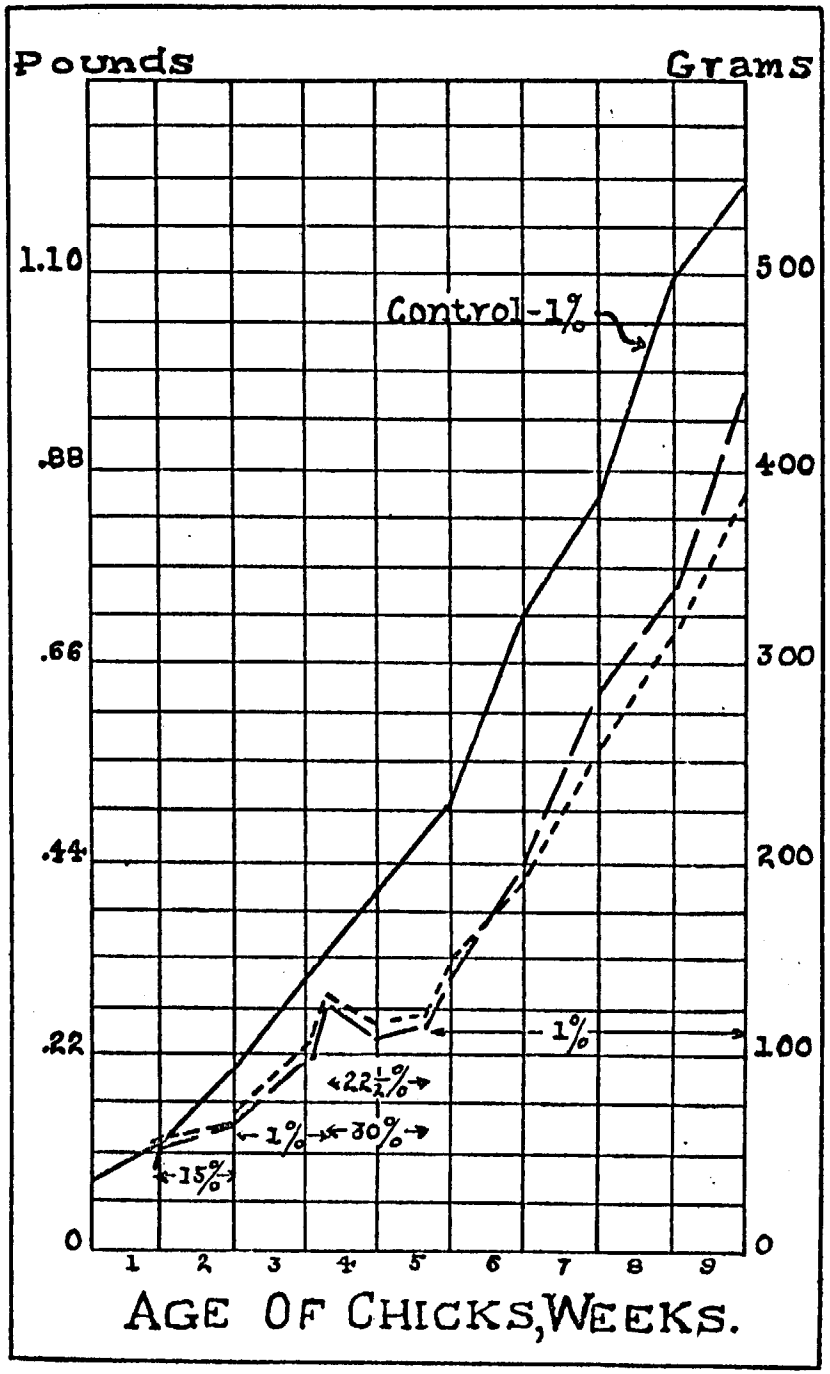

FIG. 10. *Growth Curves of the Two Lots in Which the Amount of Salt in the Ration Was Changed Several Times During the Feeding Period Compared to Control Lot.*

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Table IV shows the daily amount of salt, expressed as percentage of body weight, consumed by the various lots during the first week and thereafter by weeks. The average daily dose over the period of 9 weeks, for the various pens Nos. 1 to 6 respectively, is 0.110%, 0.295%, 0.466%, 0.813%, 0.919%, and 1.329% of

TABLE IV.

SALT CONSUMED IN TERMS OF BODY WEIGHT.

Period	Daily Consumption Salt Expressed as Percentage of Body Weight					
	Pen 1 (1% Salt)	Pen 2 (3% Salt)	Pen 3 (5% Salt)	Pen 4 (8% Salt)	Pen 5 (10% Salt)	Pen 6 (15% Salt)
1st Day	.094	.160	.253	.248	.102	.269
2nd "	.103	.129	.489	.464	1.251	.224
3rd "	.139	.453	.460	.793	.511	1.041
4th "	.127	.365	.625	.740	.885	1.408
5th "	.170	.348	.589	.862	.818	1.182
6th "	.147	.411	.558	.839	.724	1.274
7th "	.140	.287	.519	.819	.657	1.243
1st Week	.134	.316	.511	.697	.473	.998
2nd "	.152	.420	.640	1.096	.983	1.567
3rd "	.133	.383	.605	1.102	1.255	1.321
4th "	.088	.301	.483	1.342	.924	1.268
5th "	.105	.316	.431	.506	1.144	1.460
6th "	.101	.274	.462	.779	1.171	1.259
7th "	.099	.230	.390	.689	.858	1.341
8th "	.092	.227	.403	.566	.789	1.321
9th "	.086	.193	.273	.542	.680	1.425
Average for 9 Weeks....	.110	.295	.466	.813	.919	1.329

body weight. It will be shown later that these figures tend to verify results obtained in the lethal dose determination.

Results of the work with pens 7 and 8 are presented in Figures 10, 11, and 12 and in Tables V and VI. It will be seen that the birds in both pens gained slightly in weight on the 15% of salt, gained at practically a normal rate when replaced on 1% salt feed, lost weight sharply during the 22½% and 30% period, but not in proportion to the amount of salt, and that the remaining survivors—one bird in each pen—when again placed on a 1% salt ration gained at a rate practically paralleling the check lot. This experience would seem to indicate that, within the scope of

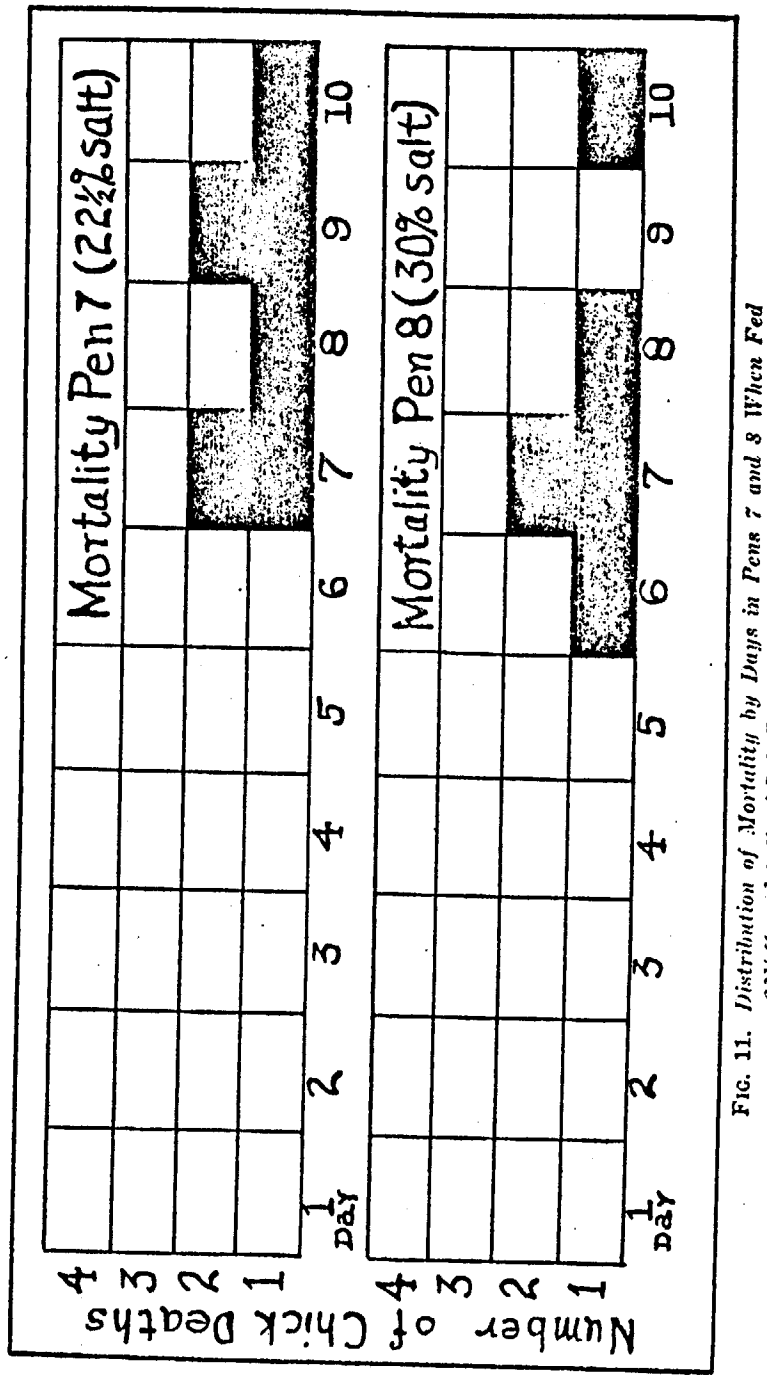

FIG. 11. *Distribution of Mortality by Days in Pens 7 and 8 When Fed 22½% and 30% of Salt Respectively.*

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TABLE V.

BODY WEIGHT AND FEED CONSUMED BY CHICKS FED DIFFERENT AMOUNTS OF SALT IN RATION. PENS 7 AND 8.

Pen 7						Pen 8				
Age Days	No. Chicks	Per Cent Salt in Feed	Body Weight Per Chick		Feed Consumed Per Chick	No. Chicks	Per Cent Salt in Feed	Body Weight Per Chick		Feed Consumed Per Chick
			Grams	Lbs.	Lbs.			Grams	Lbs.	Lbs.
6	7	15	53.1	.1171	.....	7	15	44.1	.0972	.....
7	7	15	60.0	.1323	.02	7	15	55.7	.1228	.0171
8	7	15	59.9	.1321	.0143	7	15	60.3	.1329	.0171
9	7	15	60.1	.1325	.0171	6	15	58.8	.1296	.0200
10	7	15	62.0	.1367	.0086	6	15	59.7	.1316	.0166
11	7	15	61.4	.1354	.0143	6	15	62.0	.1367	.0066
12	7	15	63.9	.1409	.0143	6	15	63.7	.1404	.0066
13	7	15	62.1	.1369	.0200	6	15	63.5	.1400	.0146
14	7	1	71.0	.1565	.0314	6	1	70.0	.1543	.0233
15	7	1	74.9	.1651	.0200	6	1	73.3	.1616	.0146
16	7	1	73.6	.1623	.0343	6	1	75.8	.1671	.0400
17	7	1	85.4	.1883	.0343	6	1	77.0	.1698	.0166
18	7	1	89.3	.1969	.0257	6	1	85.8	.1892	.0266
19	7	1	94.3	.2079	.0400	6	1	90.8	.2001	.0433
20	7	1	94.9	.2092	.0228	6	1	94.3	.2079	.0333
21	7	1	106.4	.2346	.0228	6	1	99.3	.2189	.0266
22	7	1	113.7	.2507	.0285	6	1	101.7	.2242	.0200
23	7	1	117.1	.2582	.0400	6	1	105.8	.2332	.0366
24	7	22½	126.9	.2798	.0228	6	30	124.7	.2749	.0330
25	7	22½	124.3	.2740	.0400	6	30	123.2	.2716	.0366
26	7	22½	119.4	.2632	.0143	6	30	120.0	.2646	.0166
27	7	22½	123.4	.2720	.0171	6	30	118.8	.2619	.0166
28	7	22½	114.1	.2515	.0114	6	30	110.0	.2425	.0200
29	7	22½	110.0	.2425	.0200	5	30	109.0	.2403	.0040
30	5	22½	108.0	.2381	.0160	3	30	108.0	.2381	.0000
31	4	22½	106.0	.2337	.0150	2	30	106.0	.2337	.0100
32	2	22½	100.0	.2205	.0300	2	30	104.5	.2304	.0200
33	1	22½	120.0*	.2646	.0000	1	30	116.0*	.2557	.0400
34	1	1	126.0	.2778	.0600	1	1	125.0	.2756	.0550
35	1	1	147.0	.3241	.1050	1	1	142.0	.3131	.1010
38	1	1	152.0	.3351	.0583	1	1	173.0	.3814	.1260
39	1	1	161.0	.3549	.....	1	1	175.0	.3858	.....
40	1	1	171.0	.3770	.0400	1	1	182.0	.4012	.0918
42	1	1	188.0	.4145	.1300	1	1	198.0	.4365	.1137
44	1	1	194.0	.4277	.....	1	1	194.0	.4277	.....
45	1	1	215.0	.4743	.0433	1	1	200.0	.4409	.0736
46	1	1	235.0	.5181	.....	1	1	246.0	.5423	.....
49	1	1	260.0	.5732	.1650	1	1	287.0	.6327	.1681
51	1	1	288.0	.6349	.....	1	1	285.0	.6283	.....
54	1	1	315.0	.6944	.....	1	1	322.0	.7099	.....
56	1	1	314.0	.6922	.0340	1	1	340.0	.7496	.1243
60	1	1	370.0	.8157	.....	1	1	372.0	.8201	.....
62	1	1	392.0	.8642	.0700	1	1	442.0	.9744	.1000

* This must not be understood as indicating an increase in weight, but rather that the last survivor in each pen was a large chick.

TABLE VI.

SALT CONSUMED BY LOTS FED 15%, 22½%, AND 30% OF SALT IN RATION.

Pen 7					Pen 8			
			Salt Consumed				Salt Consumed	
Day	Per Cent Salt in Feed	Body Weight Lbs.	Lbs.	Per Cent of Body Weight	Per Cent Salt in Feed	Body Weight Lbs.	Lbs.	Per Cent of Body Weight
7	15%	.1323	.003000	2.268	15%	.1228	.002565	2.089
8	"	.1321	.002145	1.624	"	.1329	.002565	1.930
9	"	.1325	.002565	1.936	"	.1296	.003000	2.315
10	"	.1367	.001290	0.944	"	.1316	.002490	1.892
11	"	.1354	.002145	1.584	"	.1367	.000990	0.724
12	"	.1409	.002145	1.522	"	.1404	.000990	0.705
13	"	.1369	.003000	2.191	"	.1400	.002190	1.564
24	22.5%	.2798	.005130	1.833	30%	.2749	.009900	3.601
25	"	.2740	.009000	3.285	"	.2716	.010980	4.043
26	"	.2632	.003218	1.223	"	.2646	.004980	1.882
27	"	.2720	.003848	1.415	"	.2619	.004980	1.901
28	"	.2515	.002565	1.020	"	.2425	.006000	2.474
29	"	.2425	.004500	1.856	"	.2403	.001200	.499
30	"	.2381	.003600	1.542	"	.2381	.000000	.000
31	"	.2337	.003375	1.444	"	.2337	.003000	1.284
32	"	.2205	.006750	3.061	"	.2304	.006000	2.604
33	"	.2646	.000000	0.000	"	.2557	.012000	4.693

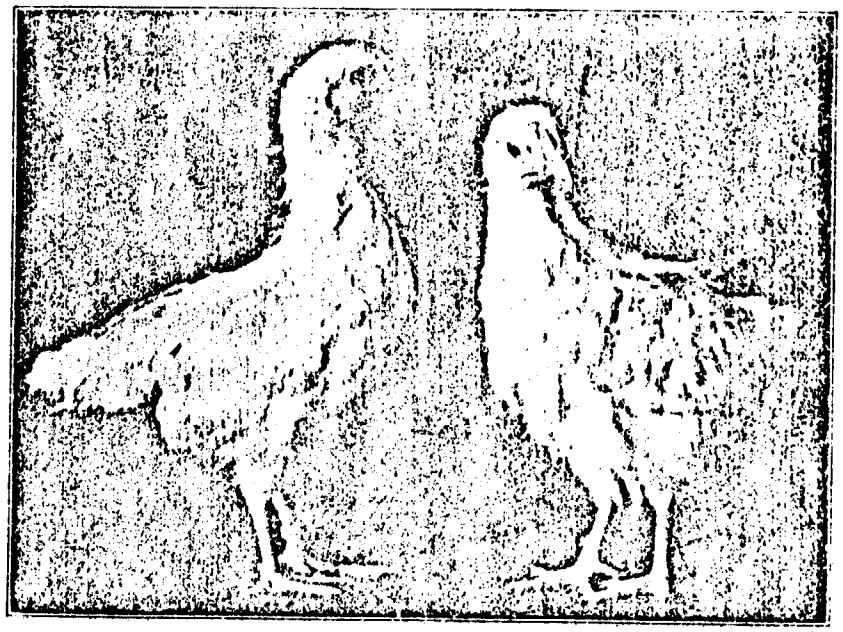

FIG. 12. *Bird on Left Lived Through 10 Days' Feeding on 22½% Salt in Ration and One on Right 10 Days on 30%. Photographed 9 Days After Return to 1% Salt Feed. Age When Heavy Salt Feeding Began, 3 Weeks.*

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the experiment, no appreciable permanent injury except loss of growing time was suffered by chicks surviving huge doses of salt in feed, running as high as 4.7% of body weight. In Fig. 13, these two survivors are shown as they appear at maturity. Both were females which may or may not indicate a difference in tol-

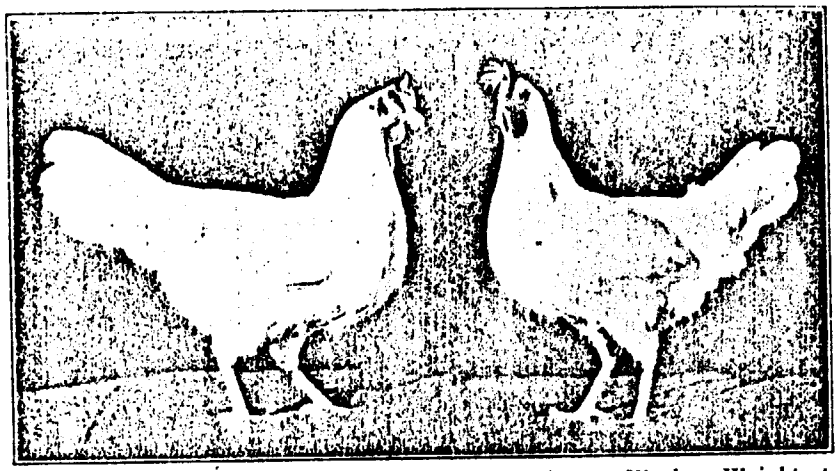

FIG. 13. *The Same Birds Shown in Fig. 12 at Age 29 Weeks. Weight at Time of Photograph, Left (22½%) 3.9 Lbs., Right (30%) 4.0 Lbs.*

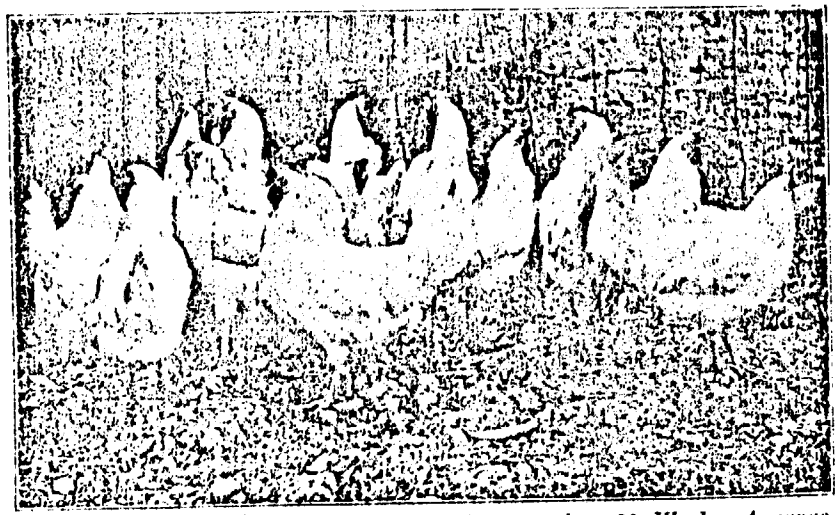

FIG. 14. *Surviving Pullets From All Lots at Age 29 Weeks, Average Weight, 3.32 Lbs.*

erance on the part of the sexes. In Fig. 14 the group of surviving pullets from the entire test is shown at 29 weeks of age.

LETHAL DOSE DETERMINATIONS

Another group of chicks, 90 in number, were utilized for individual dosing to determine the lethal amount of salt that could be administered. The chicks were kept quiet in the darkened incubator where they were hatched, for 48 hours, and were then given the same ration containing 1% of salt as used in the feeding tests. They were examined carefully for defects and vitality and the selected chicks were dosed at the fifth day. Various amounts of salt ranging from 1 to 10 grams per kilogram of body weight were given to the 90 chicks. Thirty-five chicks received their salt in a single dose, and in an attempt to simulate feeding conditions, 12 were administered their allotment in 2 equal doses, and in 43 cases the dose was divided into three equal parts. In this dosing for lethal determination no chicks were used which had previously been fed salt in excess of the 1% used in the basal ration. All chicks were in a "fed" condition when dosed. The weighed dose of dry salt was placed in the mouth of the chick by means of a small warmed cylinder. Access was given to feed and water following the dosing.

RESULTS OF LETHAL DOSE DETERMINATIONS

Results of tests with 35 S. C. W. Leghorn chicks to determine the minimum lethal single dose of salt or the minimum amount which will cause death in chicks indicate that 4 grams per kilogram of body weight, or 0.4 of 1% of body weight, which is the same as reported by Suffram, and Mitchell, Card, and Carman for adults, will produce death in from 5½ to 9 hours (Table VII).

TABLE VII.

RESULTS OF ADMINISTERING SALT TO BABY CHICKS IN SINGLE, INDIVIDUAL DOSE. AGE OF CHICKS, FIVE DAYS.

Amount of Salt Administered, Calculated in Grams Per Kilogram of Body Weight													
1 Gram Per Kilo.		2 Grams Per Kilo.		3 Grams Per Kilo.		4 Grams Per Kilo.		5 Grams Per Kilo.		7 Grams Per Kilo.		10 Grams Per Kilo.	
Wt. of Chicks, Grams	Mortality	Wt. of Chicks, Grams	Mortality	Wt. of Chicks, Grams	Mortality	Wt. of Chicks, Grams	Mortality	Wt. of Chicks, Grams	Mortality	Wt. of Chicks, Grams	Mortality	Wt. of Chicks, Grams	Mortality
27	.....	31	.....	28	.....	28	Died	32	.....	33	Died	28	Died
28	.....	32	.....	31	.....	30	Died	35	Died	35	Died	34	Died
30	.....	34	.....	34	.....	35	Died	36	Died	36	Died	35	Died
33	.....	35	.....	35	.....	37	Died	37	.....	36	Died	36	Died
38	.....	39	.....	38	.....	38	.....	37	Died	37	Died	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	37	.....	.....	.....

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Chicks given smaller doses seemed depressed, had no appetite for a few hours but displayed no other distress except a diarrhea.

The results of dosing 12 chicks where the dose was divided into two equal parts indicated that the minimum lethal dose is approximately 5 grams per kilogram of body weight, or 0.5% of body weight, while in the case of the triply divided dose administered to 43 chicks this figure was raised to 7 grams and 0.7% respectively. All portions in both the double and the triply divided doses were given at intervals of four hours. Food and water were available at all times. For summary of the data see Tables VIII and IX. It will be seen from an examination of Table IV that the chicks in the feeding experiment receiving 1%, 3%, and 5% of salt failed to consume, on the average, enough salt to bring them above the minimum lethal dose as calculated for divided doses. The fact that the chicks in pen 3 (5% salt)

TABLE VIII.

RESULTS OF ADMINISTERING SALT TO BABY CHICKS IN TWO EQUAL DOSES AT INTERVALS OF FOUR HOURS. AGE OF CHICKS, FIVE DAYS.

Amount of Salt Administered, Calculated in Grams Per Kilogram of Body Weight.							
4 Grams Per Kilo.		5 Grams Per Kilo.		7 Grams Per Kilo.		10 Grams Per Kilo.	
Wt of Chicks Grams	Mortality	Wt of Chicks Grams	Mortality	Wt of Chicks Grams	Mortality	Wt of Chicks Grams	Mortality
33	.....	37	.....	34	Died	37	Died
37	.....	38	Died	39	Died	40	Died
41	.....	43	.....	44	.....	44	Died

TABLE IX.

RESULT OF ADMINISTERING SALT TO BABY CHICKS IN THREE EQUAL DOSES AT INTERVALS OF FOUR HOURS. AGE OF CHICKS, FIVE DAYS.

Amount of Salt Administered, Calculated in Grams Per Kilogram of Body Weight.													
4 Grams Per Kilo.		5 Grams Per Kilo.		6 Grams Per Kilo.		7 Grams Per Kilo.		8 Grams Per Kilo.		9 Grams Per Kilo.		10 Grams Per Kilo.	
Wt. of Chicks Grams	Mortality	Wt. of Chicks Grams	Mortality	Wt. of Chicks Grams	Mortality	Wt. of Chicks Grams	Mortality	Wt. of Chicks Grams	Mortality	Wt. of Chicks Grams	Mortality	Wt. of Chicks Grams	Mortality
32	.....	35	.....	27	.....	31	Died	32	.....	32	Died	25	Died
33	.....	35	.....	31	.....	32	Died	34	Died	34	Died	34	Died
37	.....	38	.....	32	.....	34	.....	35	Died	34	Died	36	Died
38	.....	38	.....	32	.....	35	Died	37	Died	42	Died	38	Died
38	.....	40	.....	34	.....	36	.....	41	.....	42	.....	40	.....
				35	.....	37	.....						
				35	.....	38	.....						
				35	.....	40	.....						
				38	.....	40	.....						
				38	.....	46	Died						
				42	.....								

approached the lethal dose on several occasions without mortality would seem to indicate an ability to excrete salt until the lethal limit is reached.

General Discussion of Effect of Salt on Chicks

A study of the physiological effect of high levels of salt upon baby chicks was not within the scope of this experiment, although observations were made from time to time and autopsies performed on all chicks that died. In lots receiving above 3% salt in the general feeding tests, the first effect observed was a marked thirst which was quickly followed by a profuse, watery diarrhea. In pen 1 (1% salt) droppings were normal and in pen 2 (3% salt) droppings were only moderately moist. This increase in water consumption was noted and measured by Smith (9) in his investigations.

In the feeding tests, lots receiving a high proportion of salt, particularly pens 7 (15% and 22½% salt) and 8 (15% and 30% salt), practically ceased eating within three hours after the birds were changed to the salty feed. Consumption remained decidedly low until hunger apparently mastered the situation of obvious unpalatability and compelled further resumption of feeding. As early as 20 hours after being placed on the heavy salt ration, a puffy, bloated appearance was observed in chicks in pens 7 and 8, which was usually accompanied by difficult respiration. Bloat appeared first in the region of the crop, and gradually spread to other parts, including the head. This puffy appearance was also observed in pen 5 (10% salt) within 48 hours, and spread gradually to all pens from 2 to 6 inclusive within 6 days, see Figs. 15-16. There was but little difference between

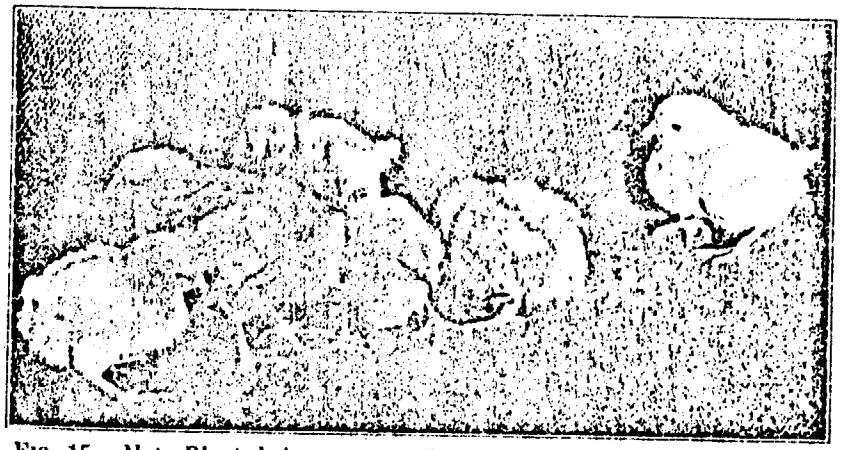

FIG. 15. *Note Bloated Appearance of Chicks From Pen 2 (3% Salt), at Age of 1 Week.*

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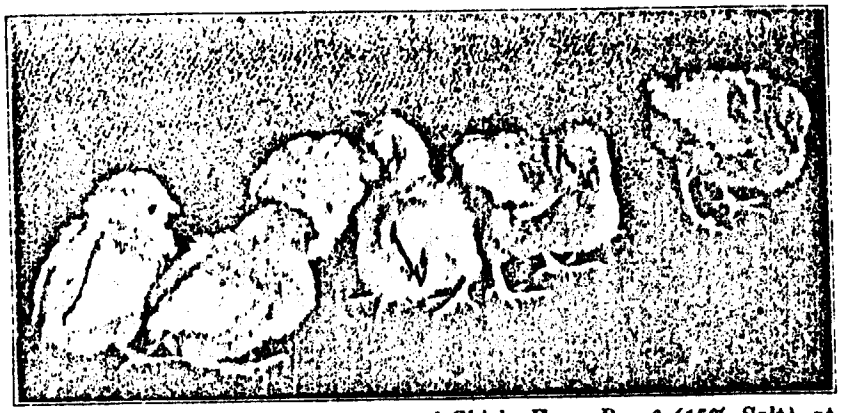

FIG. 16. *Note Bloated Appearance of Chicks From Pen 6 (15% Salt), at Age of 1 Week.*

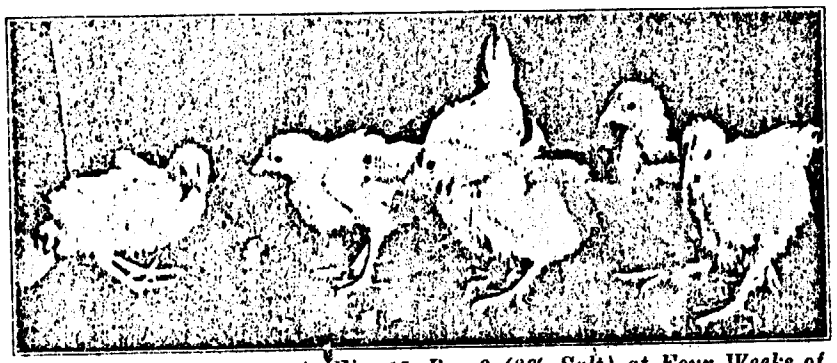

FIG. 17. *Same Group as in Fig. 15, Pen 2 (3% Salt) at Four Weeks of Age. Note Almost Complete Absence of Bloat.*

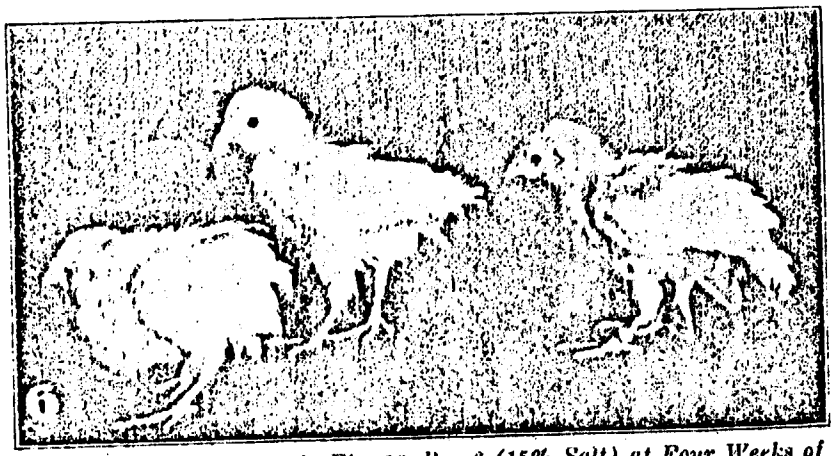

FIG. 18. *Same Group as in Fig. 16, Pen 6 (15% Salt) at Four Weeks of Age. Bloat Disappeared in Two Birds.*

pens in this regard, but the maximum of bloat seemed to be in pen 5 (10%), while pen 6 (15%) was not strikingly different from pens receiving 3%, 5%, and 8% respectively. The maximum of bloat appeared on the 8th, 9th and 10th days and became somewhat varied from that date to the fourth week, at which time most of those chicks which continued to show severe bloat had died. The survivors gradually assumed a more normal appearance in this regard up to the end of the experiment, Figs. 17 and 18. The birds of all groups continued to excrete large quantities of salt in their droppings throughout the balance of the test as indicated by the extremely watery condition of the droppings as collected in the galvanized pans under the floor of the brooding compartment. That salt was excreted in this watery discharge seems adequately proven by the fact that it readily crystalized from the droppings of pens 5 (10%) and 6 (15%).

AUTOPSY FINDINGS

Autopsy of the 37 individuals which died in the salt feeding experiment revealed in most cases, extreme congestion of the kidneys as shown by the darkened, enlarged condition of these organs; a slimy, mucoid, irritated condition of the intestine; a large hemorrhage usually at the base of the heart but occasionally enveloping it; a thick, viscous lining covering the part of the inner surface of the proventriculus near the entrance to the gizzard and sometimes extending over the entire inner surface of the proventriculus. The gizzard lining was inclined to be disjoined and to have a color ranging from pale lemon yellow to deep brown. It is not known whether the color of the gizzard was due to salt or to the feed. No material was found between the gizzard lining and the muscles of that organ. In most of the cases autopsied, effects of faulty mineral metabolism were observed such as imperfect and weakened bone formation at the proximal extremity of the femur. The mucosa of the crop did not seem visibly affected. In the various cases of puffiness previously noted, a straw-colored gelatinous exudate was found beneath the skin in the vicinity of the crop, legs, various other parts of the body, and occasionally in the neck and head.

The typical chick dying from a lethal dose of salt exhibited no changes in the crop, a large hemorrhage at the base of the heart, local congestion in the kidneys, a white slimy lining in the proventriculus, an easily detached gizzard lining, lungs normal to dark, and the intestine generally slimy but only moderately congested. Since death occurred in a relatively short time there was apparently no time for development of the gelatinous exudate as noted in birds fed the salt rations.

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CONCLUSIONS

1. We found it impossible under the conditions of the experiment to place enough salt in the all-mash ration of chicks to produce an appreciable amount of sudden mortality.

2. The unpalatability of feeds containing dangerous levels of salt, plus the tendency to consume large quantities of water when on a salty ration appear to act as a natural protection against overdose under normal conditions.

3. In this experiment mortality was not excessive until the level of salt in the ration (total ration) was greater than 5% by weight.

4. Baby chicks are able to endure salt levels as high as 30% of total ration for short periods of time.

5. It would seem that within the limits of this experiment, levels of salt as high as 30% of total ration can be fed for a period of several days without permanent injury except loss of growing time although it would seem reasonable to assume that large quantities of salt given under field conditions may heighten possible deleterious results from chilling, crowding or disease.

6. Baby chicks appear to vary considerably in their natural tolerance to salt, and in their ability to develop a resistance to it.

7. The unpalatability of high levels of salt in the feed remained more or less constant.

8. The minimum lethal single dose of dry salt, with chicks in a "fed" condition, was 4 grams per kilogram of body weight or 0.4% of body weight.

9. The minimum lethal dose when divided into two equal parts and given with an interval of 4 hours between doses, with chicks having access to feed and water, was 5 grams per kilogram, or 0.5% of body weight.

10. Under similar conditions, the minimum lethal dose when divided into 3 equal portions was 7 grams per kilogram, or 0.7% of body weight.

11. The typical appearance at autopsy of chicks dying from salt poisoning was a large hemorrhage at the base of the heart, severe bloody congestion in the kidneys, a white slimy lining in the proventriculus, an easily detached gizzard lining, and a more or less slimy appearance of the mucosa of the intestine, with occasional bloody congestion showing in the duodenum. In chronic cases a straw colored, gelatinous exudate is found beneath the skin in the region of the crop, and frequently in other portions of the body.

12. This experiment, while reversing certain popular conceptions as to salt tolerance of baby chicks, indicates no reason for changing present practices with respect to amount of salt in baby chick feeds. The present Maryland Experiment Station formula calls for one-half of one per cent of salt in the mash. Many formulae call for 1% of salt.

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* After Pearl, Surface and Curtis.

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Histological Examination of Tissue of Salt Fed Chickens*

In order to determine the effect of a high level of salt intake on immature chickens, with more exactness than is possible by the usual post-mortem examination, a series of microscopic preparations were made. It was hoped by this means to detect the histological changes, if any, which are brought about either within the tissues of the body or in the tissues lining the digestive and urinary tracts. Four chickens, each one month of age, were used in this experiment. They were placed, simultaneously, on the same basal ration which was used in the salt feeding experiments previously described in this publication, to which an amount of salt was added sufficient to bring the total of salt to 10 per cent of the feed. Tissues were taken from one chicken at the end of each of the following feeding periods: one week, two weeks, three weeks and five weeks.

In making the histological preparations the following tissues were taken as representing those in which pathological abnormalities might appear:

Crop	Lung
Proventriculus	Liver
Gizzard	Spleen
Intestine (just below gizzard)	Heart
Intestine (just below anus)	Spinal cord
Kidney	Skeletal muscle

Three complete sets of these tissues were made for each of the four chickens and fixed in Flemming's strong solution, Bouin's solution, and a 4 per cent solution of neutral formalin. The sections were stained on the slides with one of the following stains: Delafield's hematoxylin counterstained with alcoholic eosin, Mallory's triple stain, or Heidenhain's iron hematoxylin. The same general technique was used in all cases and each type of tissue was treated with the same fixer and stain.

A series of mounted sections of the tissues of a normally fed chicken was made for comparison with those of the salt fed chickens.

Results

The chicken which was killed after one week of feeding on the salt ration showed an enlargement of the crop probably due to an excessive water intake. Water also drooled from the beak at the slightest provocation. The autopsy revealed the blood vessels

* These additional studies were made subsequent to the completion of the experiment described in the fore part of this publication on a new set of older chickens with the idea of determining the microscopic changes that may occur as a result of heavy salt feeding.

of the crop slightly congested, and the intestines somewhat distended by water. Otherwise the internal organs appeared normal.

At the end of two weeks the three remaining chickens had become lean, and decidedly droopy. In the chicken killed at this time the autopsy findings were practically the same as in the chicken killed at the end of one week except that in the gizzard, a very small area of necrotic tissue was found.

At the end of three weeks the remaining two chickens had for the most part recovered and were normal in appearance and behavior, except for a condition in the legs, the normal articulation between the femur and tibia-tarsus having been lost. The left leg bent sharply laterally at this joint, while the right leg tended to curve inward.

One chicken was killed at this time (after three weeks feeding) and showed the heart and liver lobes considerably enlarged. The enlargement of the heart proved to be hyperplastic as the cells were found to be of normal size upon histological examination. A tendency to general congestion of the blood vessels prevailed and a necrotic area appeared again in the gizzard—in this case of larger dimensions than that found in the chicken killed at two weeks. The enlarged joints contained a cloudy fluid, in which a number of red blood corpuscles and a few epithelial cells were found.

The one remaining chicken was killed at the end of five weeks of salt feeding. The condition of the legs remained the same as described at three weeks but otherwise the chicken seemed normal. The autopsy showed no additional abnormal condition.

Histological Findings

In the first two chickens killed no pathological regions could be found in the sectioned tissues. They appeared perfectly normal, though some difficulty was experienced in staining the kidney tissue.

In the chicken killed at the end of three weeks the section of the gizzard taken through the afore-mentioned necrotic area showed a region in which the cells would not take the stain, and were necrotic in nature. In both this chicken, and the one killed at the end of five weeks, it was almost impossible to stain the sections of kidney tissue. The cells of the Malpighian corpuscles took the stain very ineffectively, and had the appearance of being poorly fixed. It is possible that this is due to the presence of a large amount of salt which in this case interfered with the normal action of the fixer. The optical differentiation was also poor. Another small area of a hemorrhagic nature was seen, through

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which red blood corpuscles had apparently oozed into the pelvis of the kidney. The remainder of the tissues showed nothing abnormal.

SUMMARY

The chickens became sick and lost weight in the early part of the experiment, but later recovered and seemed normal except for the condition found in the joints of the leg, which may or may not have been the result of an upset in metabolism due to the high percentage of salt in the diet.

From the study of the histological sections, no striking abnormalities or pathological conditions were observed. There was found, however, first, a small necrotic area in the gizzard of the chicken killed at the conclusion of three and five weeks of salt feeding, and second, difficulty was encountered in fixing the kidney tissue of all four chickens. It is possible that this latter condition was the result of a large amount of salt present in the kidney as the sections of the kidney tissue of the normal chicken stained clearly and without difficulty.

The autopsy, and histological preparations involved in the examination of these chickens were made by Miss E. Bray, Graduate Assistant in the department of Zoology under the directions of N. E. Phillips, Assistant Professor of Zoology. The chickens were furnished and the salt feeding was done by the Poultry Department.

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ASSOCIATION OF SWEAT SODIUM WITH ARTERIAL-BLOOD PRESSURE*

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MARACAIBO, VENEZUELA

THE salt concentration of the sweat has been observed to be low during acclimatization to heat,[1] in patients with primary hyperaldosteronism[1] and in normal persons given DCA or ACTH[2]; it is high in Addison's disease.[2] The relative concentrations of the sodium, potassium and chloride ions in the intracellular and extracellular spaces may affect the arteriolar tone.[3] There is evidence that renin and angiotensin have a direct effect on sodium exchange at the level of effector cells.[4]

An excessive secretion of aldosterone in the urine exists in malignant hypertension.[5] Direct measurements of aldosterone concentrations in plasma and urine present considerable technical difficulties owing to the small amounts of hormone present. Tests of isotope dilution using tagged radioactive metabolites of aldosterone are useful, but require material not generally available.

In view of the influence of adrenal function on the secretion of electrolytes in the sweat, and of the influence of electrolyte composition of body fluids on arteriolar tone and the existence of hypertension in primary hyperaldosteronism, it appeared worthwhile to investigate the possibility of a correlation between the blood pressure and the concentration of sodium and potassium in the sweat. The present study evaluates those variables in normal persons and in patients with hypertension of various etiologies.

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METHODS

A double-blind study of the arterial-blood pressure and sweat electrolyte concentration was made on 40 normal young adult volunteers, seventeen to thirty years of age, 149 to 180 cm. tall and between 41 and 70 kg. in weight. There were 34 women and 6 men in the group. The individual correlations between height and weight were normal. A similar double-blind study was done on 38 hypertensive patients (Tables 1-4).

The determinations of blood pressure were obtained by the Riva Rocci auscultatory method, using a standard arm cuff and mercury manometer. In each case 2 successive determinations were done by 2 different observers, the subject sitting at rest for several minutes. Each blood-pressure figure thus represents the average of 4 determinations; the value was then rounded off to the nearest 0.5 cm. of mercury. The sodium and potassium concentrations in the sweat were measured utilizing an Eppendorf Flamometer.

To determine the sodium concentration, a 1:200 dilution of a standard solution containing 143.5 milliequiv. per liter was used; 10 ml. of the dilution thus contained 7.17×10^{-3} milliequiv. of sodium

TABLE 1. *Secretion of Sodium and Potassium in the Sweat of Patients with Essential ("Primary") Hypertension.**

Case No.	Age	Sex	Blood Pressure at Time of Study	Grade of Retinopathy†	Sodium Secretion	Potassium Secretion	Treatment
	yr.				*milliequiv./liter*	*milliequiv./liter*	
5	50	F	140/85	Normal	34.16	5.27	Hydrochlorothiazide & reserpine
8	65	F	200/115	II	27.51	11.85	Trichlormethiazide & alpha-methyldopa
9	49	F	240/120	III	15.74	7.87	Trichlormethiazide & alpha-methyldopa
10	52	F	170/100	Normal	21.84	15.02	Trichlormethiazide & alpha-methyldopa
14	36	F	160/100	Normal	25.67	8.10	Reserpine
16	42	M	160/110	Normal	19.60	5.54	Hydrochlorothiazide & syrosingopine
17	39	M	160/110	Normal	31.44	4.43	Hydrochlorothiazide & reserpine
18	48	F	240/150	Normal	9.27	3.73	Trichlormethiazide & alpha-methyldopa
19	54	M	170/100	Normal	33.28	7.06	Trichlormethiazide & syrosingopine
20	47	M	200/110	Normal	23.23	7.52	Trichlormethiazide, syrosingopine & alpha-methyldopa
21	52	M	160/110	Normal	25.23	11.69	Trichlormethiazide & syrosingopine
24	43	F	180/110	Normal	22.41	10.72	Hydrochlorothiazide & syrosingopine
25	45	M	200/130	Normal	21.47	9.73	Trichlormethiazide, syrosingopine & alpha-methyldopa
28	45	F	210/120	II	19.32	5.68	Trichlormethiazide, reserpine & alpha-methyldopa
30	32	F	210/100	II	32.64	11.19	Trichlormethiazide & reserpine; thyroid replacement for myxedema
32	44	F	180/120	Normal	32.40	5.65	Hydrochlorothiazide & alpha-methyldopa
35	53	F	190/110	Normal	23.50	8.50	Hydrochlormethiazide & alpha-methyldopa
36	43	F	230/120	II	15.01	8.66	Trichlormethiazide, syrosingopine & alpha-methyldopa
37	47	F	160/110	Normal	24.30	9.57	Trichlormethiazide, syrosingopine & alpha-methyldopa
38	41	M	220/120	I	24.56	20.36	Trichlormethiazide, reserpine & alpha-methyldopa

*None of these patients had proteinuria; none had serum creatinine levels over 1.2 mg./100 ml.; pyelogram normal in all; none had evidence of coarctation of the aorta; determination of 24-hour excretion of vanillylmandelic acid, done on several, normal; all patients on salt-restricted diets; none in heart failure; most had radiologic evidence of cardiac enlargement, with electrocardiographic signs of hypertrophy of left ventricle; & in few cases in which checked, serum sodium ranged between 134 & 145 milliequiv., serum potassium between 3.1 & 4.5 milliequiv./liter; figures for arterial-blood pressure rounded off to nearest 0.5 cm. Hg.

†Keith–Wagener classification.

The Flamometer was adjusted so that this diluted standard solution read 143.5. The sweat sample was obtained from the skin of the anterior forearm after standard iontophoretic stimulation with a Buchler Sweat Inducer, a 0.1 per cent pilocarpine solution and a current of 2 milliamperes for five minutes being used. The sample was first weighed and then suspended in triple-distilled water to a volume of 10 ml. before the Flamometer reading was taken. In this way, if P represents the weight in grams of the sweat sample collected, and X represents the unknown sodium concentration reading of the Flamometer, the concentration of sodium in milliequivalents of sweat per liter is given by the formula

$$\frac{X}{2 \times P}$$

For the determination of the potassium concen-

TABLE 2. *Secretion of Sodium and Potassium in the Sweat of Patients with Renal Hypertension.**

Case No.	Age	Sex	Blood Pressure at Time of Study	Grade of Retinopathy†	Serum Creatinine	Findings on Renal Biopsy (Needle)	Urine Culture	Sodium Secretion	Potassium Secretion	Treatment
	yr.				*mg./100 ml.*			*milliequiv./liter*	*milliequiv./liter*	
11	57	M	170/110	III	4.4	Chronic glomerulonephritis	Negative	35.62	7.25	Hydralazine
12	35	F	170/120	III	18.3	Chronic glomerulonephritis	Not done	14.32	11.31	Alpha-methyldopa
22	22	M	200/120	II	13.0	Chronic glomerulonephritis	Not done	14.40	14.87	Trichlormethiazide & alpha-methyldopa
26	65	M	160/100	I	4.0	Not done	Positive	12.88	8.75	Hydralazine & alpha-methyldopa (pyelogram compatible with chronic bilateral pyelonephritis)
33	46	M	200/120	II	12.0	Chronic glomerulonephritis	Negative	27.94	13.19	Trichlormethiazide, alpha-methyldopa & digitalis (patient in heart failure)
34	23	F	170/110	II	12.0	Not done	Positive	18.42	11.18	Trichlormethiazide, hydralazine & tetracycline (pyelogram compatible with chronic bilateral pyelonephritis)

*All patients had documented attacks of acute glomerulonephritis, pyelonephritis or latent nephritis (persistent proteinuria) before onset of hypertension; diagnosis of chronic glomerulonephritis in Cases 11, 12, 22 & 33 & chronic pyelonephritis in cases 26 and 34; all on salt-restricted diet; figures for arterial-blood pressure rounded off to nearest 0.5 cm. Hg.

†Keith–Wagener classification.

TABLE 3. *Secretion of Sodium and Potassium in the Sweat of Patients with Advanced Essential or Renal Hypertension.**

Case No.	Age	Sex	Blood Pressure at Time of Study	Serum Creatinine	Urine Test for Protein	Urine Culture	Pyelogram	Sodium Secretion	Potassium Secretion	Treatment
	yr.			*mg./100 ml.*				*milliequiv./liter*	*milliequiv./liter*	
1	46	F	220/120	1.12	++	Positive	Compatible with pyelonephritis	14.76	6.88	Hydrochlorothiazide & alpha-methyldopa (patient diabetic)
15	37	F	150/100	1.0	+	Positive	Compatible with pyelonephritis	31.91	17.29	Trichlormethiazide, reserpine & digitalis (patient in heart failure)
23	51	M	160/100	1.0	++	Positive	Compatible with pyelonephritis	36.40	6.73	Hydrochlorothiazide & reserpine
27	50	F	200/120	1.1	Trace	Positive	Compatible with pyelonephritis	13.26	7.88	Trichlormethiazide & alpha-methyldopa (patient had mild diabetes)
29	50	M	190/120	1.2	Trace	Not done	Compatible with pyelonephritis	17.19	12.0	Trichlormethiazide, syrosingopine & alpha-methyldopa
31	44	M	200/140	1.1	Trace	Not done	Normal	22.50	8.30	Trichlormethiazide, syrosingopine & alpha-methyldopa (patient diabetic)

*Evidence did not warrant further discrimination; all patients on salt-restricted diet; figures for arterial-blood pressure rounded off to nearest 0.5 cm. Hg.

tration a 1:20 dilution of a standard solution containing 3.84 milliequiv. per liter was used; 10 ml. of this dilution contained 1.92×10^{-3} milliequiv. of potassium. The Flamometer was adjusted so that this diluted standard potassium solution read 3.84. The sweat sample was handled as for the sodium determination. In this way, if P again represents the weight in grams of the sweat sample, and X represents the unknown potassium concentration reading of the Flamometer, the concentration in milliequivalents of sweat per liter is given by the formula

$$\frac{X}{20 \times P}.$$

RESULTS

The sodium concentration in the sweat of the 40 normal subjects varied between 16.26 and 70.61 milliequiv., with an average of 40.63 milliequiv. per liter and a standard deviation of ±15.96. The systolic pressure ranged from 80 to 130 mm., with an average of 104 mm. of mercury ±10.11. The diastolic pressure varied between 40 and 90 mm., with an average of 62 mm. of mercury.

When the arterial systolic blood pressure of this normal group was compared with the concentration of sodium in the sweat, a correlation was found, with an r of −0.63. This is statistically significant (p less than 0.001). The regression line and the 95 per cent confidence limits are shown in Figure 1.

Similarly, there is a correlation between mean arterial blood pressure and the concentration of sodium in sweat. The r is −0.59, which is statistically significant (p less than 0.001). The regression line and 95 per cent confidence limits are shown in Figure 2.

No statistically significant correlation was found when the arterial-blood pressure was compared with the total sodium eliminated in the sweat by this normal group.

Among the hypertensive subjects the sodium concentration in the sweat ranged between 9.27 and 36.40 milliequiv. per liter and a standard deviation of ±7.54. The systolic arterial-blood pressure of this group varied between 140 and 240 mm., with an average of 187 mm. of mercury and a standard deviation of ±27.10. The diastolic blood pressure varied be-

TABLE 4. *Secretion of Sodium and Potassium in the Sweat of Patients with Renovascular Hypertension.**

Case No.	Age	Sex	Blood Pressure at Time of Study	Serum Creatinine	Urine Test for Protein	Renal Angiogram (Aortogram)	Sodium Secretion	Potassium Secretion	Treatment
	yr.			*mg./100 ml.*			*milliequiv./liter*	*milliequiv./liter*	
2	35	F	155/90	1.15	Trace	Occlusion of right renal artery	33.1	8.0	Reserpine
3	42	F	200/130	1.5	++	Stenosis of right renal artery	20.81	12.54	Hydrochlorothiazide, reserpine & guanethidine
4	50	F	160/85	1.2	++	Stenosis of left renal artery	34.78	9.23	Hydrochlorothiazide & alpha-methyldopa
6	44	F	200/145	1.0	Negative	Stenosis of right renal artery	28.31	45.5	Trichlormethiazide, syrosingopine & alpha-methyldopa
7	51	M	240/150	2.1	Trace	Occlusion of right renal artery	24.90	7.28	Guanethidine
13	5	M	200/120	1.0	Negative	Both renal arteries elongated, with lumens of corkscrew appearance	14.18	6.02	Reserpine (autopsy showed hyperplasia of zona glomerularis in both adrenal cortexes)

*Reduction of 50 per cent or more in diameter of lumen of renal artery demonstrated in all cases; all patients on salt-restricted diets; figures for arterial-blood pressure rounded off to nearest 0.5 cm. Hg.

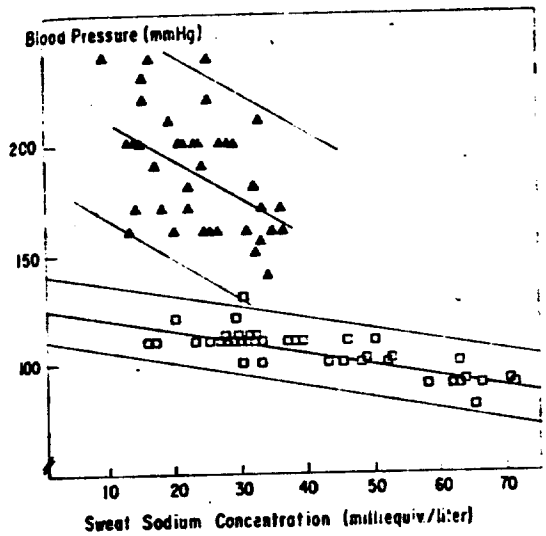

FIGURE 1. *Correlation between the Sweat Sodium Concentration and the Systolic Arterial-Blood Pressure in Normal (□) and Hypertensive (▲) subjects.*

Each set of parallel lines represents the regression line and the 95 per cent confidence limits.

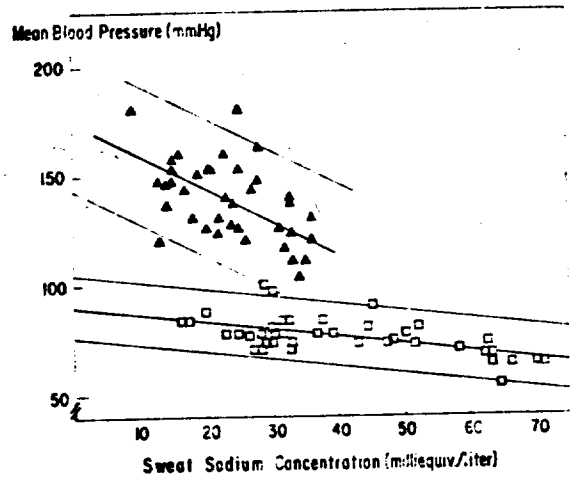

FIGURE 2. *Correlation between the Sweat Sodium Concentration and the Mean Arterial-Blood Pressure in Normal (□) and Hypertensive (▲) Subjects.*

The mean arterial blood pressure is taken as the diastolic pressure plus one third of the pulse pressure. Each set of parallel lines represents the regression line and the 95 per cent confidence limits.

tween 85 and 150 mm., with an average of 114.47 mm. of mercury. High diastolic blood pressures had been detected in all these patients before the time of our study; those who were receiving treatment are identified in Tables 1-4, with the type of therapy used.

When the concentrations of sodium in the sweat of the subjects comprising the hypertensive group were compared with their arterial systolic blood pressures, a correlation was found, with an r of −0.51. This is statistically significant (p less than 0.001). The regression line and the 95 per cent confidence limits for this group are also shown in Figure 1.

When the mean arterial-blood pressure of the hypertensive subjects are compared with the concentration of sodium in the sweat, the correlation has an r equal to −0.50, with p less than 0.001. The regression line and the 95 per cent confidence limits are shown in Figure 2.

When the sodium concentration in the sweat of the hypertensive patients is compared with that of the normal subjects by means of the Student "t" test, the difference is again significant (p less than 0.001). The sodium is less concentrated in the sweat of the hypertensive than in that of the normal subjects.

When the total sodium eliminated in the sweat by each of the two groups is compared by means of the "t" test, a significant difference is also found (p less than 0.001). The hypertensive patients excreted less sodium than the normal subjects.

No significant correlation was found between the arterial systolic or mean arterial-blood pressure and the potassium concentrations in the sweat of subjects of either group. However, a tendency to such a correlation was evident, as it was for the total sodium and potassium eliminated.

Lastly, there was no statistically significant correlation between diastolic blood pressure and the sodium or potassium in the sweat of either group; here, again, a tendency to a correlation appeared to exist.

DISCUSSION

The sweat electrolyte determinations performed on the group of normal persons were done for the purpose of obtaining base-line controls for comparison with hypertensive patients. Unexpectedly, they exhibited the existence of a significant inverse correlation between the concentrations of sodium in the sweat and the blood-pressure levels within the normal range.

A similar inverse correlation has been noted between adrenocortical activity and sweat sodium levels[1,2]; however, no correlation has been noted between aldosterone excretion in the urine and blood-pressure levels, except for malignant hypertension.[4] A secondary aldosteronism has been reported to accompany renovascular hypertension,[6] and evidence given for increased angiotensin-aldosterone activity in essential hypertension.[7] It is therefore impossible to state whether the decreased sodium concentration in the sweat of hypertensive patients reflects increased aldosterone action; the possibility remains that other factors are active. The inverse correlation between the sweat sodium concentration and the levels of arterial-blood pressure, even when the latter are in the normal range, sug-

gests that these variables are somehow intimately related; the factors that intervene in this interrelation have not yet been defined.

Among other studies it may be of interest to investigate simultaneously the effects of diuretics on blood-pressure levels and on sweat sodium concentrations and to re-evaluate the activities of aldosterone, angiotensin and renin in relation to blood pressure and sweat electrolytes, in tropical and temperate climates.

Summary and Conclusions

The data presented suggest a significant correlation between the arterial-blood pressure and the ability of the sweat glands to concentrate sodium, both in normal and in hypertensive subjects. There is less sodium in the sweat of patients with higher blood pressures.

With the small number of hypertensive patients in some of the etiologic groups of our study it is not possible to state whether there are differences in the handling of sweat electrolytes by the various groups.

We are indebted to Dr. A. Renato Acosta for permission to study the hypertensive patients from his Renal Service included in this report; the normal volunteers were students from the Maracaibo School of Nursing.

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Preservation and Packaging of Indian Foods

II. Storage Studies on Preserved Chapaties

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SUMMARY

Preserved chapaties packaged and heat-sealed in paper-foil-polyethylene pouches kept satisfactorily and remained acceptable for six months under diverse tropical conditions. During storage the fat acidity increased slightly, the reducing sugars increased, and nonreducing sugars decreased. Free sorbic acid was found to be a better preservative than its potassium salt.

INTRODUCTION

In an earlier communication, the authors (Kameswara Rao *et al.*, 1964) presented methods for the preservation of chapaties. The present paper presents packaging and storage studies on preserved chapaties.

Chapati making is a manual process, involving: a) making the dough; b) dividing into portions, or 'paras'; c) rolling each 'para' into a disc; d) baking the disc on a hot plate (first on one side and then on the other); and e) puffing the disc to give the chapati. As a first step in this study it was found necessary to standardize

Table 3. Results of analysis of stored chapaties in different flexible packs under different climatic conditions[a] (on 30% moisture basis).

Age	Packaging	Fat acidity[b] mg/KOH (%)	Extract soluble in cold water (%)	Reducing sugars (%)			Total sugars as sucrose			Sodium chloride content (%)
				AOAC method for wheat flour	AOAC method for bread	From extract soluble in cold water	AOAC method for wheat flour	AOAC method for bread	From extract soluble in cold water	
Fresh	.....	0.012	7.4	1.5	1.6	1.6	3.5	3.4	3.3	1.6
1 month	Polyethylene (500)	0.013	7.3	1.8	1.5	1.7	3.8	....	3.7	1.5
	Paper/foil/ polyethylene	0.0128	6.8	1.7	1.6	1.6	4.2	3.9	....	....
3 months	Polyethylene (500)	0.0130	7.1	1.9	2.0	1.8	....	....	3.4	1.45
	Paper/foil/ polyethylene	0.0125	7.4	2.2	....	2.2	3.8	3.5	3.8	....
4 months	Polyethylene (500)	0.0146	7.6	2.1	2.2	2.1	3.3	3.4	3.4	1.6
	Paper/foil/ polyethylene	0.0132	8.9	2.2	2.3	2.2	3.5	3.6	3.5	....
5 months	Polyethylene (500)	0.0156	7.8	2.5	....	2.3	3.7	..	3.7	....
	Paper/foil/ polyethylene	0.0135	8.3	2.6	2.6	2.5	3.9	3.6	3.9	....
6 months	Polyethylene (500)	0.018	8.2	2.5		....	3.6	....	....	1.7
	Paper/foil/ polyethylene	0.015	8.5	2.7	2.6	2.6	3.8	3.6	3.9	1.6

[a] The flexible packs were stored under different climatic conditions, e.g. hot-dry (16–39°C; 16–79% RH), hot-humid (16–35°C; 27–95% RH) and cold-dry (0–15°C; 27–54% RH). No significant differences were observed in the values between the different storage conditions. Average figures are reported.

[b] After allowing for the acidity contributed by the sorbic acid.

Table 4. Results of acceptability tests (appearance, taste and texture) on preserved chapaties (as compared with freshly made ones).

Age	Preserved chapaties plain	Preserved chapaties containing: Hydrogenated vegetable oil (6%)	Sorbitol liquid (5%)	Glycerine (5%)
3 months	Like fresh chapaties	Like fresh chapaties	Like fresh chapaties; slightly sweetish	Like fresh chapaties; slightly sweetish
4 months	Like fresh but slightly salty after-taste	Like fresh; good in taste	Like fresh; slightly sweetish	Like fresh; slightly sweetish in the end
5 months	Like fresh in appearance and texture; slightly bitter after-taste	Like fresh; good in taste	Like fresh; slightly sweetish	Like fresh; slightly sweetish
6 months	Almost like fresh chapaties in appearance and taste; texture slightly parchment-like, tends to harden soon	Like fresh; soft and good in taste; liked most	Almost like fresh; slightly peculiar after-taste	Almost like fresh in appearance and taste; texture slightly parchment-like

sorbic acid, calculated on the basis of sorbic acid content of the chapati, these results have to be interpreted with caution. Extract soluble in cold water and reducing sugars increased progressively, while nonreducing sugars decreased, indicating progressive hydrolysis of the starches and disaccharides. This is reflected in the slightly sweeter taste of the preserved chapaties. There is fairly close agreement in reducing and total sugars determined by the three different methods.

The preserved chapaties were found to be acceptable by soldiers for up to 6 months (Table 4). Those containing hydrogenated vegetable oil (6%) were liked most, followed in acceptability by those containing sorbitol (5%), glycerine (5%), and plain ones.

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the processing conditions of chapati making.

EXPERIMENTAL METHODS

Standardization of processing conditions. In the packaging and storage studies, large quantities of chapaties had to be made in different lots. For the chemical data to be comparable during the storage studies it was important to minimize inherent variations in values that may be caused by variations in process conditions. Toward this end the following conditions were standardized; only one type of wheat, purchased at one time, was used. It was ground into flour, and the rough bran removed. The water required for the dough making was kept at 625–650 ml per kilo of wheat flour. The weight of each para was kept constant at 45 g, and the diameter of the disc at 7 inches, which automatically fixed the thickness of each disc. The temperature of the hot plate was kept at 220–240°C, and total time was 2 min for baking and 1 min for puffing (which was also done on the hot plate).

Chapaties were made from 2 kg of wheat flour, each lot consisting of 72–73 chapaties. Six chapaties were packed in a flexible pouch while still hot. For storage studies the lots were distributed evenly among the different storage conditions. For analysis, one pack was opened and all 6 chapaties were made into small bits and thoroughly mixed to make a sample.

To determine the variation in the test results after standardizing the process conditions, a lot of chapaties (72) was made from 2 kg of wheat flour and packaged in 12 polyethylene pouches each containing 6 chapaties. Six pouches were drawn as samples, and each sample was uniformly mixed and analyzed for fat acidity, extract soluble in cold water, and sugar contents.

Optimum NaCl and sorbic acid; effect of potassium sorbate. To determine the optimum concentrations of sodium chloride and sorbic acid, chapaties were made from 2-kg lots of flour, with the concentration of sodium chloride varied between zero and 4% and that of sorbic acid between 0.48 and 0.2%, both calculated on the basis of flour weight. The chapaties were packed in polyethylene bags, six in each, and stored under ambient conditions (14–38°C and 24–95% RH).

Potassium sorbate is the recommended preservative in foodstuffs that contain a high percentage of water. It was therefore substituted for sorbic acid in equivalent quantity (0.64%, by flour weight), keeping the sodium chloride at 2% by flour weight.

Effect of packaging on moisture content. Chapaties which contain about 30–35% moisture and have a surface area of about 480 sq cm tend to lose moisture very fast under normal ambient conditions and become stiff since their ERH is about 92%. From the logistic viewpoint the chapati was required to be packaged in light-weight packs. So pouches made of polyethylene (500- and 700-gauge), laminate of paper-foil (vinyl resin coated), and paper-foil-polyethylene were used for retail packing (6 chapaties in each). For bulk packing, the initial packaging of 6 chapaties in polyethylene (500-gauge) pouches was followed by final packing of 10 such packs in waxed cartons sealed with adhesive tape. The packs were stored under three controlled-humidity conditions, and the moisture changes in chapaties so packed were determined.

Effect of storage on chemical constituents. Chapaties packaged in polyethylene and paper-foil-poly laminate pouches were stored at different places in the country representing different climatic zones, such as hot-dry (16–39°C and 16–79% RH), hot-humid (16–35°C and 27–95% RH), and cold-dry (10–15°C and 27–54% RH). Samples of the stored chapaties were tested periodically. Zeleny and Coleman (1938) reported that acidities in wheat flour due to acid phosphates and amino acids do not increase significantly during storage but that free fat acidity increases, thereby being an index of storage deterioration. Hence, only this acidity was determined. The other chemical tests done on the chapaties during storage included extract soluble in cold water, reducing and total sugars, and sorbic acid and sodium chloride contents. All results were expressed on 30%-moisture basis. The fat acidity and extract soluble in cold water were determined by the AOAC method (1960) for wheat flour; allowance was made for acidity due to sorbic acid. The reducing sugars and total sugars (as sucrose) were estimated by: a) the AOAC method for wheat flour (1960); b) the AOAC method for bread (1960), but following the Lane and Eynon general volumetric method given in AOAC for sugars and sugar products (1960) instead of the Munsen Walker method specified; and c) the Lane and Eynon general volumetric method given in AOAC for sugars and sugar products (1960), starting from the "extract soluble in cold water" and adopting the acid inversion specified in AOAC for bread (1960) for determining the total sugars as sucrose. Sorbic acid was estimated by the colorimetric method of Schmidt (1960).

Acceptability. Acceptability tests were conducted over the storage period of six months, with soldiers used as the subjects. With a view to improving taste and texture, humectants (sorbitol and glycerine) and hydrogenated vegetable oil were incorporated into some lots of the chapaties. The units to which the samples were sent for storage were asked to evaluate the different preserved chapaties (i.e. those with and without the above taste improvers) using troops to determine acceptability, by comparison with freshly made chapaties. They were advised to warm the preserved chapaties on their mess tins, smear a little hydrogenated vegetable oil (except on those ones already containing it), and then eat according to normal practice.

Microbiological status of preserved chapaties. The spoilage of chapaties is due to mold growth; bacterial spoilage can scarcely precede it, in view of the nature of the foodstuffs. Spoilage due to molds and fungi is readily visible to the naked eye. Nevertheless, mycological and bacteriological tests were carried out on the preserved chapaties periodically, using Czapek's agar and enriched nutrient agar media.

RESULTS AND DISCUSSION

Variations in chemical constituents were reduced considerably by standardizing the process conditions: the variation for fat acidity being between 0.016 and 0.018%, for extract soluble in cold water between 6.4 and 7.3%, for reducing sugars between 1.03 and 1.15%, and for total sugars between 2.08 and 2.36%, all the results having been calculated on 30%-moisture basis. This has facilitated proper interpretation of the results during storage.

The sorbic acid content in the stored chapaties was found to vary between 0.23 and 0.27% (average 0.25%), and the sorbic acid content in the dough was between 0.3 and 0.315%. Blanks carried out on chapaties without sorbic acid gave zero values.

Chapaties prepared with only sorbic acid incorporated into the dough at the 0.3% level did not keep satisfactorily, for mold growth occurred within a month in some of them. This established that sodium chloride, which was originally added only to improve the taste, is required for satisfactory preservation with 0.3% sorbic acid in the dough. Table 1 shows that the minimum concentration of sodium chloride required with 0.3% sorbic acid in the

Table 1. Optimum concentrations of sodium chloride and sorbic acid required for preservation and effect of potassium sorbate on preservation of chapaties (temperature 14–38°C; RH 25–95%).

Sample No.	Sodium chloride added, on: Flour basis (%)	Sodium chloride added, on: Dough basis (%)	Sorbic acid added, on: Flour basis (%)	Sorbic acid added, on: Dough basis (%)	Potassium sorbate added, on: Flour basis (%)	Potassium sorbate added, on: Dough basis (%)	Minimum number of days preserved [a]	Remarks
1	0	0	0.48	0.3	.....	.....	12	A few preserved for 30 days
2	0.65	0.4	0.48	0.3	.....	.....	20	A few preserved for 60 days
3	0.96	0.6	0.48	0.3	.....	.....	110	A few preserved for 180 days
4	1.6	1.0	0.48	0.3	.....	.....	over 180	.....
5	2.0	1.2	0.48	0.3	.....	.....	over 180	.....
6	2.0	1.2	0.4	0.24	.....	.....	40	Some preserved for 180 days
7	2.0	1.2	0.3	0.18	.....	.....	20	Some preserved for 180 days
8	2.5	1.5	0.3	0.18	.....	.....	180	.....
9	3.0	1.8	0.2	0.12	.....	.....	30	Few preserved for 180 days but slightly salty to taste
10	3.0	1.8	0.3	0.18	.....	.....	180	Very slightly salty to taste
11	3.0	1.8	0.4	0.24	.....	.....	180	Very slightly salty to taste
12	4.0	2.4	0.2	0.12	.....	.....	30	A few preserved for 180 days but salty to taste
13	4.0	2.4	0.3	0.18	.....	.....	180	Salty to taste
14	4.0	2.4	0.4	0.24	.....	.....	180	Salty to taste
15	2.0	1.2	.....	.....	0.64	0.4	60	.....

[a] Absence of mold growth.

dough (0.48% on the wheat flour) is 1%. But it was found that the chapaties taste better with 1.25% sodium chloride in the dough (or 2% on the wheat flour). Gooding and Melnick (1955) showed that the efficiency of sorbic acid is increased remarkably by the addition of salt and sugar. Besides its synergistic action, sodium chloride may have contributed to the preservation by its antibacterial action, suppressing initial bacterial attack that may be necessary to facilitate the subsequent fungal growth. It was therefore considered that the effective sorbic acid content could be reduced by increasing the sodium chloride content up to the limit of acceptability. Table 1 shows that sorbic acid could be reduced from 0.48% to 0.3% by increasing the sodium chloride from 2% to 2.5%, both on the flour basis, for preserving the chapaties for six months. This slight increase in sodium chloride is not detectable by taste.

Another factor facilitating the preservative action of sorbic acid is the slightly acid pH of the chapati. The pH of the chapaties remained fairly constant between 5.3 and 5.5 when freshly made with the preservative and during storage. It has been reported that the higher the acidity the larger the amount of undissociated acid and the greater the preservative effect. At pH 5.5, considerable amounts of undissociated acid are still available (Anon., 1958).

Potassium sorbate, though highly soluble in water, did not exert preservative action in a concentration of 0.4%, which is equivalent to 0.3% sorbic acid (Table 1). One explanation for this failure may be that sorbic acid, being volatile, mixes with the steam generated during baking and gets evenly distributed in the chapati, with a little escaping: the sorbic acid ultimately remaining in the chapati is about 0.25%, with practically no further loss during storage when 0.3% has been incorporated into the dough. Potassium sorbate, on the other hand, not being volatile, is partially decomposed at the surface in contact with the hot plate, its decomposition temperature (270°C) being very near that of the hot plate (220–240°C). These spots where the potassium sorbate is decomposed may well serve as foci for mold growth. Gooding and Melnick 1955) reported that in foods wrapped with sorbic-acid-treated material, the sorbic acid sublimes and diffuses into the food when exposed to about 300°C for 7 seconds. The inner sides of the flexible pouches, in which the chapaties were packaged while still hot were found to contain on the average 1.5 mg of sorbic acid per pouch of 800 sq cm; this was not the case with chapaties treated with potassium sorbate. It would thus appear that the sorbic acid has not only diffused into the chapati but also has formed a thin protective film, as it were, around it. The microbiological tests indicate that the chapaties were well preserved. There were no molds. Two thousand to four thousand colonies per g of gram-positive micrococci and occasional streptococci were observed and these may have been present in the starting flour or have come from stray contamination during handling at the time of packing.

Table 2 shows that the paper-foil-poly laminate pouch (heat sealed) afforded good protection against loss of moisture from the chapati over a period of six months even under ambient conditions of low humidity. It has also not imparted any off-flavors. This laminate was therefore finally chosen for packaging the preserved chapaties, since it also meets the logistic requirements. Polyethylene by itself did not afford enough protection, and the vinyl-resin-coated foil did not give strong seals.

On storage, chapaties showed a very slight increase in fat acidity, being less in the paper-foil-poly packs (Table 3). In view of the fact that slight variations can occur in the allowance made for the acidity contributed by

Table 2. Moisture changes in chapaties during six months of storage in different flexible packages (6 in each) under three controlled relative humidities (temperature 14–38°C).

Pack	I: Initial moisture content (%)	I: % moisture loss when stored under 60% RH	II: Initial moisture content (%)	II: % moisture loss when stored under 75% RH	III: Initial moisture content (%)	III: % moisture gain when stored under 97–100% RH
Polyethylene (500)	32.8	13.2	28.9	11.2	33.9	3.5
Polyethylene (700)	34.8	9.6	31.0	7.5	30.4	2.8
Paper foil (0.02), vinyl resin coated	32.8	2.5	33.2	2.0	34.3	1.5
Paper-foil (0.02) polyethylene	34.2	1.5	36.5	1.0	34.9	0.8
Bulk pack (6 in polyethylene pouch and 10 such pouches in waxed carton sealed with tape)	35.6	Negligible	31.8	0.3	33.2	0.3

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THE INSTITUTE OF EXPERIMENTAL PHARMACOLOGY OF THE REGIUS UNIVERSITY OF PISA.

Director: Prof. ITALO SIMON

By: Dr. Giorgio Ravasini

The minimum distant lethal dose of sodium fluoride, sodium bromide, sodium chloride, and sodium iodide.

The results of many studies carried out at this School show that the best method for determining the minimum lethal dose of drugs is that of determining the minimum distant lethal dose, this expression meaning the smallest dose which, when injected into the veins of the animal, kills the animal at a certain distance (in time) from the injection. This research ought always to be accompanied by histological examination of the organs, which allows the establishment, when possible, of the cause of death. (1)

In accordance with these concepts, I have made a comparative study of the toxicity of the halogenated salts of sodium, using the rabbit as the experimental animal.

I ought to state in advance that, in determining the minimum distant lethal dose of two of the drugs studied (i.e., fluoride and iodide), I have only determined more precisely the data already known by preceding studies made in this institute by BOLLECTINO [for fluoride (1)] and by me [for iodide(2)].

(*) (sic)
A study carried out in the institute of pharmacology of Padua, in the academic year 1931-32.

For this reason I will not report the records of the experiements, the results of which are summarized ~~weekly~~ briefly in the following Tables 1-4.

The minimum distant lethal dose is therefore:

For the NaF of eq. gr. . . . . 0.00250 per Kg.

For the NaI " " ". . . . . 0.0146 " "

For the NaBr " " ". . . . . 0.0390 " "

For the NaCl " " ". . . . . 0.0500 " "

* * *

The results of these experiements show us that NaF is the most toxic of all the four drugs studied, and not only is this true, but it is also the drug that is not subject to the general law that regulates the toxicity of the other drugs. In fact, NaCl is less toxic than NaBr, which in its turn is less toxic than NaI: i.e., their toxicity is in direct relationship with the atomic weights of their anion so that toxicity increases as the atomic weight increases. NaF instead of being, according to this law, less toxic than NaCl, is shown to be instead 20 times more toxic. It is perhaps necessary to seek the reason for this anomaly in the fact that NaF is a reagent of the calcium-ion that it removes from the tissues, forming a not very dissociated compound.

With regard to the cause of death for each individual drug in our experiment, we have been able to make the following observations:

Sodium Fluoride. In the rabbit that died after nine days with the minimum lethal dose (eq. gr. 0.00250 per Kg.), there was noticed a progressive decrease in liveliness, appetite, and body weight (from 1650 gr. that was the initial weight, it decreased to 1100 gr. on the day of death). Upon autopsy, there was noted only a congestion affecting the liver and the kidney, limited in this latter organ almost exclusively to the passage zone between the cortex and the medullary substance. At the histological examination of the organs, there were observed slight instances of degeneration affecting the hepatic and renal parenchymes. Therefore, in conclusion, the anatomical and histological changes observed are not such as to allow affirmation that death was due to the harmful effect of the drug upon a definite organ, for which reason it would appear that we ought to deduce that the harmful effect is to be sought in a damaging effect on the whole organism and more especially on the trophic nervous centers as a result of modifications of the saline balances (fixation of the calcium-ion through the working of the NaF).

SODIUM CHLORIDE. The symptomatology that is observed with lethal doses of this drug is of two types according to the dose administered. With doses of equivalent gr. 0.0515 and higher, on the basis of the symptomological and anatomo-pathological data, I believe that I am able to state that the cause of death ought to be sought in changes in the central nervous system; in fact, in all the rabbits that died with doses of eq. gr. 0.0515 to 0.0575, I have been able to observe, during the period of survival,

the phenomena of nervous excitement (tonic-clonic contractions, opisthotonos) which were followed by a very intense depressive stage, with flaccid paralysis of the muscles that led imperceptively to death in the space of 9-12 hours. The phenomena of nervous excitement were manifested also in the surviving rabbits, but the rabbits did not move from the stage of nervous excitement to a depressive stage but recovered and thereafter appeared normal. This statement of mine on the cause of death by changes in the nervous system is further supported by the fact that the symptomological data as well as the autopsy findings permitted me always to notice a noteworthy cerebral edema, accompanied by hyperemia and hemorrhaging of the meninge vessels.

There is a second type of death with doses of 0.0510-0.0500, with which the animals died after 7-9 days. In the animals that thus died, there was found no anatomo-pathological change worthy of note and sufficient to explain to us death with profound modifications of the parenchyme of the organs needed for the normal carrying on of life. The only noteworthy fact was the extreme state of emaciation of the animals. This emaciation was also noticed in the animals injected with doses lower than eq. gr. 0.0500. This emaciation persisted for about ten days, after which the animals regained in two weeks' time the weight lost. In this case, death could only be explained by profound changes of the trophic centers that would especially lead to a disorder in the mechanism of the saline balance [a decrease of calcium in the brain as stated by NOVI (5)].

SODIUM BROMIDE. In these experiments, the most interesting facts that could be observed regarding the symptomatology and the autopsy findings are in respect to the central nervous sytem. With regard to the symptomatology, in fact, in all the rabbits that died, there occurred immediately a very intense state of drowsiness that was prolonged, perhaps (the experiment with eq. gr. 0.0450) up to 29 hours, during which cardiac and respiratory activity slowly and progressively were attenuated, leading imperceptively to death. Symptoms of nervous excitement were always lacking. The sense of pain was always preserved for a long time. For confirmation of an effect of the drug on the central nervous sytem, we also have the anatomo- and histo-pathological findings that showed an intense cerebral edema with congestion and hemorrhaging of the meningeal vessels, while no changes adequate to explain death were found in the other organs. On the other hand, from the experiments by SCREMIN (1)on the effect of sodium bromide on breathing, it is known that death with high doses occurs by paralysis of the respiratory system in which arrest occurs before cardiac arrest.

SODIUM IODIDE. The symptomatology and histo-anatomo-pathological findings for the rabbits that died with doses equal to and higher than eq. gr. 0.0149 per Kg. were minutely described in the article that I published cited above, and I consider it superfluous to repeat them. The facts then observed lead us to conclude that with this drug as well, death

occurring relatively shortly after injection (15 hours at most) is due to a general toxic effect on the whole organism, which is especially manifested on the nervous centers.

With the minimum distant lethal dose, instead, (the rabbit being dead after 17 days with eq. gr. 0.0146 per Kg.), the mechanism of death ought probably to be sought in the same causes mentioned to explain death due to small doses of NaCl and which I mentioned above. In this rabbit as well, indeed, the only important fact observed is the extreme emaciation by which, from 1670 gr. that was the animal's weight at the beginning of the experiment, it decreased to 1100 gr. on the day of death.

CONCLUSIONS.

1°-The minimum distant lethal dose given endovenously of NaF is eq. gr. 0.00250 per kilogram of the rabbit, as weight; the corresponding dose of NaI is eq. gr. 0.0146 per kilogram; the minimum distant lethal dose of NaBr is eq. gr. 0.0390; the minimum distant lethal dose of NaCl is eq. gr. 0.0500 per Kg.

2°-The cause of death by this dose is always an effect on the central nervous system as a result of which the animal falls into a state of deep depression, eats inadequately, and suffers a progressive loss of weight up to the point of death, which occurs within a time of nine to seventeen days. The histological examination of the abdominal and thoracic organs does not in fact show anything that might explain the mechanism of death.

Table I

TABELLA I.

Tossicità del fluoruro di sodio Toxicity of sodium fluoride

Solution used

weight of rabbit in g

Peso coniglio g. per Kg.	Soluzione impiegata %	Soluzione impiegata cc. inietta (inject.)	Fluoruro di sodio somministrato (administered) in g.	in g/eq.	in g/eq. per Kg.	Esito Result
*) 1070	2,099	13,4	0,28142	0,00775	0,00724	Muore durante l'iniezione dies during injection
*) 1000	„	12,0	0,25194	0,00600	0,00660	„ pochi minuti dopo l'iniez. dies a few minutes after injection
*) 1060	„	16,8	0,17855	0,00425	0,00400	„ „ „ „
*) 1380	„	16,7	0,23178	0,00552	0,00400	„ „ „ „
*) 850	0,420	25,5	0,10749	0,00256	0,00301	„ dopo 2 giorni dies after 2 days
1650	„	41,2	0,17330	0,00412	0,00250	„ dopo 9 giorni after 9 days
1570	„	31,4	0,13184	0,00314	0,00200	Vive lives
*) 970	„	15,0	0,06298	0,00150	0,00133	„
*) 1120	„	9,6	0,04034	0,00097	0,00100	„

N.B. — Le esperienze segnate con asterisco sono ricavate dal lavoro di Bollettino (2).

The experiments marked with asterisk taken from research by Bollettino (2)

TABELLA II. Table II

Tossicità del cloruro di sodio Toxicity of sodium chloride

Solution used

weight of rabbit in g

Peso del coniglio g	Soluzione impiegata %	Soluzione impiegata cc. inietta (inject.)	Cloruro di sodio somministrato (administered) in g	in g/eq	in g/eq. per Kg.	Esito Result
1200	4,815	83	4,0333	0,0690	0,0575	Muore dopo 11 ore dies after 11 hours
1050	„	70	3,3610	0,0575	0,0550	„ „ 9 „ 9 „
1000	„	64	3,0980	0,0530	0,0530	„ „ 10 „ 10 „
1400	„	96	4,6554	0,0728	0,0520	„ „ 10 „ 10 „
1550	„	96	4,6616	0,0798	0,0515	„ „ 12 „ 12 „
1200	„	53	2,5773	0,0612	0,0510	„ „ 7 giorni 7 days
1500	„	90	4,3840	0,0750	0,0500	„ „ 9 giorni 9 days
1250	„	75	3,5803	0,0612	0,0490	Vive lives
1200	„	69	3,3669	0,0576	0,0480	„
1400	„	80	3,8871	0,0665	0,0475	„

Table III

TABELLA III.

Tossicità del bromuro di sodio Toxicity of sodium bromide

Solution used sodium bromide injected

weight of rabbit in gr

Peso del coniglio g	Soluzione impiegata		Bromuro di sodio iniettato			Esito Result
	%	cc. inlett. inject.	in g	in g/eq.	in g/eq. per Kg.	
1300	10,29	65	6,6893	0,0650	0,0500	dies after 6 hours Muore dopo 6 ore
1250	„	56	5,7888	0,0562	0,0450	„ „ 22 „ 22 hours
1100	„	44	5,5281	0,0440	0,0400	„ „ 10 „ 10 hours
950	„	37	3,8129	0,0370	0,0390	„ „ 14 „ 14 hours
1000	„	38	3,9621	0,0385	0,0385	Vive lives
1200	„	64	6,6310	0,0450	0,0375	„
1300	„	45	4,6825	0,0455	0,0350	„
1450	„	44	4,5767	0,0435	0,0300	„

TABELLA IV. Table IV

Tossicità dell' ioduro di sodio Toxicity of sodium iodide

Solution used sodium iodide administered

weight of rabbit in gr

Peso del coniglio g	Soluzione impiegata		Ioduro di sodio somministrato			Esito Result
	%	cc. inlett. inject.	in g	in g/eq.	in g/eq. per kg.	
*) 850	14,7	34,7	5,10	0,0240	0,0400	dies after 4 hours & 35 min. Muore dopo 4 ore e 35'
*) 850	„	25,7	3,79	0,0252	0,0296	„ „ 6 „ 45' 6 hours & 45 min.
*) 815	„	17,1	2,44	0,0162	0,0198	„ „ 8 „ 15' 8 hours & 15 min.
*) 920	„	16,0	2,37	0,0158	0,0171	„ „ 8 „ 25' 8 hours & 25 min.
*) 1550	„	23.6	3,48	0,0232	0,0149	„ „ 15 „ 15 hours
1670	„	25,0	3,67	0,0244	0,0146	„ „ 17 giorni 17 days
1900	„	27,1	3,99	0,0266	0,0140	Vive lives
*) 1120	„	15,2	2,24	0,0149	0,0133	„
*) 1200	„	14,2	2,10	0,0140	0,0119	„

The data marked with asterisk are taken from my preceding experiments (3)

N.B. — I dati segnati con asterisco sono ricavati dalle precedenti mie esperienze (3).

ISTITUTO DI FARMACOLOGIA SPERIMENTALE DELLA R. UNIVERSITÀ DI PISA
DIRETTORE: PROF. ITALO SIMON

Dott. GIORGIO RAVASINI

LA DOSE MINIMA LETALE LONTANA DEL FLUORURO, BROMURO, CLORURO E IODURO DI SODIO

Da molti lavori usciti da questa Scuola risulta che il miglior metodo per fissare la dose minima letale dei farmaci è quello di cercare la *dose minima letale lontana*, intendendo con questa espressione la dose più piccola che iniettata nelle vene dell'animale lo uccide ad una certa distanza dall'iniezione. Questa ricerca deve esser sempre accompagnata da un esame istologico degli organi, il quale permette di stabilire, quando è possibile, la causa della morte. (1)

Seguendo questi concetti io ho studiato comparativamente la tossicità dei sali alogenati del sodio, servendomi come animale di esperimento del coniglio.

Debbo premettere subito che nella determinazione della dose minima letale lontana di due dei farmaci studiati (del fluoruro, cioè, e dell'ioduro) non ho fatto altro che fissare con maggior precisione i dati già noti per precedenti ricerche fatte in questo Istituto da BOLLETTINO [per il fluoruro (1)] e da me [per l'ioduro (2)].

Per questo non riporto i protocolli delle esperienze, i risultati delle quali sono riassunti brevemente nelle seguenti Tabelle 1 - 4.

(*) Lavoro fatto nell'Istituto di Farmacologia di Padova, nell'anno accademico 1931-32.

TABELLA I.

Tossicità del fluoruro di sodio

Peso coniglio g. per Kg.	Soluzione impiegata		Fluoruro di sodio somministrato			Esito
	al %	cc. iniett.	in g.	in g eq.	in g eq. per Kg.	
*) 1070	2,099	13,1	0,28142	0,00775	0,00724	Muore durante l'iniezione
*) 1000	„	12,0	0,25194	0,00600	0,00600	„ pochi minuti dopo l'iniez.
*) 1060	„	16,8	0,17855	0,00425	0,00400	„ „ „ „
*) 1380	„	16,7	0,23178	0,00552	0,00400	„ „ „ „
*) 850	0,420	25,5	0,10749	0,00256	0,00301	„ dopo 2 giorni
1650	„	41,2	0,17330	0,00412	0,00250	„ dopo 9 giorni
1570	„	31,4	0,13184	0,00314	0,00200	Vive
*) 970	„	15,0	0,06298	0,00150	0,00133	„
*) 1120	„	9,6	0,04034	0,00097	0,00100	„

N.B. — Le esperienze segnate con asterisco sono ricavate dal lavoro di Bollettino (2).

TABELLA II.

Tossicità del cloruro di sodio

Peso del coniglio g	Soluzione impiegata		Cloruro di sodio somministrato			Esito
	al %	cc. iniett.	in g	in g eq.	in g eq. per Kg.	
1200	4,845	83	4,0333	0,0690	0,0575	Muore dopo 11 ore
1050	„	70	3,3640	0,0575	0,0550	„ „ 9 „
1000	„	64	3,0980	0,0530	0,0530	„ „ 10 „
1400	„	96	4,6554	0,0728	0,0520	„ „ 10 „
1550	„	96	4,6616	0,0798	0,0515	„ „ 12 „
1200	„	53	2,5773	0,0612	0,0510	„ „ 7 giorni
1500	„	90	4,3840	0,0750	0,0500	„ „ 9 giorni
1250	„	75	3,5803	0,0612	0,0490	Vive
1200	„	69	3,3669	0,0576	0,0480	„
1400	„	80	3,3871	0,0665	0,0475	„

TABELLA III.

Tossicità del bromuro di sodio

Peso del coniglio g	Soluzione impiegata		Bromuro di sodio iniettato			Esito
	al %	cc. iniett	in g	in g eq.	in g eq. per Kg.	
1300	10,29	65	6,6893	0,0650	0.0500	Muore dopo 6 ore
1250	„	56	5,7888	0,0562	0,0450	„ „ 22 „
1100	„	44	5,5281	0,0440	0,0400	„ „ 10 „
950	„	37	3,8129	0,0370	0,0390	„ „ 14 „
1000	„	38	3,9621	0,0385	0,0385	Vive
1200	„	64	6,6310	0,0450	0,0375	„
1300	„	45	4,6825	0,0455	0,0350	„
1450	„	44	4,5767	0,0435	0,0300	„

TABELLA IV.

Tossicità dell' ioduro di sodio

Peso del coniglio g	Soluzione impiegata		Ioduro di sodio somministrato			Esito
	al %	cc. iniett	in g	in g eq.	in g eq. per Kg.	
*) 850	14,7	34,7	5,10	0,0240	0,0400	Muore dopo 4 ore e 35'
*) 850	„	25,7	3,79	0,0252	0,0296	„ „ 6 „ 45'
*) 815	„	17,1	2,44	0,0162	0,0198	„ „ 8 „ 15'
*) 920	„	16,0	2,37	0,0158	0,0171	„ „ 8 „ 25'
*) 1550	„	23.6	3,48	0,0232	0,0149	„ „ 15 „
1670	„	25,0	3,67	0,0244	0,0146	„ „ 17 giorni
1900	„	27,1	3,99	0,0266	0,0140	Vive
*) 1120	„	15,2	2,24	0,0149	0,0133	„
*) 1200	„	14.2	2,10	0,0140	6,0119	„

N.B. — I dati segnati con asterisco sono ricavati dalle precedenti mie esperienze (3).

La dose minima letale lontana è perciò:

per l'NaF	di	gr.	eq.	. . . . .	0,00250	per	Kg.
» l'NaI	»	»	»	. . . . .	0,0146	»	»
» l'NaBr	»	»	»	. . . . .	0,0390	»	»
» l'NaCl	»	»	»	. . . . .	0,0500	»	»

* * *

I risultati di queste esperienze ci dimostrano che l'NaF è il più tossico di tutti i quattro farmaci studiati, non solo, ma che esso è quello che si stacca dalla legge generale che regola la tossicità degli altri farmaci. Infatti l'NaCl è meno tossico dell'NaBr, che a sua volta è meno tossico dell'NaI: la loro tossicità cioè è in rapporto diretto dei pesi atomici del loro anione in quanto col crescere del peso atomico cresce la tossicità. L'NaF invece di essere, secondo questa legge, meno tossico dell'NaCl, si dimostra, invece, 20 volte più tossico. La ragione di questa anomalia bisogna forse cercarla nel fatto che l'NaF è un reattivo del calcio-ione, che sottrae ai tessuti formando un composto poco dissociato.

Per quanto riguarda la causa della morte per ogni singolo farmaco nelle nostre esperienze si sono potute fare le seguenti osservazioni:

Fluoruro di sodio. Nel coniglio venuto a morte dopo 9 giorni con la d. m. l. (g. eq. 0,00250 per Kg.) si constatò una progressiva diminuzione della vivacità, dell'appetito e del peso corporeo (da 1650 g. che era il peso iniziale, si arrivò a 1100 g. il giorno della morte). All'autopsia si notò soltanto una congestione a carico del fegato e del rene, limitata in quest'ultimo organo quasi esclusivamente alla zona di passaggio fra sostanze corticale e midollare. All'esame istologico degli organi si rilevarono leggeri fatti degenerativi a carico dei parenchimi epatico e renale. In conclusione dunque le alterazioni anatomiche ed istologiche osservate non sono tali da permettere di affermare che la morte sia stata dovuta all'azione nociva del farmaco su di un determinato organo, per cui parrebbe di dover dedurre che essa sia da ricercarsi in un'azione dannosa su tutto l'organismo e più specialmente sui centri nervosi trofici in seguito a modificazioni degli equilibri salini (fissazione del calcio-ione per opera dell'NaF).

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Cloruro di sodio. La sintomatologia che si osserva con dosi letali di questo farmaco è di due tipi, a seconda della dose somministrata. Con dosi di gr. equivalenti 0,0515 e superiori, in base ai dati sintomatologici e anatomo-patologici, credo di poter affermare che la causa della morte debba ricercarsi in alterazioni del sistema nervoso centrale; infatti in tutti i conigli morti con dosi da gr. eq. 0,0515 a 0,0575 ho potuto osservare durante il periodo di sopravvivenza dei fenomeni di eccitamento nervoso (contrazioni tonico-cloniche, opistotono) ai quali succedeva uno stadio depressivo molto intenso, con paralisi flaccida dei muscoli che portava insensibilmente alla morte nello spazio di 9 - 12 ore. I fenomeni di eccitamento nervoso furono manifesti anche nei conigli sopravvissuti, soltanto che dallo stadio di eccitamento nervoso i conigli non entravano in uno stadio depressivo, ma si rimettevano ed apparivano in seguito normali. Questa mia affermazione sulla causa della morte per alterazioni del sistema nervoso è suffragata oltre che dai dati sintomatologici, anche dai reperti di autopsia che mi permisero di constatare sempre un edema cerebrale notevole, accompagnato da iperemia ed emorragia dei vasi meningei.

Un secondo tipo di morte si ha con le dosi 0,0510 - 0,0500 con le quali gli animali morivano dopo 7 - 9 giorni. Negli animali venuti così a morte non si riscontrò alcuna alterazione anatomo-patologica degna di nota e sufficiente a spiegarci la morte con profonde alterazioni del parenchima di organi necessari al normale svolgersi della vita. Solo fatto rimarchevole fu il forte stato di dimagramento in cui si trovavano gli animali. Dimagramento che fu constatato anche negli animali cui si iniettarono dosi inferiori a gr. eq. 0,0500. Tale dimagramento persistette per una diecina di giorni, dopo di che gli animali riacquistavano nello spazio di circa 2 settimane il peso perduto. In questo caso la morte potrebbe solo spiegarsi con profonde alterazioni dei centri trofici che porterebbero specialmente ad un disordine nel meccanismo del ricambio salino [diminuzione di calcio nel cervello come afferma il Novi (5)].

Bromuro di sodio. In queste esperienze i fatti più interessanti che si sono potuti osservare nei riguardi della sintomatologia e dei reperti d'autopsia sono a carico del sistema nervoso centrale. Quanto alla sintomatologia infatti, in tutti i conigli venuti a morte

si stabilì immediatamente uno stato di sopore molto intenso che si prolungò, talvolta (esperienza con gr. eq. 0,0150), fino a 29 ore, durante il quale le attività cardiaca e respiratoria venivano lentamente e progressivamente attenuandosi portando alla morte, insensibilmente. Sono sempre mancati fatti di eccitamento nervoso. La sensibilità dolorifica si conservò sempre per molto tempo. Per confermare un'azione del farmaco sul sistema nervoso centrale stanno anche i reperti anatomo - e isto-patologici che misero in evidenza un edema cerebrale intenso con congestione ed emorragia dei vasi meningei, mentre che negli altri organi non si riscontrarono alterazioni sufficienti a spiegare la morte. D'altra parte dalle esperienze di SCREMIN (1) sull'azione del bromuro di sodio sul respiro si sa che la morte con dosi alte avviene per paralisi del centro del respiro, che si arresta prima del cuore.

Ioduro di sodio. La sintomatologia ed i reperti isto-anatomo-patologici dei conigli venuti a morte con dosi uguali e superiori a g. eq. 0,0149 per Kg. è stata minutamente descritta nel mio lavoro citato precedentemente e credo superfluo ripeterli. I fatti allora osservati ci portarono a concludere che anche con questo farmaco la morte, che interviene a distanza relativamente breve dall'iniezione (15 ore al massimo), è dovuta ad un'azione tossica generale su tutto l'organismo, la quale si fa risentire specialmente sui centri nervosi.

Con la dose minima letale lontana, invece, (coniglio morto dopo 17 giorni con g. eq. 0,0146 per Kg.) il meccanismo della morte si deve ricercare probabilmente nelle stesse cause invocate per spiegare la morte dovuta alle dosi piccole di NaCl e alle quali ho accennato più sopra. Anche in questo coniglio infatti l'unico fatto importante osservato è il forte dimagramento per cui da 1670 gr. che era il peso dell'animale al momento dell'esperienza si arrivò a 1100 gr. il giorno della morte.

CONCLUSIONI.

1° - La dose minima letale lontana per via endovenosa di NaF è di g. eq. 0,00250 per Kg. di coniglio; la dose corrispondente di NaJ è di g. eq. 0,0146 per Kg.; quella di NaBr di g. eq. 0,0390; quella di NaCl, infine, di g. eq. 0,0509 per Kg.

2° - La causa della morte per questa dose è sempre un'azione sul sistema nervoso centrale in conseguenza della quale l'animale cade in un stato di grave depressione, si nutrisce insufficientemente e soffre una progressiva perdita di peso fino alla morte, che avviene in un tempo che va da 9 a 17 giorni. L'esame istologico degli organi addominali e toracici nulla rivela infatti che possa spiegarci il meccanismo della morte.

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RIASSUNTO

Le dosi minime letali lontane per via endovenosa trovate nel coniglio sono le seguenti: fluoruro di sodio eq. gr. 0,0025 per Kgr.; joduro di sodio eq. gr. 0,0146 per Kgr.; bromuro di sodio eq. gr. 0,0390 per Kgr.; cloruro di sodio eq. gr. 0,05 per Kgr. La causa della morte si deve cercare in lesioni del sistema nervoso centrale.

RÉSUMÉ

Les doses minimes mortelles à distance par injections intraveineuses dans le lapin ce sont NaFl g. eq. 0,0025 par Kgr.; NaJ g. eq. 0,0146 par Kgr.; Na Br. g. eq. 0,0390 par Kgr.; NaCl. g. eq. 0,05 par Kgr. La cause de la mort être recherchés dans les alterations du systhéme nerveux central.

ZUSAMMENFASSUNG

Die kleinsten minimal langwirkenden tödliche Dosis durch Veneneinspritzung im Kaninchen sind: Fluornatrium gr. eq. 0,0025 pro kilo; Jodnatrium gr. eq. 0,0146 pro kilo; Bromnatrium gr. eq. 0,0390 pro kilo; Chlornatrium gr. eq. 0,05 pro kilo. Der Tod ist bedingt durch Beschädigungen des Centralnervensystems.

SUMMARY

The minimal lethal doses in the rabbits for intravenous injections are: fluoride sodium eq. gr. 0,0025 for Kilogram; iodide sodium eq. gr. 0,0146 for Kilogram; bromide sodium eq. gr. 0,0390 for Kilogram; chloride sodium eq. gr. 0,05 for Kilogram; Dead is due to alterations of the central nerveus system.

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Maximum Sodium Chloride Intake and Thirst in Domesticated and Wild Norway Rats[1]

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THE FOLLOWING STUDIES are concerned with the questions of a) how much salt can be put in a rat's food before it stops eating; b) how much salt can a rat ingest before toxic symptoms set in; and c) the extent to which salt toxicity depends on the amounts of water that a rat drinks in relation to the salt content of its diet.

These studies were originally undertaken because of observations made during an investigation of the effects of domestication on the Norway rat (1). In the course of that investigation comparisons between recently trapped wild Norway rats and domesticated Norway rats from our colony showed: a) that the adrenals are definitely smaller and less active in the domesticated rat; b) that 2 or 4% salt added to the diet resulted in a greater increase of water intake in wild than in domesticated rats; and c) that about 30% of wild rats trapped from the streets have an abnormally high water intake in relation to body weight. These observations indicated that wild and domesticated rats may differ in their salt and water metabolism, perhaps as a direct or indirect consequence of differences in the size and activity of their adrenals.

Further experiments have now been undertaken to determine just what differences in salt and water metabolism actually exist between these two strains. The results, which are presented in this report, show that marked differences do exist between the two, but more importantly, they indicate principles that may be involved in the ingestion and metabolism of very large amounts of salt and water by animals in general.

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[1] This work was carried out under a contract between the Office of Naval Research and the Johns Hopkins University.

[2] Henry Strong Denison Scholar, 1951–1952.

METHOD

The 36 adult domesticated Norway rats (27 males, 9 females) used for these experiments came from our colony, which was started 30 years ago with albino rats from the Wistar Institute. A few piebald and hooded rats from the colony of Dr. E. V. McCollum were added 28 years ago. Since then no new strains have been added. Their weights ranged from 155–366 gm. The 36 adult wild Norway rats (18 males, 18 females) were all recently trapped in the radius of six residental blocks in Baltimore. The traps have been described elsewhere (2). Their weights ranged from 178–363 gm. Only healthy appearing rats were used in these experiments.

The domesticated rats were kept in individual regular stock metal cages, 8 x 12 x 10 inches, each equipped with a screen bottom, a nonspillable food cup, and 1–3 inverted graduated 100-cc water bottles. The wild rats were kept in specially designed metal cages, also equipped with a screen bottom, a removable tray with a nonspillable food-cup, one or more inverted graduated 100-cc water bottles, and a sliding door through which the rats could safely be transferred to the weighing cage or to the device used for holding wild rats (3). The food-cup and guard fit into a recess built into the corner of the tray. A small hole in the screen floor over this cup provides access to the food. The tray can be slipped out for cleaning or for removing the food-cup, without giving the rat an opportunity to escape.

All food-cups were removed, weighed, and refilled daily between 9 and 11 A.M. At the same time the level of fluid in the water bottles was read and then each was refilled. The bottles were removed and cleaned every other day. All rats were weighed weekly.

External conditions were maintained as nearly constant as possible. The room housing the rats was kept at a temperature near 75°F, and it was illuminated with electric lights during the day and was kept dark at night. Daily procedures such as cleaning and record-taking, with their constant noises, were carried out as uniformly as possible.

The domesticated rats received our stock diet continuously from the time of weaning to 15–30 days before the start of the experimental diets, at which time they were given a low-salt diet. The stock diet contained graham flour 72.5%, skim milk powder 10.0%, casein 10.0%, butter 5.0%, calcium carbonate 1.5% and sodium chloride 1.0%; the same ingredients were used for the low-salt diet except for the omission of the salt and the use of saltless butter in place of the regular salted butter. In most instances the wild rats were

started on the low-salt diet within 1–2 days after capture, but were not changed to the several experimental diets until their water intakes had reached fairly constant levels, which usually required 30–50 days.

Twelve groups of rats (3 per group) of each strain received the low-salt diet plus 0, 2, 4, 6, 8, 10, 15, 20, 25, 35, 50 and 70%, respectively, of added salt (as percentage of diet). Unless they died earlier, or were killed in a moribund condition, all of the animals were kept under observation for 72–92 days. At the termination of this experimental period the rats were sacrificed by crushing the cervical cord; at autopsy the adrenals, thymus, pituitary, spleen and kidneys were weighed and preserved for histological and histochemical studies. The results of studies of adrenals from these animals will be reported in detail in another paper.

RESULTS

Survival Times. Table 1 summarizes the results. It shows that on concentrations of salt from 0–25% inclusive, all domesticated rats were alive and in good health at the end of the 89–92 day period, with the exception of one rat on 4% salt diet that was killed at 24 days because of hematuria. On these concentrations all of the wild rats with the exception of two, also were alive and in good health at the end of the 72–92 day experimental period. On the 35% concentrations, two domesticated and one wild rat died; the other three were killed after 41–42 days in a moribund condition. All 12 rats on the 50 and 70% concentrations died early. It is noteworthy, however, that a domesticated rat survived 17 days on the 70% concentration.

Acceptance of Salt Diet and Food Intake. Almost all rats started at once to eat the salt diets, at least in small amounts; some ate them as freely as the low-salt diet. With the exception of the rats on the 50 and 70% salt diets, all the others gradually increased their intake until, after 5–15 days, they were eating as much or more than they had previously eaten of the low-salt diets. Figure 1 gives typical food intake records for rats on each of the 11 concentrations of salt for the last 10 days on the low-salt diet and the first 10 days on the high-salt diets. The rats on the 2 and 4% concentrations ate these salt diets as freely as the low-salt diet; the rat on the 6% concentration reduced its intake for only 1 day; the rat on the 8% concentration ate almost normal amounts at once; the rat on the 10% concentration reduced its intake for 3 days and then ate even larger amounts than before; and the rats on concentrations from 15 to 50% showed that with increasing salt concentrations there is a progressively greater reduction in food intake on the first day and a progressively longer time interval before return to the intake levels on the low-salt diet. Of special interest however is the fact that the rat on the 70% diet ate 5 gm on the first day and continued to eat much the same amounts throughout the first 10-day period.

There were no definite differences between wild and domesticated rats with respect to their acceptance of the high salt diets during the first 10-day period.

Figure 2 summarizes the results for the entire experimental period. The ordinates show average daily food intake in gm/kg body weight for the three rats on each of the salt diets, and for a control group on the low-salt diet; abscissas give time in days. The intake averages are given for the last 10 days on the low-salt diet and for the subsequent 70–90 day experimental period in 10 day intervals. Records are shown for domesticated (*A*) and wild (*B*) rats. These curves show that with the exception of the rats on the 35, 50 and 70% concentrations, both wild and domesticated rats continued to eat the high-salt diets in large amounts throughout the 70–90 days of the experiment; and that there were no differences in intake between the wild and domesticated rats.

The curves show that in almost all instances

TABLE 1. LENGTH OF OBSERVATION PERIODS IN DAYS

Conc. of Salt in Diet, %	Domesticated Norways	Wild Norways
0	91, 91, 91	88, 88, 92
2	91, 91, 91	72, 72, 72
4	92, 92, 24†	75, 75, 62*
6	92, 92, 92	72, 72, 72
8	92, 92, 92	75, 75, 75
10	91, 91, 91	72, 72, 72
15	89, 89, 89	90, 90, 89*
20	89, 89, 89	90, 90, 90
25	89, 89, 89	90, 90, 90
35	41†, 41*, 41*	18,* 42,† 42†
50	13,* 15,* 15*	6,* 14,* 14*
70	13,* 15,* 17*	9,* 9,* 11*

* Died. † Killed in moribund state.

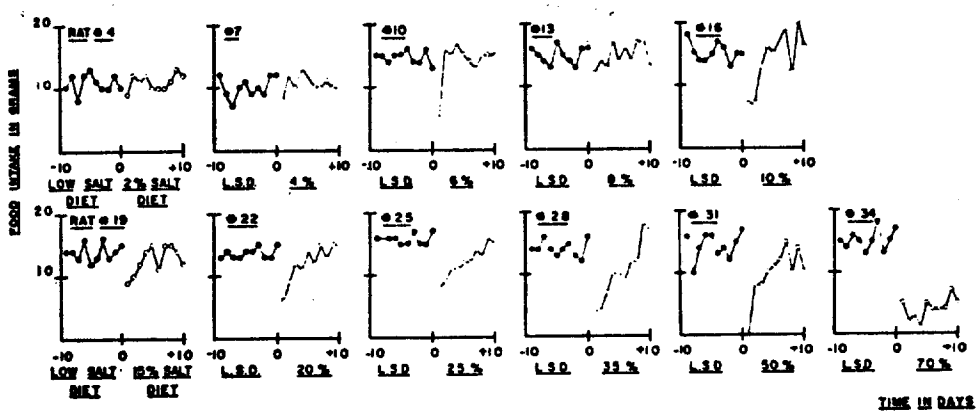

FIG. 1. Daily food intake for individual domesticated rats, one for each of the diets with the various concentrations of salt for the last 10 days on the low-salt diets, and for the first 10 days on the salt diet. Ordinates show food intake in grams; abscissas time in days.

during the second 10-day interval, food-intake increased well above its level on the low-salt diet and that in the succeeding 10-day periods it tended to decrease at a slow rate. They show further that with only two exceptions the salt diets were eaten in larger amounts (as much as 25% more) than the control low-salt diet.

A few wild rats on salt diets of 15% and above learned to spill their food. This was very unusual since we have found through many years of experience with our food-cups that none of the domesticated rats succeeded in spilling food. These wild rats may not have been adjusted to the high-salt diets as well as the other rats and so continued to search for plain unsalted food. Their records have been omitted from the averages in figure 2.

Body Weight. Figure 3*A* shows the average body weight curves for the 3 domesticated rats on each concentration of salt for the last 10 days on the low-salt diet and for 7–9 subsequent 10-day periods on the experimental diets. On all concentrations up to and including 25%, the domesticated rats either maintained their weights during the experimental period, or gained at about the same rate as the controls. On the three highest concentrations all of the rats rapidly lost weight.

Figure 3*B* shows the body weight records for the wild rats. The records are quite similar to those of the domesticated rats except that during the last two 10-day periods the rats on the 4, 10 and 15% concentrations showed small losses.

Sodium Chloride Intake. Figure 4 shows the average daily amounts of salt in gm/kg body weight that the domesticated (*A*) and wild (*B*) rats on the various salt diets ingested with their food. For the domesticated rats the salt intake varied roughly in proportion to the percentage of salt in the diet on all concentrations up to and including 35%; on the 50–70% concentrations the salt intake in gm/kg body weight decreased promptly. Furthermore, in the lower concentrations the intake of sodium chloride at each concentration remained extraordinarily constant throughout the experimental period. The intake levels ranged from slightly over 1 gm/kg body weight at the 2% level to 23 gm/kg body weight on the 35% concentrations.

The wild rats had essentially similar records except for the smaller amounts ingested by the wild rats on the 20 and 35% concentrations during the first 10-day period.

Thirst. Figures 5*A* and 5*B* summarize the observations on the domesticated and wild rats respectively. The ordinates show the average daily water intake for the three rats in each experimental group in cc/m² body surface. Earlier experiments had shown that water intake is a function of body surface, averaging 800 cc/m² for the rats from this colony on our stock diet containing 1% added salt (4). The intakes for the 12 groups of domesticated rats for the last 10-day period

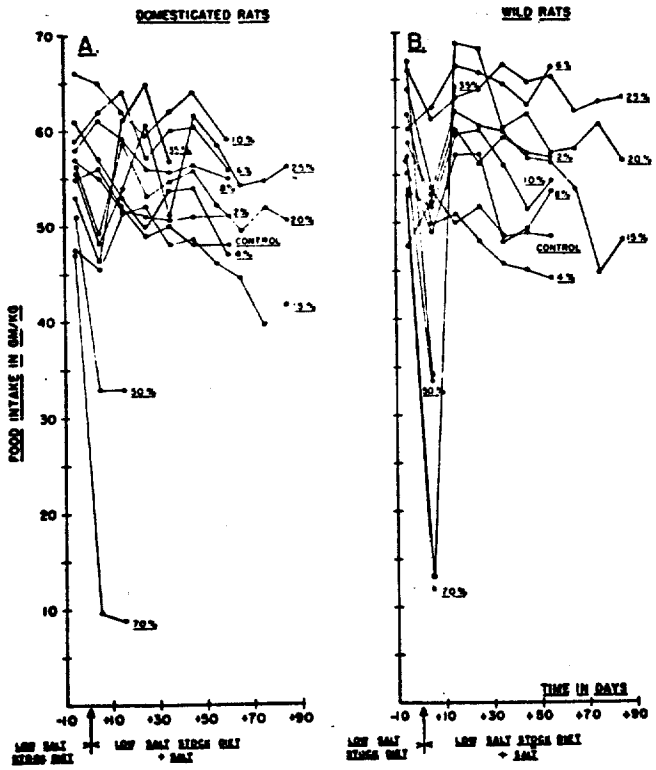

FIG. 2. Average daily food intake for the 3 rats on each of the diets of the various concentrations of salt and for a control group of 3 rats on the low-salt diet. Ordinates give food intake in gm/kg body wt.; abscissas time in days.

on the low-salt diets ranged from 450–820 cc/m², most of them falling below the normal average for rats on the full stock diet. The controls that remained on the low-salt diet throughout the experiment drank constant and very small amounts of water, averaging 620 cc/m². The rats on the 2 and 4% diets increased their water intake only a small amount, approximately to the average level for rats on the full stock diet containing 1% salt. On the 6 and 8% concentrations, the rats showed an immediate and significant increase in water intake, which was then maintained at almost constant levels for each group. On the higher concentrations the water increased still more abruptly and at each concentration after the short initial adjustment period, was maintained at a nearly constant level throughout the experimental period. The rats on the 25% sodium chloride concentration drank the largest amounts of water—5,500 cc/m² body surface which is about 9 times as much as the average for the controls.

Figure 5*B* summarizes the observations on the daily voluntary water intake of wild rats. When first brought into the laboratory about one-third of the wild rats drank very large amounts of water. In most instances water intake decreased at a steady rate during the 20–50 days on the low-salt diets, reaching fairly constant levels between 800 and 2,150 cc/m² body surface by the end of the first 50 days. With one or two exceptions these levels were well above those for the domesticated rats. The intake for the control wild rats averaged 1,700 cc/m². These 3 rats happened to have a much higher water intake than any of the rats in the experimental groups.

On all concentrations with the exception of 25%, the wild rats increased their water intake to levels that were not only higher in absolute amounts than those of the domesticated rats, but higher in relation to their own pre-experimental levels.

The voluntary water-intake levels for the two strains of rats may also be expressed

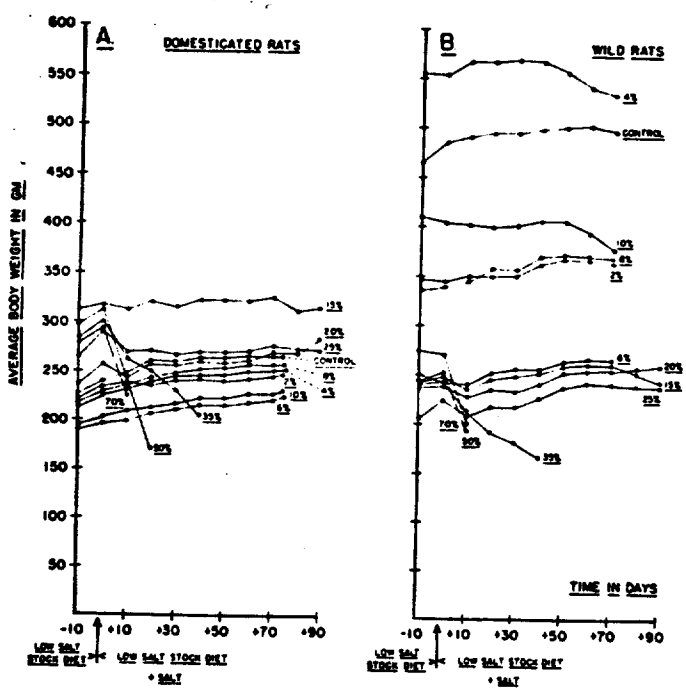

FIG. 3. Average body weight of the 3 rats on each of the various salt diets and for a control group of 3 rats on a low-salt diet. Ordinates give wt. in gm; abscissas time in days.

terms of the amounts of water ingested per gram of salt. Figure 6 summarizes the results. The ordinates give the water intake in cc/gm of salt; the abscissas the various concentrations of salt in the diets.

Figure 6*A* shows that at salt concentrations of 4, 6, 8, and 10%, the domesticated rats drank about the same amounts of water per gram of salt, approximately 60 cc. It is noteworthy that at these concentrations the rats gained weight. Even at 25% the intake still averaged 51 cc/gm of salt. A sharp drop occurred at the 35, 50 and 70% concentrations on which the rats lost weight rapidly and died.

Figure 6*B* shows that the water intake of the wild rats per gram of ingested salt was definitely higher than that of the domesticated rats at concentrations up to and including 15%, and that at the higher concentrations the differences were smaller. The intake at the different concentrations from 4–15% decreased progressively as the concentrations of salt increased rather than leveling off at 50–60 cc as it did in the domesticated rats.

Physiological Effects. No edema, ascites or diarrhea was noticed at any time, even in the rats receiving the very high salt diets. NaCl content of blood serum was determined in 12 additional domesticated rats, 2 on the low-salt diet and 2 on each of the diets with the following concentrations of salt: 2, 4, 6, 8, and 10%. Blood was taken from the abdominal vena cava while the rats were under ether 20 days after the start of the diets. Table 2 gives the results. Values were only slightly higher for the rats on the high-salt diets than for those on the control diets, as was to be expected from the relative constancy of the water intake per gram of salt ingested with these concentrations.

Anatomical Effects. The adrenal weights of the domesticated rats remained fairly constant at all concentrations of salt; for the wild rats they tended to decrease as the concentrations of salt increased, and also showed a wider range of variation.

The kidneys of both strains increased in weight as the salt concentrations increased. Histological studies of the kidneys failed to reveal any pathological changes. The domesticated rats showed some distention of the tubules. The results of the histological and histochemical studies of the adrenals and other

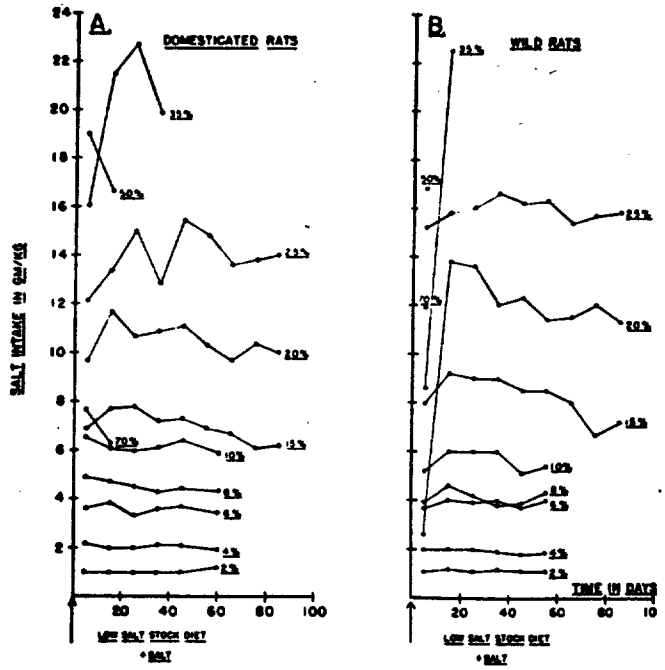

FIG. 4. Average daily salt intake for the 3 rats on each of the various salt diets. Ordinates give salt intake in gm/kg body weight; abscissas time in days.

organs will be reported in detail in a later paper.

DISCUSSION

The results of these experiments have demonstrated that Norway rats are able to handle very large amounts of salt and over long periods of time apparently without ill effects. That rats have this ability was demonstrated by Gamble *et al.* (5). In their experiments each rat was tested with a diet containing progressively higher amounts of salt each week; the concentrations of salt were increased in six steps over a 6-week period from 2.9–17.4%. Apparently the rats thrived on this regimen. On the highest concentrations they received an average of 7.5 gm/kg body weight of salt/day. The present experiments showed that the rats can consume even larger amounts and over longer periods without ill effects. Some of our rats survived the ingestion of as much as 30 gm/kg body weight of salt in 1 day.[3]

Viewed by itself this observation that rats are able to thrive on such high amounts of salt would indicate that salt in any amount is not toxic to rats. When viewed, however, in relation to other observations, it becomes clear that salt may be ingested to the point of toxicity, depending on the amount of water with which it is accompanied.

To obtain information on the toxicity of salt without water, a few observations were made on the effects of giving salt by stomach tube in single doses, to rats without access to water. It was found that under these circumstances a dose of 3–4 gm/kg body weight regularly kills domesticated rats from our colony in just a few hours. This agrees well with the values determined by Mitchell *et al.* for chickens (6). (Salt given by injection into the tail vein (25% solution) killed our domesticated rats almost instantaneously in doses as low as 0.6 gm/kg.) It is clear that salt is toxic to our rats when thus administered.

Since in the present experiments, in which water was accessible at all times, the rats got along well after ingesting very high amounts of salt over long periods of time, it must be concluded that salt, when given with adequate amounts of water, is not toxic to rats.

The rats continued to gain weight at their

[3] Fowl are also able to ingest very large amounts of salt (6–8).

normal rate when they drank from 50–60 cc of water/gm of salt. They were able to get this amount of water on diets containing salt in concentrations up to 15–25%. On higher concentrations of salt they apparently did not thrive because of their inability to get adequate calories from the high salt diets and their inability to get adequate amounts of water to take care of the salt. For instance, a rat weighing 250 gm and requiring 15 gm of stock diet/day for maintenance, would have to eat 30 gm/day of a 50% salt diet. In order to properly excrete the excess salt it would have to drink 50–60 cc of water/day for each gram of ingested salt, or 750–900 cc of water every day, which is approximately 3–4 times the animal's body weight. This may represent an almost impossible task for the rat. In these present experiments only a few rats drank amounts of water equivalent to, but not exceeding, their own body weights.

Our finding that 50–60 cc of water are needed to handle each gram of salt confirms the findings of Gamble *et al.* (5) and Adolph (9, 10).

From the point of view of studies on the rat's ability to self-regulate its own dietary requirements the present studies are in need of some comment (11). As a background for these comments it must be kept in mind that when given free choice of distilled water and solutions of various concentrations of salt, rats show preference for concentrations of 0.8–0.9% which closely approximate the salt content of normal blood. They drink about 60 cc of these concentrations on the average, that is, they ingest about 0.5 gm of salt/day, or 2.0–2.5 gm/kg body weight. This is a considerably smaller amount of salt than was re-

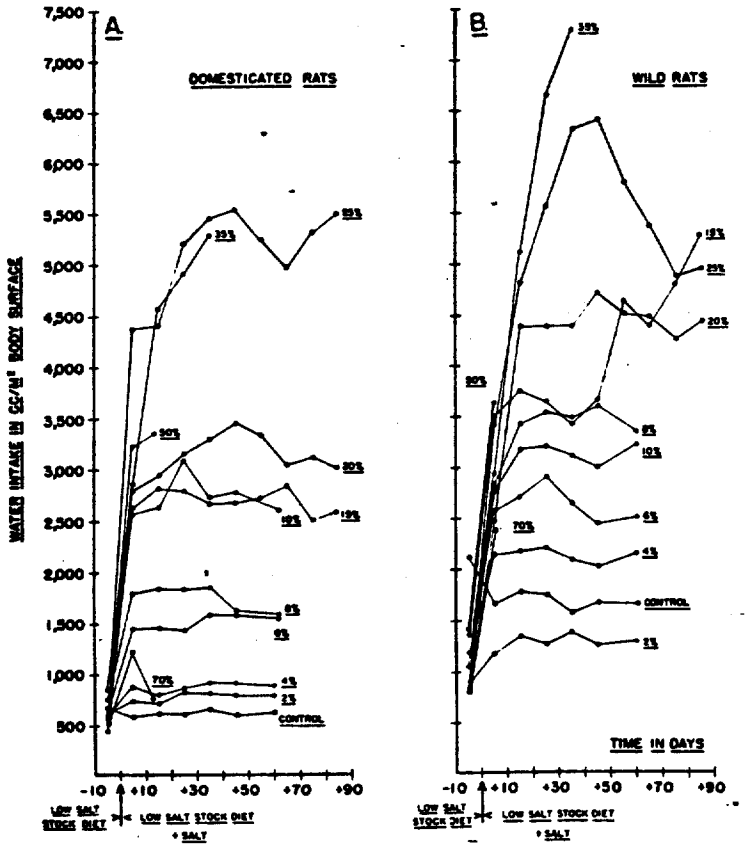

FIG. 5. Average daily voluntary water intake for the 3 rats on each of the various salt diets and for a control group of 3 rats on a low-salt diet (10-day averages). Ordinates give water intake in cc per square meter body surface; abscissas time in days.

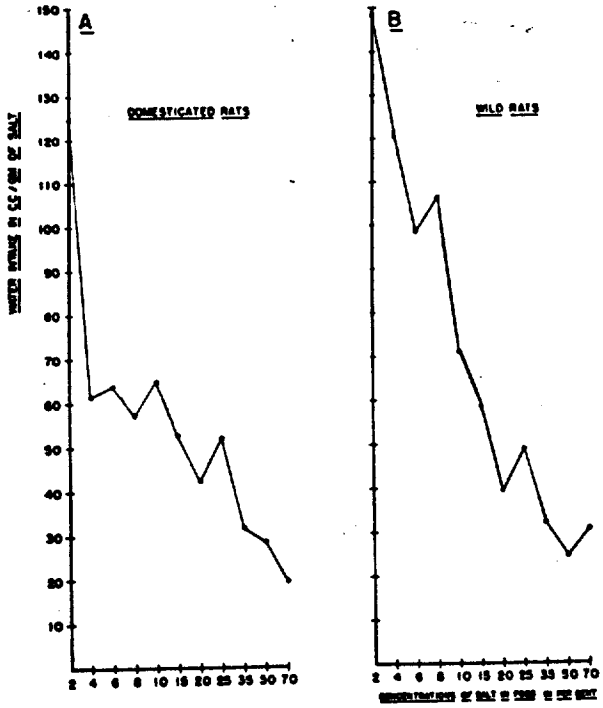

FIG. 6. Average daily voluntary water intake in cc/gm of ingested salt (for 3 rats on each of the various salt diets) for last 20 days of experimental period.

ceived from several of the diets in the present experiments. It is higher, however, than the amounts of salt present in the stock diet from which rats receive approximately 0.6 gm/kg body weight of salt/day.

In the present experiments in order to satisfy their caloric needs, the rats were forced to eat diets containing high amounts of salt. That they did not regard the salt as a poison in any sense is shown by the fact that they ate these diets in large amounts with very little hesitation. This behavior contrasts sharply with their reactions to foods containing only minute amounts of poison such as strychnine or morphine which they refuse altogether and so literally starve themselves to death (12).

The importance of the supply of water has further been brought out by the observation that the rats will immediately stop eating salt food when the supply of water is removed.

Of further interest here is the fact that the rats closely regulated their water intake to the amounts of ingested salt, that is, up to the point at which the salt content of the diets became too high to permit adequate amounts of water to be ingested.

One other aspect of the part played by salt may also be worthy of comment: that is that the amount of this all-important substance remains pegged at such a constant level in the blood despite the wide range and difference in the amounts that are ingested. On the one hand, it is difficult to reduce the salt content of a diet low enough to produce visible signs of nutritional deficiency. Osborne and Mendel (13) found that young rats grew normally on diets containing only 0.035% of salt, making a daily intake of only 20 mg/kg. On the other hand, the present experiments have shown that rats may thrive on diets containing 15–25% salt or daily intakes of 15–20 gm/kg, a thousand-fold difference.

Since these experiments originally were undertaken as part of the study of the effects produced by domestication in the Norway rat, we should like to comment also on this aspect of the results. It was found that the wild rats apparently have a greater need for water when they ingest excess salt than do the domesticated rats. Through the range of lower concentrations of salt in the diet they consistently drank more water; also their water

intake was more erratic. In explanation of the higher water intake of the wild rats we may consider results of experiments on the effects of treating normal rats with desoxycorticosterone (14). In such animals thirst is a function of both the amount of injected DCA and the amount of ingested salt. It is possible then that the wild rats with their larger and more active adrenals may secrete more DCA or similar compounds in order to conserve what salt is available to them, and as a result show a corresponding increase in thirst. This same mechanism may explain at least part of the high-water intake found in so many of the wild rats when they are brought into the laboratory and placed on the regular stock diet. In the wild state rats live largely on grain and seeds and probably have only a very low salt intake. Rats that have survived under these conditions may have inherited a highly efficient salt conserving mechanism. When they are brought into the laboratory and placed on a diet containing 1–2% salt, they may need more water, just as the domesticated rats do when they are treated with higher amounts of DCA.

On the basis of these observations on rats, we should now like to make a few remarks about the use of salt in man.

Man, like the rat, is able to adjust to widely varying intakes of salt. In some parts of the world he receives little or no added salt, in other parts very large amounts. Since in European countries salt is used in such large amounts, a high salt intake may be considered to be a characteristic of civilization. Mahatma Gandhi in his *Monograph on Common Salt* has given an excellent statement of the part played by salt in a modern civilization (15). It has been estimated that the average European takes from 12–20 gm of salt/day. Many individuals eat much larger amounts. Based on results of the rat experiments it would seem likely that man also is able to ingest many times his usual intake of salt, providing he takes sufficient amounts of water. His failure to do this probably gets him into difficulty. Owing to cultural ideas about the use of water for quenching thirst, he may not get adequate amounts of water in relation to salt intake. This would certainly be true in countries where thirst is quenched only with wine or beer. In some instances when the salt intake is very high, it would not be possible to take adequate amounts of fluid without becoming intoxicated.

TABLE 2. SODIUM CHLORIDE CONTENT IN BLOOD
Individual Domesticated Rats

Salt Diets, %	mEq/l	
Controls (low salt)	155	160
2	170	169
4	161	162
6	160	151
8	169	160
10	162	161

It is likely that when individuals with diseased kidneys or adrenals are forced to take much salt they may readily develop toxic manifestations indirectly attributable to the salt, e.g. hypertension, edema and adrenal dysfunction, even when otherwise normal amounts of water are ingested.

SUMMARY

Rats are able to handle very large amounts of salt, 15–20 gm/kg body weight/day, providing they are permitted to ingest sufficiently large amounts of water. Domesticated rats need from 50–60 cc of water/gm of salt ingested to maintain health. No such statement can be made for wild rats since other functions apparently influence their water intake. The ingestion of large amounts of salt over 2–3 months time did not produce any visible dietary deficiencies, nor did it produce any detectable pathological changes in the kidneys. When given access to water, rats will freely eat diets containing as much as 35% salt; they will eat fair amounts of a diet containing 50–70% salt. Salt does not produce as much thirst in domesticated Norway rats as in their wild ancestors. A greater secretion of a salt-conserving hormone from the larger and more active adrenals of the latter may account for this difference. On the basis of the results of these experiments it was suggested that difficulties encountered by man in using salt may result from his failure, for cultural and social reasons, to drink adequate amounts of water for excreting the excess of salt ingested.

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THE EFFECTS OF METALS ON THE CHICK EMBRYO: TOXICITY AND PRODUCTION OF ABNORMALITIES IN DEVELOPMENT*

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The stimulus for a study of the effects of the various elements on the development of the chick embryo was initiated by the striking effect produced by thallium sulfate injected into the yolk sac of the four-day-old embryo.[1] In order to determine the specificity of this effect, 53 other elements were assayed against the chick embryo. The current status of the work is presented in this report.

Materials and Methods

The effects of the elements were studied principally in the four- and eight-day-old embryo since developmental abnormalities are often grossly evident at these older ages. Furthermore, elements generally toxic to embryos early in development may produce selective growth effects when given to more differentiated embryos. Elements found to produce specific and consistent abnormalities when injected at four or eight days were studied further in younger and older embryos.

Commercially supplied White Leghorn eggs were used. They were maintained at a temperature of 38°C, and candled daily for viability. The volume of the test agent injected per egg ranged from .05 cc. to 0.2 cc. with an occasional yolk sac injection of 0.3 or 0.4 cc. When possible, water soluble salts were used, but, in rare cases, insoluble salts were ground in distilled water and injected as a suspension. The injections were made by two routes: into the yolk, or onto the chorioallantoic membrane (CAM) of eight-day eggs. Yolk sac injections were made through a hole drilled in the blunt end of the egg, and the opening then was sealed with melted paraffin. The CAM was injected through a small window cut in the shell directly over the membrane and the opening was sealed with Scotch tape after the injection.

The preliminary toxicity tests were carried out in the four-day-old embryo. Four eggs were injected at each of three of four dose levels, and, after several trials, the LD_{50} dose was approximated. Groups of six to ten eggs were injected at several dose levels above and below the estimated LD_{50} in order to obtain a more precise figure. In many instances, several confirmatory toxicity runs were necessary to obtain a reasonably consistent LD_{50} figure. With the four-day yolk sac data as a guide, similar toxicity studies were completed in the eight-day embryo by the yolk sac and CAM routes.

The embryos were candled daily and, each day, the dead embryos were recorded and examined. Occasionally, embryos were sacrificed for specific

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reasons during incubation but, in most instances, toxicity experiments continued until the 18th day of incubation, when all embryos were sacrificed. At this time, the embryos were weighed and examined grossly for abnormalities of the skin, feathers, bone, brain and internal organs. The membranes and yolk were also inspected.

Results

Toxicity data. The LD_{50} of 54 elements in various forms, when injected into the chick embryo at four and eight days of development, are summarized in TABLE 1. When more than one salt of an element was tested, they are listed with the most toxic salt of that element. There are numerous factors which influence the toxicity of an element in the fertile chicken egg, besides the inherent or direct toxicity of the chemical at its locus of action. These factors are related to:

(*a*) *The egg.* The egg varies in size and chemical composition, the latter being related to the diet of the hen to a considerable extent. Certain excesses or deficiencies of minerals or vitamins, for example, may modify the response of the embryo. An extremely important factor is the developmental age of the embryo at the time the chemical is injected, since the presence of specific organs or metabolic systems may be critical in determining the effect of a chemical.

(*b*) *The state and properties of the injected chemical.* The route of injection and the vehicle used may be very important in the rate of absorption of the chemical. Certain elements may react with non-embryo constituents of the egg, may be precipitated at the site of injection, or may be converted by combining with substances in the egg to form a substance of greater physiological activity.

(*c*) *Distribution and metabolism of the injected chemical.* The rate of absorption and the toxicity of high blood levels of an element are important factors in toxicity. Some chemicals are concentrated in the albumen or in the allantoic sac, and thus detoxified as far as the embryo is concerned. Other chemicals may be deposited in inactive form in some parts of the embryo, as the bones, and thus be rendered less toxic.

All of these factors must be taken into account in interpreting the toxicity or teratogenic effect of an element. In FIGURE 1, the elements have been divided into four groups, based on their toxicity in the four- and eight-day embryo, by the yolk sac and CAM routes. These groupings suggest how some of the factors mentioned above influence the activity of specific chemicals.

Specific Effects on Embryonic Development. Under appropriate circumstances, it is probable that an excess of any element can be shown to have some specific effect on embryonic development. In many instances, however, the element appears to be generally toxic and the embryo either dies promptly or, if the embryo survives, it will develop normally. On the other hand, situations have been found in which some elements will exhibit specific biological activity in the chick embryo. Some elements exert their effect when given in any chemical form, at any time during incubation, and

TABLE 1

TOXICITY OF SALTS OF VARIOUS ELEMENTS IN FOUR- AND EIGHT-DAY CHICK EMBRYOS

Element	Formula of Salt	Estimated LD_{50}			
		Mgs/egg	Microatoms/egg*		
		4-day	4-day	8-day	8-day CAM route
		Yolk sac route			
Arsenic	$KAsO_2$	0.02	0.10	0.34	0.07
Arsenic	$Na_2HAsO_4 \cdot 7H_2O$	0.30	0.96	2.60	0.32
Arsenic	As_2O_5	0.16	1.40		
Cadmium	$CdCl_2$	0.02	0.13	0.22	0.09
Selenium	H_2SeO_4	0.03	0.17	0.55	0.11
Platinum	$PtCl_4 \cdot 2HCl \cdot 6H_2O$	0.13	0.25	>19.0	0.17
Lead	$Pb(NO_3)_2$	0.10	0.30	4.50	3.00
Mercury	$HgCl_2$	0.08	0.30	3.10	0.21
Chromium	$Na_2Cr_2O_7 \cdot 2H_2O$	0.06	0.40	0.30	1.02
Chromium	$Cr(NO_3)_3 \cdot 9H_2O$	0.80	2.00	>50.0	7.50
Antimony	$SbCl_3$	0.10	0.44	2.20	0.08
Silver	$AgNO_3$	0.10	0.59	0.30	0.50
Nickel	$NiCl_2 \cdot 6H_2O$	0.20	0.84	10.0	1.40
Vanadium	$VO(CH_3COO)_2$	0.20	1.10	0.65	0.24
Tungsten	Na_2WO_4	0.78	2.60	8.50	6.30
Molybdenum	$Na_2MoO_4 \cdot 2H_2O$	0.80	3.30	2.90	14.0
Copper	$CuCl_2 \cdot 2H_2O$	0.60	3.50	29.0	0.71
Strontium	$Sr(NO_3)_2$	0.80	3.80	>94.0	54.0
Cobalt	$CoCl_2$	0.50	3.80	7.70	2.30
Tellurium	Na_2TeO_3	1.00	4.50	18.0	0.12
Osmium	OsO_4	1.20	4.70	9.90	0.99
Thallium	Tl_2SO_4	1.30	5.20	5.50	2.80
Lanthanum	$LaCl_3 \cdot 7H_2O$	6.00	16.0	54.0	2.80
Zinc	$ZnSO_4 \cdot 7H_2O$	5.00	17.0	>69.0	1.20
Cerium	$Ce(NO_3)_3 \cdot 6H_2O$	8.00	18.0	>46.0	3.00
Manganese	$MnSO_4 \cdot 4H_2O$	5.00	21.0	>86.0	1.50
Uranium	$UO_2(C_2H_3O_2)_2 \cdot 2H_2O$	15.0	22.0	>29.0	0.44
Indium	$InCl_3$	5.00	23.0	>91.0	21.0
Ruthenium	$RuBr_3$	7.00	27.0	>76.0	9.50
Iron	$FeSO_4 \cdot 7H_2O$	8.0	29.0	25.0	2.00
Iron	$FePO_4 \cdot 2H_2O$	15.0	80.0	75.0	2.90
Thorium	$Th(NO_3)_4 \cdot 4H_2O$	20.0	38.0	>38.0	14.0
Fluorine	NaF	1.75	42.0	71.0	30.0
Iridium	$IrCl_3$	14.0	47.0	13.0	1.30
Iodine	KI	8.0	48.0	60.0	78.0
Tantalum	K_2TaF_7	20.0	51.0	36.0	6.40
Tin	$SnCl_2$	10.0	53.0	>105.0	7.40
Tin	$SnCL_4$	20.0	77.0	77.0	5.40
Gallium	$GaC_6H_5O_7$	15.0	58.0	77.0	2.70
Magnesium	$MgSO_4 \cdot 7H_2O$	15.0	61.0	>121.0	105.0
Lithium	$LiSO_4 \cdot H_2O$	8.0	62.0	78.0	64.0
Zircon	$ZrOH(C_3H_3O_2)_3$	18.0	63.0	>70.0	18.0
Boron	H_3BO_3	5.0	81.0	113.0	81.0
Aluminum	$AlCl_3$	15.0	112.0	>150.0	16.0
Silicon	$Na_2Si_3O_7$	20.0	247.0	>247.0	31.0
Germanium	GeI_2	>10.0	>31.0	31.0	3.10
Gold	$AuCl_3HCl \cdot 4H_2O$	>20.0	>41.0	>41.0	1.20
Rhodium	$RhCl_3$	>10.0	>48.0	72.0	4.30
Bismuth	$BiCl_3$	>20.0	>63.0	>63.0	11.0
Yttrium	$Y(NO_3)_3 \cdot 6H_2O$	>30.0	>78.0	>52.0	2.90

TABLE 1 (CONTINUED)

Element	Formula of Salt	Estimated LD_{50}			
		Mgs/egg	Microatoms/egg*		
		4-day	4-day	8-day	8-day CAM route
		Yolk sac route			
Barium	$BaCl_2 \cdot 2H_2O$	>20.0	>82.0	45.0	3.30
Palladium	$PdCl_2 \cdot 2H_2O$	>20.0	>94.0	>94.0	19.0
Titanium	$Ti_2(SO_4)_3$	>20.0	>104.0	89.0	29.0
Beryllium	$BeSo_4 \cdot 4H_2O$	>20.0	>113.0	>113.0	5.60
Cesium	CsCl	>20.0	>119.0	>119.0	77.0
Rubidium	RbCl	>30.0	>248.0	>248.0	124.0
Calcium	$CaCl_2$	>30.0	>270.0	270.0	23.0
Potassium	KCl	>50.0	>671.0	>537.0	134.0
Sodium	NaCl	>50.0	>855.0	>685.0	145.0

* Microatoms/egg = $\frac{LD_{50} \text{ in mg/egg} \times 1000}{\text{Mol. wt. of salt in gms.}} \times$ no. atoms of element in salt. The LD_{50} in mg/egg for each salt for the eight-day embryros is calculated easily from the relationship between the mg/egg and microatoms/egg data presented for the four day embryo.

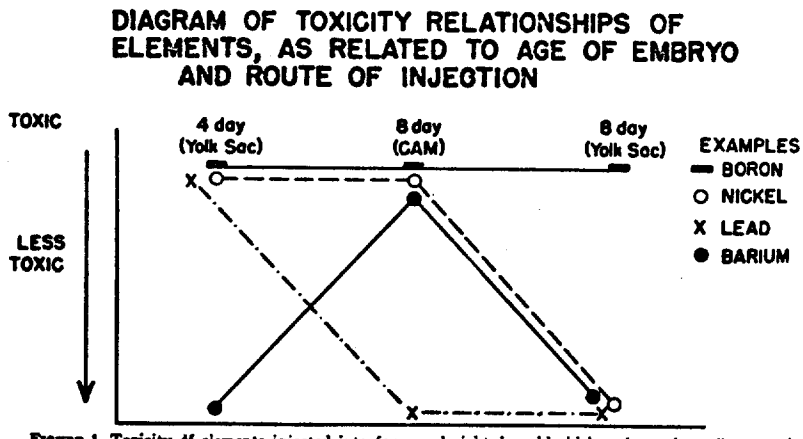

FIGURE 1. Toxicity of elements injected into four- and eight-day-old chick embryos by yolk sac and CAM routes. The four groupings cover the majority of results summarized in TABLE 1. The elements, illustrated by boron require approximately the same LD_{50} dose, irrespective of the age or route of administration. This may indicate that the chemical is readily absorbed, and that it acts specifically on a certain system as it appears in the embryo. If this system is not present at the time of injection the chemical remains biologically inactive until the proper susceptible mechanism appears. The elements, illustrated by nickel, show decreased toxicity at eight days as compared to four days by yolk sac injection, but they are toxic on the CAM at eight days. This may be interpreted as indicating that absorption of the salt is more efficient by the CAM route, and as the embryo increases in size more of the salt is necessary to poison it. About 16 per cent of the chemicals tested fell in this category. The elements, illustrated by lead, are most toxic at four days, and less toxic at eight days, by both yolk sac and CAM routes. This suggests that the salt is readily absorbed from the yolk sac as well as the CAM, and the tolerance of the embryo to the chemical increases with its increase in size. The elements, illustrated by barium, are more toxic by the CAM than by the yolk sac route. This pattern was found in about 50 per cent of the elements tested. Chemicals in this group may be precipitated in the yolk sac or they may react with the yolk components so that the embryo is not exposed to the full injected dose. Another possibility is that if the chemical is rapidly absorbed from the CAM, the embryo may be acutely susceptible to a high dose; although if the substance is absorbed slowly from the yolk sac, the embryo may detoxify or excrete it before a lethal concentration develops.

by any route of administration, while others are active only when given at specific stages of development, or by a certain route of administration, or in a definite chemical form. During the course of our toxicity studies, if gross and definite evidence of embryonic abnormality was produced by a given element, the effect was analyzed in more detail. If nothing specific occurred, possibly because an abnormality was missed by our gross methods of examination, or because the embryo was not treated properly to bring out such an effect, further studies have not been carried out. It does not mean, of course, that the element is completely without teratogenic activity. Nine elements thus far have been found to produce a specific and reproducible effect on embryonic development, and these are summarized in TABLE 2.

TABLE 2
SUMMARY OF ELEMENTS PRODUCING ABNORMALITIES IN EMBRYONIC DEVELOPMENT

Element	*Abnormality*	*Maximum activity*		*Approximate consistency of effect, per cent treated embryo*
		Ages of embryo at injection	*Route of injection*	
Thallium	achondroplasia	4–10	yolk sac, CAM	90–100
Chromium	mild achondroplasia	8	CAM	60
Lead	brain injury	3–15	yolk sac, CAM	80–90
Cobalt	brain injury	4–12	yolk sac	80–90
Boron	pale and edematous, feather inhibition	0–8	yolk sac, CAM	70–85
Arsenic	stunted, micromelia, abdominal edema	4	yolk sac	50
Rhodium	stunted, feather inhibition, micromelia, mild edema	8	CAM	80
Barium	abnormal feet	8	yolk sac, CAM	50
Selenium	bizarre abnormalities, stunting	0–8	yolk sac, CAM	10–20

(*a*) *Thallium.* Thallium sulfate produced a severe and consistent picture of achondroplasia in the chick embryo,[1] which was evident in embryos sacrificed or dying at ten days of incubation or later. The most marked effects were seen when thallium was given into the yolk sac at four to ten days of incubation, and the LD_{50} dose during this period was between one and two mg/egg. Thallium did not produce achondroplasia when given at the maximum tolerated dose (0.3 mg/egg) to the two day embryo, presumably because thallium had a generally toxic effect at higher doses at two days, and lower doses were insufficient to produce achondroplasia. The LD_{50} of thallium sulfate on the CAM at eight days is 0.7 mg/egg, and achondroplasia occurs consistently at doses of 0.4 to 0.7 mg/egg by this route. Thallium produces a less severe achondroplasia in embryos injected after the tenth day.

When sacrificed at 18 to 20 days, the embryo treated with thallium at four to eight days is severely achondroplastic, the limbs are short and the

long bones curved, the trunk is thickened, the head is small and the beak resembles a parrot's. On gross examination of the head, the eyes and orbit appear smaller than normal. The brain is small, sometimes contains cysts and the cerebellum may be atrophied. The abnormalities in the central nervous system are presumably due to compression of the brain and eyes as a result of defective growth of the skull. The feathers appear normal (FIGURE 2A). Roentgenograms of the thallium-treated embryos show the conspicuous interference in bone growth (FIGURE 2D, E). Microscopic section of the toe of the thallium-treated embryos shows defective cartilage growth, with failure of longitudinal bone growth, but the shaft is widened and thickened by the deposition of bone in the shaft (FIGURE 2B, C).

(*b*) *Chromium.* Sodium dichromate ($Na_2Cr_2O_7 \cdot 2H_2O$) produced no apparent abnormalities when injected into the yolk sac of the four-day embryo. When sodium dichromate was injected onto the CAM, however, about 60 per cent of the embryos developed moderate achondroplasia, parrot beaks, slight stunting in growth, paleness and, in some instances, sparse feather formation (FIGURE 7C). Achondroplasia was never as severe as that produced by thallium. Injection of sodium dichromate into the yolk sac at eight days, and chromium nitrate ($Cr(NO_3)_3 \cdot 9H_2O$) into the four- and eight-day embryo failed to produce gross developmental abnormalities.

(*c*) *Lead.* The effect of lead on the early development of the chick embryo has been the subject of several studies, including those of Hammett and Wallace[2] and Catizone and Gray[3]; the latter group, working in the 0 to 48 hour of incubation period, concluded that lead can specifically affect the morphogenesis of the central nervous system of the chick. Our studies were carried out in older embryos, and lead was found to have a remarkable effect on the central nervous system. Four-day-old embryos injected with 0.15 mg. of lead nitrate showed an extreme degree of hydrocephalus when examined at 18 days of age[4]. In most instances, following the yolk sac injection of 0.15 mg/egg of lead nitrate at four days, embryos dying or sacrificed within the next four days exhibited brain hemorrhage (FIGURES 3A, B). Embryos examined after eight days of incubation presented varying degrees of hydrocephalus, ranging from a small herniation of the meninges to massive enlargement of the head (FIGURE 4A, B). After the tenth day of incubation, some of the brains showed evidence of regeneration of nervous tissue, particularly at the base and anterior portion.

Lead produced brain hemorrhage when injected into the yolk sac over the four- to 15-day incubation period. At eight days, doses of 0.1 mg/egg or higher of lead nitrate produced brain injury in 96 per cent of the embryos. These embryos usually showed necrosis of the brain substance at 18 days, and only a thin shell of nervous tissue remained, distended by a bloody fluid. Although the tolerance of the embryo for lead increased rapidly, the dose injuring the brain was fairly constant during the fourth to eighth day. At the tenth day and beyond, larger doses of lead nitrate, up to two mg/egg, were necessary to induce brain injury. This was attributed to the fact that other systems, such as bone, were appearing in the chick embryo, and the lead may have been competitively fixed by these new systems. The

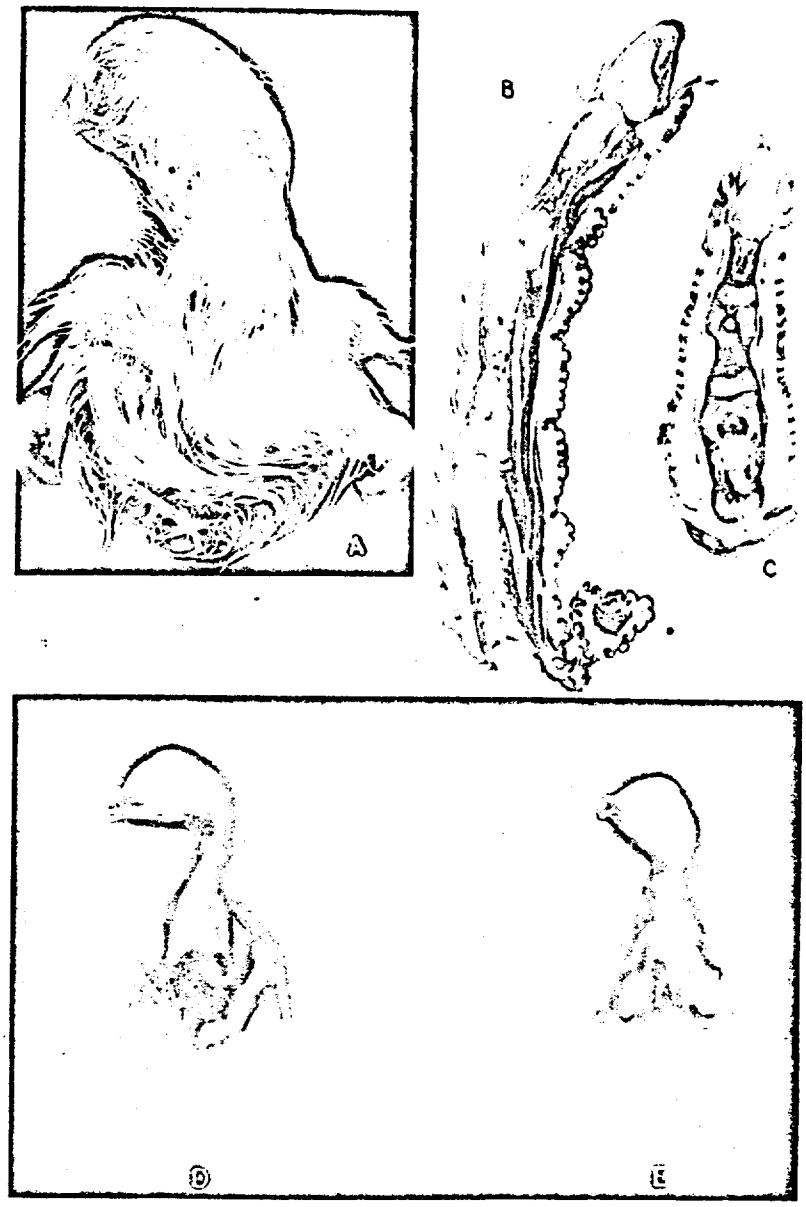

FIGURE 2. Effect of thallium on the chick embryo. A—Embryo, sacrificed at 18 days, injected with one mg. of thallium sulfate into the yolk sac at four days. Embryo shows characteristic picture of achondroplasia, with shortened extremities and parrot beak. B—Microscopic section of toe of 18-day-old embryo. C—Toe of 18-day embryo treated at four days with thallium. The thallium-treated toe is shortened, cartilage formation impaired, but there is some bone formation in the shaft. D—Roentgenogram of a normal 18-day embryo. E—An 18-day thallium-treated embryo. The bones of the extremities are shortened, curved and appear denser than the control.

histopathology of the brain injury induced by lead is now under study,[5] but the sequence of events, as they are seen grossly, are extensive hemorrhage throughout the brain substance within 48 hours after injection, followed by

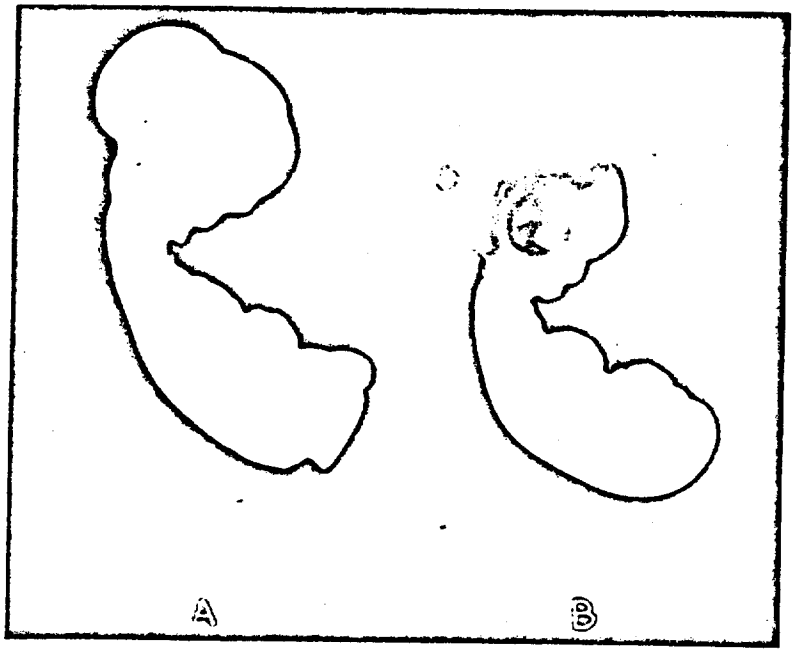

FIGURE 3. A—Control. B—Lead-treated embryo six days of age. Injected in the yolk sac with 0.15 mg. lead nitrate at four days of age. Marked hemorrhage is present in the brain.

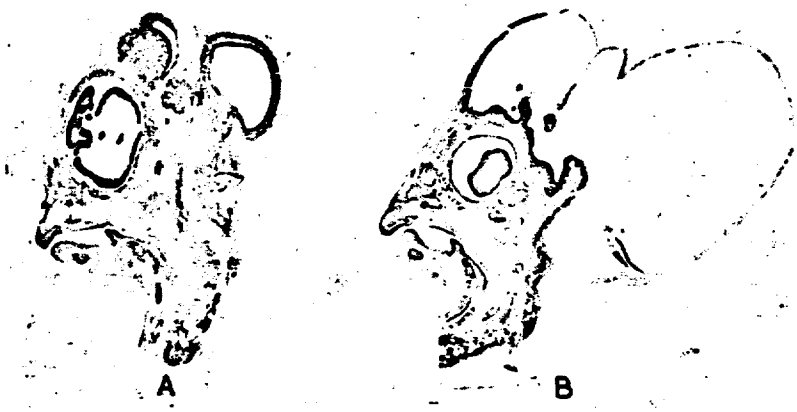

FIGURE 4. Microscopic sections of head of control A, and lead-treated embryo B, eight days of age. B was injected in the yolk sac with 0.15 mg. lead nitrate at four days. The cranium of the lead-treated embryo is enlarged and empty, as a result of the hemorrhagic necrosis in the brain induced by lead.

necrosis of the tissue. Some recovery is seen later, particularly if brain injury was induced early in development.

Lead nitrate failed to induce gross evidence of brain injury when injected into the yolk sac at two days of incubation, whereas in the three-day embryo, brain injury occurred in 70 per cent, and, in the four-day embryo, in 85 to 90 per cent of the treated embryos. It is of interest that the period of development when lead begins to induce its effects on the brain coincided with the appearance of blood vessels in the nervous system.[6]

The effects of lead and thallium could be induced by the simultaneous

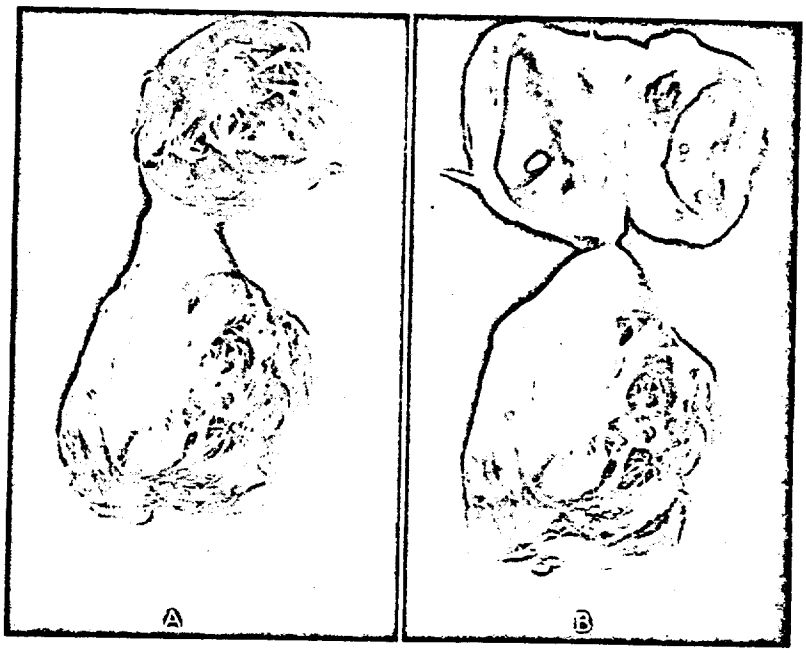

FIGURE 5. Embryo, 18 days of age, injected with one mg. of thallium sulfate into the yolk sac at four days, and 0.15 mg. of lead nitrate into the yolk sac at eight days of age. Note the achondroplasia and hydrocephalus (A), and the distended empty cranium (B). A similar picture followed the simultaneous injection of both agents at eight days of age.

injection of lead nitrate and thallium sulfate into the same embryo (FIGURES 5A, B).

(*d*) *Cobalt.* Gray and Scully[7] reported that cobalt chloride, injected into the albumen of the egg before incubation, did not induce any specific abnormalities in the embryo, but it appeared to inhibit the growth of the amnion. In our work, cobalt has been used later in development. Cobalt chloride injected into the yolk sac of eight-day embryos consistently induced softening and some hemorrhage in the brain, when it was examined at 16 to 18 days (FIGURE 6A, B). The embryos also were pale, slightly edematous and the livers were enlarged. The nature of the brain injury is under study. Cobalt induced brain injury, apparently less severe than at eight days, when given at four or 12 days of development.

(*e*) *Boron.* When given in the yolk sac of the four-day-old embryo as boric acid, boron caused marked growth inhibition, and embryos surviving

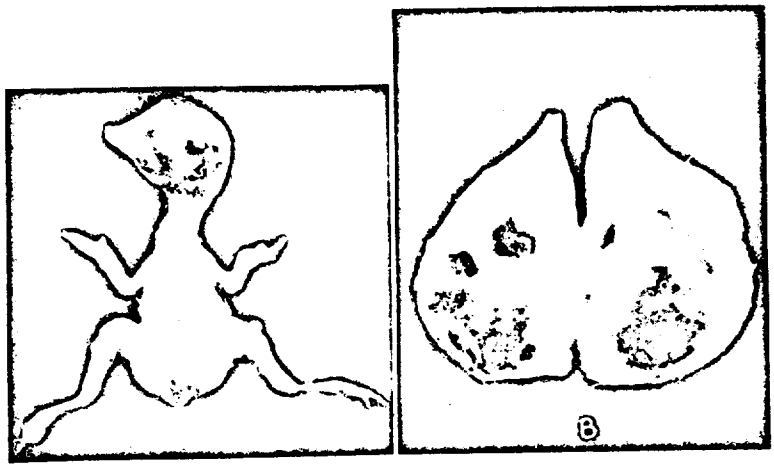

FIGURE 6. A. 18-day-embryo, after injection in the yolk sac at eight days with two mg. cobalt chloride. Embryo shows redness and puffiness of the head, impaired feather growth, mild generalized edema and slight micromelia. B—Splitting the head reveals a soft brain with some hemorrhagic areas.

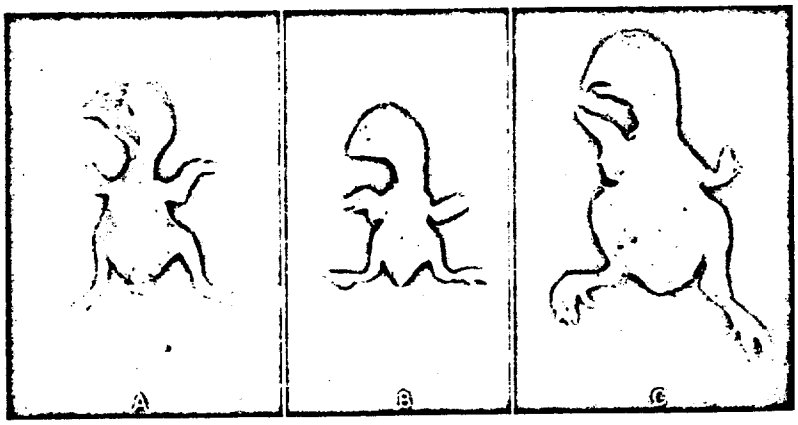

FIGURE 7. A—Embryo, sacrificed at 18 days, injected on the CAM at eight days with five mg. boric acid. The embryo is pale and stunted, and shows an over-all gelatinous edema and scanty and inhibited feather growth. B—Embryo, sacrificed at 18 days of age, was injected with two mg. of rhodium chloride onto the CAM at eight days. The embryo is severely stunted with mild micromelia and impaired feather formation. C—Embryo, sacrificed at 18 days, was injected onto the CAM with 0.1 mg. of sodium dichromate ($Na_2Cr_2O_7 \cdot 2H_2O$) at eight days of age. The embryo shows a moderate achondroplasia with parrot beak, and slight growth inhibition.

to 18 days were also very pale, showed definite interference in feather growth and occasional beak deformities. Boric acid, by the yolk sac and CAM routes at eight days, caused a similar effect on the embryos, and those

surviving to 18 days also showed an over-all gelatinous edema (FIGURE 7A). The LD_{50} dose of boric acid given into the yolk sac at zero and two days of age was in the same range as the four- and eight-day dosage, five mg/egg, and the effects on the embryo when examined at 18 days were somewhat similar, irrespective of the age of the embryo when injected. Boron, therefore, caused a specific and consistent effect on the chick embryo when given at zero to eight days at a fairly uniform dose level.

Arsenic. Sodium ortho arsenate ($Na_2HAsO_4 \cdot 7H_2O$), a pentavalent arsenical, produced definite growth inhibition when injected into the yolk sac

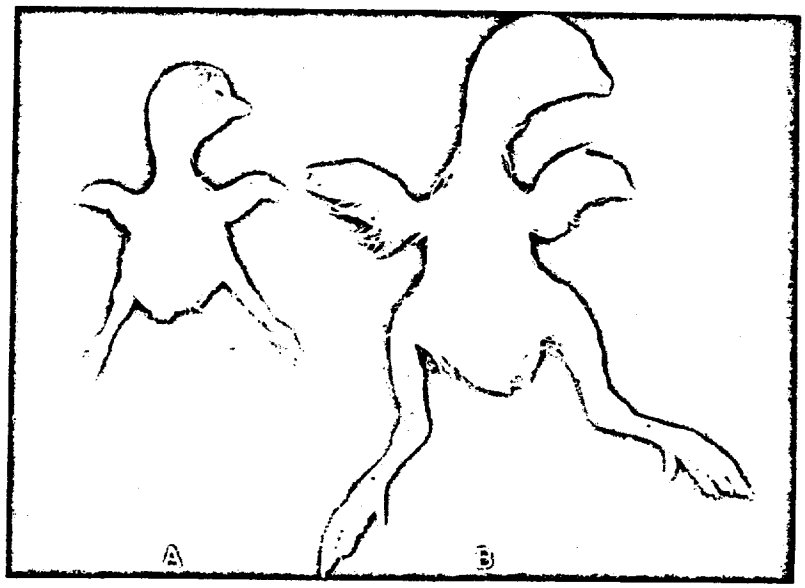

FIGURE 8. A—Embryo, sacrificed at 18 days, injected into the yolk sac at four days of age with 0.20 mg. of an arsenical salt ($Na_2HAsO_4 \cdot 7H_2O$). The embryo is markedly stunted, shows mild micromelia, impaired feather growth and swelling of the abdomen, as compared with B, a normal 18-day-old chick embryo.

of four-day embryos, but not in the eight-day embryo. At 18 days, the embryos were small, showed mild micromelia and abdominal edema (FIGURE 8A). Potassium arsenite ($KAsO_2$) and arsenic pentoxide (As_2O_5) did not produce this picture when given at four or eight days.

Rhodium. Rhodium chloride produced abnormal embryos when injected on the CAM at eight days. The embryos surviving to 18 days were somewhat similar to those treated with sodium ortho arsenate, in that they were stunted, with mild micromelia and edema, but the rhodium treated embryos showed greater interference with feather growth (FIGURE 7B).

Barium. Barium chloride, injected into the yolk sac or onto the CAM of the eight-day embryo, produced a remarkable inhibition in the growth of the toes (FIGURE 9A). This abnormality occurred irregularly, but was seen in about 50 per cent of the treated embryos surviving to 18 days.

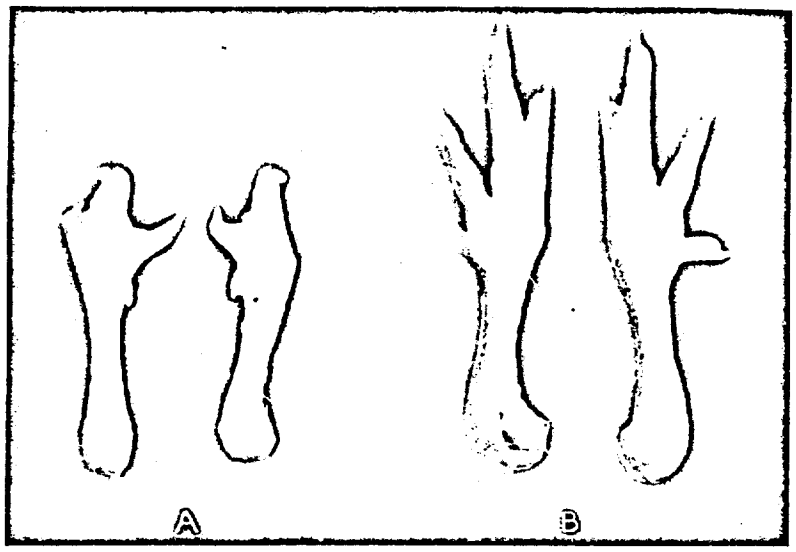

FIGURE 9. Feet of 18 day embryos. A After injection in the yolk sac at eight days with 20 mg. barium chloride, and showing deformity and inhibition in toe development as compared with B - control normal toes.

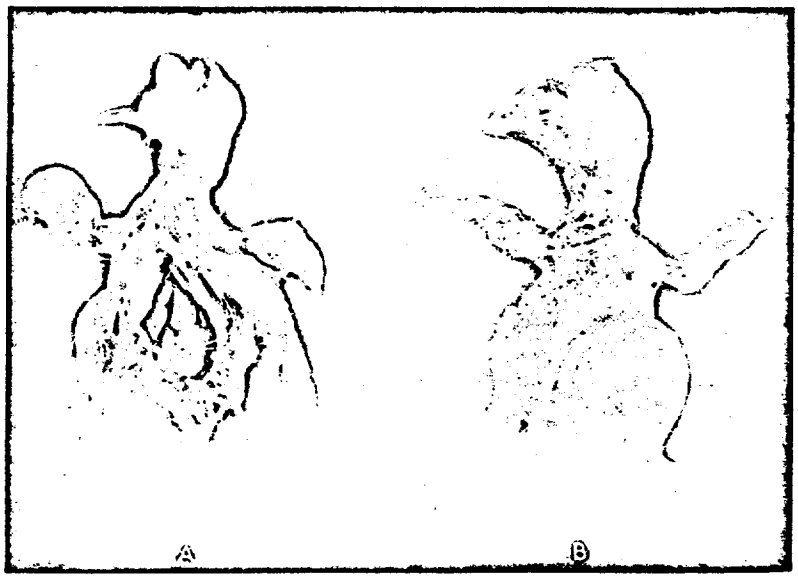

FIGURE 10. Effect of selenium on the chick embryo. A—18-day embryo injected in the yolk sac before incubation with 0.01 mg. selenious acid (H_2SeO_4). This embryo shows malformation of head and upper beak. B—18-day embryo injected in the yolk sac at eight days with 0.08 mg. of selenious acid. The embryo developed apparently normally, except for the enormous cysts on the rump.

There were no other gross effects noted on the embryo. None of the embryos injected at 4 days showed toe abnormalities.

Selenium. Franke, *et al.*[8] obtained a variety of monsters following the injection of sodium selenate into the air sac prior to incubation. We obtained several abnormal embryos, two of which are shown in FIGURE 10A, B, when selenious acid (H_2SeO_4) was injected into the yolk sac of embryos from zero to eight days of age. The incidence of abnormalities in our experience was low, in the range of 10 to 20 per cent of the embryos surviving to 18 days.

Conclusion

The effects of excessive concentrations of elements on the developing chick embryo may be a useful method for studying the biological properties of the elements. It is apparent that some elements, at least, induce a characteristic effect on the embryo when given at the proper period of development, and two elements, given simultaneously, may each induce their specific abnormality in the embryo. Some of the elements have caused destruction and regression in an organ already well-developed at the time of treatment, thus demonstrating a teratogenic effect by retrogression of an established organ, rather than by damage to the anlage of a developing organ. The specific affinity of some elements and embryonic organs, as suggested by selective injury to these organs, may suggest approaches and methods for damaging neoplastic growths arising from those organs.

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J. Pediatr. 79: 877

A further warning on the use of salt as an emetic agent

To the Editor:

DeGenaro and Nyhan's condemnation of salt as an emetic agent (J. PEDIAT. 78: 1048, 1971) is indeed most timely. As will be reported in detail by Brown and colleagues, our community has also just witnessed the death of a 23-year-old woman consequent to administration of salt in an attempt to induce vomiting. After she had ingested some 30 Librium capsules, her husband was allegedly advised to give salt and water. Some four hours later her serum sodium was recorded as 211 mEq.; upon transfer to our medical center, it continued at 178 mEq. Subsequent dialysis proved of no avail, and death occurred in 18 hours.

Recently Laurence and Hopkins[1] also recorded the death of an adult following use of hypertonic saline as an emetic agent. And of considerable importance is the most recent paper of Carter and Fotheringham[2] detailing two deaths in young children consequent to the use of hypertonic saline as the washwater during the process of gastric lavage.

The message is clear. Salt—as with virtually any other element or compound in our environment—does constitute a "poison." Its use as an emetic agent should be solely a tale of history.

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Hemodynamic effects of hypertonic sodium chloride

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ROWE, GEORGE G., DAVID H. McKENNA, ROBERT J. CORLISS, AND SALVADOR SIALER. *Hemodynamic effects of hypertonic sodium chloride.* J. Appl. Physiol. 32(2): 182-184. 1972.—The systemic and coronary hemodynamic effects of an infusion of hypertonic sodium chloride solution were studied in intact anesthetized mongrel dogs. The experiment produced hemodilution accompanied by an increase in cardiac output and stroke volume and a decrease in peripheral and pulmonary vascular resistance. Changes in the coronary circulation were variable and did not reach statistical significance although flow and vascular resistance tended in the same direction as the changes in peripheral and pulmonary circulation. There was a considerable increase in cardiac output and stroke volume with no significant change in systemic blood pressure; therefore calculated external left ventricular work increased. However, since left ventricular oxygen consumption did not increase, cardiac efficiency, as calculated from the external left ventricular work per unit of oxygen consumed by the left ventricle, increased during the administration of hypertonic sodium chloride.

cardiac output; coronary blood flow; vascular resistance and NaCl

HYPERTONIC SODIUM BICARBONATE administered intravenously increases cardiac output and coronary blood flow (13). Similar changes did not occur when comparable increases in pH were produced by tromethamine (8) or hyperventilation (10) but did tend to occur subsequent to administration of sodium lactate (12) and to some extent 50% glucose (7). Furthermore, profound hemodynamic effects have been reported to occur subsequent to the rapid administration of hypertonic contrast materials (1-6, 11, 14-16, 18, 19). Although numerous studies have been made of the systemic hemodynamic effects of hypertonic solutions, there is little information concerning the effects of such solutions on the coronary circulation. Therefore, the present study was undertaken.

MATERIALS AND METHODS

Systemic and coronary hemodynamic effects of hypertonic sodium chloride were investigated in 10 intact mongrel dogs varying in weight between 19 and 29 kg, with an average weight of 23.3 kg. They were anesthetized with 3 mg/kg of morphine sulfate subcutaneously, followed 1 hr later by 0.25 ml/kg of a 50–50 mixture of pentobarbital and Dial-urethane.[1] After anesthesia was obtained cardiac catheters were maneuvered fluoroscopically into the pulmonary artery, right atrium, and coronary sinus, and a Cournand needle was placed percutaneously in the femoral artery. A vein was exposed in the foreleg and cannulated for administration of sodium chloride. Cardiac output was determined by the direct Fick principle collecting air in the Tissot spirometer via flutter valves and a cuffed endotracheal tube attached to a large-bore low-resistance tubing. To reduce respiratory dead space the nonrebreathing (Kirchoff) valve was inserved into the oropharynx. Analyses for oxygen and carbon dioxide were done in blood specimens by the Van Slyke-Neill method and in expired air by the method of Scholander. The nitrous oxide saturation technique was used for determining coronary blood flow and the partition coefficient between blood and myocardium was assumed to be one. Arterial and coronary sinus blood specimens were analyzed for nitrous oxide by the method of Orcutt and Waters. Blood pH was measured in the femoral arterial and coronary sinus whole blood specimens utilizing the Radiometer model 22 pH meter. Blood pressure in the pulmonary artery, right atrium, and femoral artery were recorded by Statham strain-gauge pressure transducers as transcribed on the Gilson macropolygraph. Mean pressures were determined by electrical damping. Standard formulas were used for all calculations. In calculating cardiac work neither right nor left atrial pressure was subtracted from the appropriate arterial pressure since the left atrial pressure was not determined and the error introduced by this omission is relatively small. Comparison of values before and after administration of sodium chloride were made by the t test for paired values.

Control determinations of cardiac output and coronary blood flow were made in each animal. Fifteen minutes after the determination of coronary blood flow, sodium chloride administration was begun intravenously in a dose of 0.3 g/kg of 30% sodium chloride during approximately 3 min. The dog was then given an infusion of 0.01 g/kg per min of hypertonic sodium chloride throughout the second determination of cardiac output and coronary blood flow. The salt solution was made up as 30% but minor adjustments

[1] Dial-urethane contains Dial (diallylbarbituric acid), 100 mg/ml; monoethylurea, 400 mg/ml; and urethan, 400 mg/ml. Veterinary pentobarbital contains 60 mg/ml of pentobarbital.

were made in its volume to permit accurate rates of infusion with the Harvard Apparatus Company infusion pump.

RESULTS

As can be seen in Table 1, administration of hypertonic sodium chloride was associated with a 16% increase in heart rate. No significant change occurred in the systemic or pulmonary arterial pressure, nor in the mean right atrial pressure. The minute volume of respiration and the quantity of carbon dioxide eliminated increased significantly, whereas the oxygen consumption did not change significantly. There were significant decreases in the arterial hematocrit and hemoglobin, and the arterial and coronary sinus blood oxygen content, accompanied by a decrease in the arteriovenous oxygen difference for the systemic circulation and the coronary circulation. Significant decreases occurred in the mixed venous, arterial, and coronary sinus blood carbon dioxide content. The cardiac output increased significantly. While the total pulmonary and total peripheral resistance decreased, both right and left ventricular work were increased. Femoral arterial pH decreased slightly but consistently.

Coronary blood flow was variably affected, increasing in six, decreasing in three, and remaining unchanged in one of the animals. The overall trend was a slight increase (+10.8%) but this was not statistically significant. Coronary vascular resistance tended to be decreased (−12.8%) but again this was variable and not significant. The myocardial oxygen and carbon dioxide consumption did not change significantly. Consequently, in the face of increased cardiac work attributable to an increased cardiac output and elevated stroke volume, cardiac efficiency was significantly increased.

TABLE 1. *Hemodynamic effects of hypertonic sodium chloride solution*

Parameter	Control ± SD	Study ± SD	% Change	P Value
Heart rate, beats/min	73±16	85±17	+16.4	<0.01
Mean arterial blood press, mm Hg	130±20	127±15	−2.3	<0.4
Mean pulmonary arterial blood press, mm Hg	18±2	19±3	+5.6	>0.9
Mean right atrial blood press, mm Hg	5.3±2.1	5.3±1.6	0	0
Oxygen consumption, ml/min	122±21	127±22	+4.1	<0.2
Arterial oxygen content, ml/100 ml blood	17.5±1.8	15.4±1.8	−12.0	<0.001
Arterial-venous oxygen diff, ml/100 ml blood	4.3±1.0	3.0±0.6	−30.2	<0.01
Arterial hematocrit, %	42±5	35±4	−16.7	<0.001
Coronary sinus oxygen content, ml/100 ml blood	6.7±1.9	5.5±2.1	−17.9	<0.01
Cardiac output, liters/min	2.97±0.88	4.41±1.30	+48.5	<0.001
Stroke vol, ml	40±6	51±8	+27.5	<0.001
Left ventricular work, kg-m/min	5.3±2.1	7.8±3.0	+47.2	<0.01
Right ventricular work, kg-m/min	0.8±0.3	1.1±0.4	+37.5	<0.001
Total peripheral resistance, cgs units	3707±1064	2417±428	−34.8	<0.01
Total pulmonary resistance, cgs units	530±143	363±119	−31.5	<0.01
Coronary blood flow, ml/100 g per min	111±46	123±52	+10.8	<0.5
Coronary vascular resistance, units	1.33±0.56	1.16±0.37	−12.8	<0.4
Left ventricular oxygen usage, ml/100 g per min	12.1±4.9	11.8±5.0	−2.5	<0.9
Index of efficiency, LVW/LVO_2 usage	0.45±0.06	0.68±0.19	+51.1	<0.01

DISCUSSION

Hypertonic sodium chloride as administered in this investigation clearly has significant hemodynamic effects. Although the pH was changed slightly, it has been established that under the conditions of the present study, such a minor change does not reproduce these results. Thus, very minor changes occurred in cardiac output and coronary blood flow of dogs in response to a much greater pH change induced by administration of tromethamine (THAM) (8) and by mechanical hyperventilation (10).

The systemic hemodynamic effects of the sodium chloride solution given in this study could be explained at least in part by hemodilution, since there was a significant decrease in the hematocrit and in hemoglobin. The hemodilution is probably due to the osmotic effect of the hypertonic solution. It is possible that the change in cardiac output can also be explained in part by the Starling mechanism of increased myocardial stretching even though the right atrial and pulmonary arterial pressure remained unchanged. Since the end-diastolic pressure in the two ventricles was not measured, accurate data on their filling pressure are not available.

It is of interest that no real changes occurred in systemic or pulmonary arterial pressure with the administration of this quantity of hypertonic sodium chloride. This is particularly of interest since direct injection of hypertonic solutions into the peripheral arteries of dogs is known to produce an increase in pressure during constant perfusion (14). Although this was originally attributed to vasoconstriction (14), subsequent experiments would appear to have established that agglutination of red blood cells is a better explanation for the change in resistance to blood flow (6, 16). The fact that we found no evidence to support red cell agglutination in the pulmonary artery with pulmonary hypertension in the present experiments is very probably due to the rate of saline administration. Thus, in the present study, the rate of saline infusion was such that its dilution by blood passing the site of needle entry might well prevent the osmolarity of the blood and saline mixture from reaching a level which produces red cell agglutination (6).

The changes in the coronary circuit, although tending in the same direction as those in the systemic circuit, were not as pronounced and did not reach statistical significance. It is noted that the trends were similar with administration of 50% glucose (7) and molar sodium succinate (9), whereas the effects of molar sodium lactate (12) and sodium bicarbonate (13) were more pronounced on the coronary vessels. In these latter two agents a significant increase in coronary blood flow was produced, whereas in the two former agents significant increases in coronary flow did not occur. The difference would appear to be quantitative rather than qualitative since the trend was toward increase with each of these agents.

The present study also confirms the previous demonstration that a considerable increase in cardiac output and stroke volume may occur without a comparable increase in left ventricular oxygen consumption (17). Indeed, if the systemic arterial pressure is lowered at the same time as output is increased, left ventricular oxygen consumption may actually decrease and thus rather striking changes in calculated cardiac efficiency may be produced (17).

The administration of sodium chloride to hyponatremic human subjects produces a rapid rise in blood pressure; however, it is clear that the response of the circulatory system when passing from a low to a normonatremic state need not predict the response produced by passage from a normonatremic to hypernatremic state.

In normal anesthetized animals the total hemodynamic response was less with hypertonic sodium chloride than with administration of a similar quantity of hypertonic sodium bicarbonate. Such a response encourages the continued clinical use of sodium bicarbonate for hypotensive states related to inadequate cardiac output rather than other hypertonic solutions studied to date (7, 8, 11).

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D. H. McKenna and R. J. Corliss are both Postdoctoral Fe[illegible]s.

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EXPERIMENTAL MORPHOLOGY

THE ACTION OF AN EXCESS OF Na, K, Ca-CHLORIDES ON THE CYTOPLASMIC STRUCTURE OF EMBRYONIC TISSUE

By A. RUMYANTZEW

(Communicated by I. I. Schmalhausen, Member of the Academy, II. XI. 1938)

In an earlier report [1] we have found that excess of any kind of chloride differently affects cells growing in vitro: $CaCl_2$ induces a compression of the protoplasm, which leads to a 40% loss in size of the nuclei, while KCl provokes a rapid vacuolization of the protoplasm and swelling of the chondriome. A similar tendency towards vacuolization and swelling is observed also in the case of an excess of NaCl.

We regarded the vacuolization of cells as the result of swelling, and the compression of protoplasm as a process of its inspissation. This assumption needed verification. Since the alterations arising in the cellular structures during the swelling and unswelling of protoplasm have been ascertained with some precision, in particular by the author [2], we decided to study these alterations as they arise in our experiments. Material: bits of tissue of the myocardium of a 6—8 day old chicken embryo. Media: I series—one drop of the chloride solution under test + the smallest possible drop of plasm; II series—1 part of plasm + 2 parts of the solution under test. The tissue cultures were grown on slides and examined after 2—3 days in passing light or in the dark field. Intravital staining with neutral red (NR) or Janus green (JG). The total number of cultures studied in each series equalized 250, of which 50 were controls.

The first series of cultures supplied us with indications as to the specifity of the effect and thus supplemented the second, or the main experimental series. In the first series the tissue grew practically in a liquid medium. In the presence of KCl and $CaCl_2$ the growth was very scanty, more or less considerable zones of growth being observed only with NaCl. All the cultures degenerated in a short time. We were able to notice the following alterations.

NaCl. Growth spreading on the slide. The zone of growth is composed of flattened out cells. Their protoplasm is transparent, the fat droplets small and their number is less than in the controls. The shape of the cells is in some polygonal, in others rounded, in the others again elongated. The protoplasm becomes vacuolized already in the course of the first day. On the second day almost every cell is vacuolized and manifests evident signs that it is dying. The chondriosomes have a typical, i. e. band-like, appearance on the first day, while on the second day they are represented by rounded formations or have the shape of thick rods. Intravital staining is quickly achieved. On the second day NR staining gives rise to granules

of 2 types: fine ones with caps, and larger ones staining allover an orange tint. These larger granules are vacuoles which were visible also without staining. In the dark field the protoplasm appears optically empty. Protoplasm staining produces no great amount of new granulation. On the second day there appear alterations in the nuclei of the cells. These alterations consist in the loss of a regular shape and in the arising of small brilliant bodies.

In cultures of the 2nd series vacuolization appeared only on the second day. The vacuoles were usually small. The chondriosomes of the vacuolized cells showed alterations. The vacuoles and granules stained with NR similarly to those in the 1st series, but the amount of granulation was also less than in the controls. No alterations were observed in the nuclei. In some cases the growing cells formed—as the result of the fusion of the ectoplasm—a continuous membrane. Thus, so far as the character and the trend of the alterations were the same in both series, we are free to assume that the alterations which arise in cytoplasmic structures in the case of an excess of NaCl are the same as in that of swelling.

KCl. Series I. Most of the cells emerging into the medium possess no stellar shape, neither do they show any process. They are either round or polygonal, their protoplasm is visibly vacuolized. On the second day and sometimes already on the first isolated cells unite and form large vacuolized layers with altered nuclei. Sometimes, however, these layers fail to form, but the protoplasm of the cells spread out on the slide is always greatly vacuolized, the vacuoles reaching the size of the nucleus, as this has been also observed by Möllendorf ([3]). After staining such cultures it can be established that the chondriome is represented by thick rods and round swollen granules. NR-granulations are few, but the vacuoles of the protoplasm are intensely stained, the stain deposited in them acquiring an orange-red tint. No new granules or vacuoles are formed if the action of NR is prolonged, only the stain regularly flocculates in the vacuoles. In the dark field the protoplasm is optically empty and displays an intense motion of fat particles. The latter are not numerous and very fine.

Similar alterations, though not so marked, are observed in the second series. On the second day the protoplasm of most cells is vacuolized and there is a tendency towards the formation of acellular layer. The protoplasm is optically empty and liquified; the Brownian motion of fat particles is well visible. The chondriome is altered and increases in size, but does not form such clods and swollen blocks as in series I. After prolonged staining with NR there appear no additional stained granules in the strongly vacuolized cells. All this considered together suggests that in KCl as well there is a considerable hydration of the protoplasm; it is expressed in a great increase of the hydrous phases, and this results in the arising of vacuoles of considerable size.

$CaCl_2$. Growth always scanty. In the small growth zone of the culture of series I, if it does form, one sees almost no flattened polygonal cells. The cells are usually elongated or angular and are always supplied with thin processi. Among the angular cells one encounters smaller ones with short branching processi; the fat droplets in the protoplasm are always small. A fine vacuolization is sometimes noticeable in the protoplasm of the cells; at the borders of the growth zone compressed rounded cells are observed; they are small and show evident signs of degeneration. After staining with NR and JG it is easy to see, that NR accumulates in the form of fine granules in the endoplasm; their number increases after prolonged staining, but their size remains unchanged. The chondriosomes have the shape of fine granules or of thin rods; band-like chondriosomes, similar to those in normal cultures or in cultures with NaCl, are extremely scarce. In the dark field the proto-

plasm shines a milky, dull light; there is no Brownian motion, nor any flowing motions of the protoplasm.

In the second series the cells are mostly spindle-shaped, or angular, though flattened out cells are also encountered. The cells are linked by thin processi. The chondriosomes are almost normal. The fat droplets are fine. The numerous NR-granulations are fine and have an evident tendency towards increasing under a prolonged action of the stain. Such alteration, particularly if one takes into account the decrease of the cell size described earlier ([1]), obviously point to a dehydration of the protoplasm, which sets in in the case of an excess of $CaCl_2$ in the medium. On the second day there are many degenerating cells at the border of the zone of growth.

Comparing the results obtained with what was already known concerning the action of cations on protoplasm, we arrive at the conclusion that our observations are in good agreement with the views of Spek ([4]), Chambers ([5]), Fouré-Fremiet ([6]), Gellhorn ([7]) et al., who affirm that Na and K provoke liquefaction, while Ca leads to an inspissation of the protoplasm. It would be added that the above effect is observed not only when these cations are tested separately, but also when one of them is in excess, as this was the case in our experiments. Moreover, after the works by Spek ([8]), Chambers-Reznikoff ([5]), Lepeschkin ([9]) et al. it can be considered, that turbidity of the protoplasm is an indication of beginning coagulation under the influence of salt penetrating into the protoplasm. In $CaCl_2$ (ser. I) turbidity was observed already in the course of the first 24 hours, while in NaCl (ser. I) only after the cells began to die off, i. e. on the 2nd or 3rd day. Vacuolization of the protoplasm was observed in every case. This points to the fact that Na, K and Ca act not only on the surface of the cells, but penetrate into them. We know very little of the mechanism of the action of the penetrating cations on the particles of protoplasm, yet we can affirm that different cations affect them in a different way.

The difference in the staining with NR and in deposition (rapid staining in KCl and NaCl and a slower one in $CaCl_2$) and the inability to form new granules (Krinom according to Khlopin) (in KCl and NaCl contrary to $CaCl_2$) suffice to show that the condition of the protoplasm is different in these cases and its permeability is not the same.

Without attempting to analyze these conditions of the protoplasm, we decided to examine the only question, accessible to our investigation, i. e. whether the observed phenomena of demixing and coagulation are reversible or they are a sign of beginning irreversible degeneration. To solve this question we cut the bits of tissue, typically altered by the action of the salt under test, into pieces on the 3rd day of cultivation and transferred them into a fresh portion of the normal medium (1 part of plasm + 1 part of embryonic extract). After 24 hours we already could observe in the zone of growth healthy cells, differing in nothing from those of the control cultures. No great quantity of cell debris or of evidently degenerating cells was to be seen on the 2nd day neither. This seems to show the complete reversibility of such phenomena as vacuolization of the protoplasm, swelling and partial disruption of the chondriome, arising of fine granularity, etc. Only in the subcultures of those tissue cultures, in which we observed the formation of layers (cultures in KCl) or in which the nuclei of the cells acquired an angular or irregular shape, there was no growth, i. e. the protoplasm in these cases had suffered irreversible alterations.

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The relationship of salt intake to hypertension in the Japanese

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It is epidemiologically interesting that the patterns of death differ with various races. The time when the predominant causes of death were contagious diseases has passed, and now the chronic diseases which develop with advanced age have become important. At such time, it seems especially important to investigate the relationship between these chronic diseases and the habitual pattern of life of each race.

In Japan, too, deaths from contagious diseases such as tuberculosis decreased rapidly after World War II, and chronic diseases such as apoplexy, cardiac diseases, and cancer have become the main causes of death. There is an epidemiologically interesting problem in the pattern of death of the Japanese. The first of the causes of death is apoplexy, and deaths from cardiac diseases are comparatively few. This is the reverse of the situation in Europe and America. Scholars who have the opinion that atherosclerosis, the basis of much adult cardiac disease, is closely related to fat intake refer to the fact that deaths from cardiac diseases are fewer in Japan as evidence for their theory. However, no definite explanation has been given for the fact that apoplexy is common in Japan. There seems to be an opinion among some that in Japan all of acute deaths of older people are apt to be attributed to apoplexy because the diagnostic techniques are poor, leading to the results shown in the vital statistics. Histopathological examinations, however, have revealed that the order of apoplexy and cardiac diseases as causes of death is the same in autopsy series as in clinical diagnosis, and the mortality from apoplexy is still high.[1] Actually, the death from apoplexy reflects a variety of processes. In Japan, it remains to be solved whether the death from apoplexy is due to the cerebral infarction, intracranial hemorrhage, or other diseases, but it is assumed that the death from apoplexy of the Japanese, especially in

young adults, reflects hypertensive intracranial hemorrhage. This assumption is made because of the fact that severe atherosclerosis is rarely to be found and because of histopathological examinations and clinical pictures. Furthermore, recent investigations have brought out that there are fairly numerous persons suffering from hemiplegia, the aftereffect of an apoplectic attack. These facts suggest that the mortality from apoplexy is high in Japan.

The relationship of salt intake to hypertension in the Japanese

Features of mortalities from apoplexy Observations of mortality rates from apoplexy, the most dominant cause of death, have revealed extreme regional differences in Japan. The authors previously investigated the regional differences in mortality from apoplexy in Japan.[2] Comparative investigations were made on mortality classified by age between the Akita prefecture, showing the highest mortality from apoplexy in the northeastern district where the mortality was generally high, and the Okayama prefecture in the southwestern district where the mortality was low (Fig. 1, Table 1). In the Akita prefecture, the mortality was high even in the fourth and fifth decades. Investigations of the further details by causes of death revealed the following difference between the 2 prefectures. Difference in the mortality from B22 (vascular lesions affecting central nervous system) of the International Statistical Classification of Diseases and Causes of Death was most marked. Differences were hardly noted in the mortalities from the other diseases except those of the cardiovascular system which were related to B22. Thus, the representative picture of regional differences in the mortality from apoplexy in Japan was shown. This fact suggests that there is some factor leading people to early death and presenting a public health problem. Since it is recognized

Table 1 **Death rate in Akita and Okayama prefectures, 1960 (death rate per 100,000 males by each age group)***

	Akita prefecture							
Age	*30-34*	*35-39*	*40-44*	*45-49*	*50-54*	*55-59*	*60-64*	*65-69*
Total death rate	214.6	300.5	462.8	728.1	1,308.4	2,323.8	3,710.1	5,664.2
B22	20.5	37.9	93.7	206.3	496.8	1,045.1	1,700.5	2,559.7
B25-27	5.6	17.7	35.8	68.8	120.1	200.8	430.0	597.0
B28, 29	1.9	5.1	5.5	9.4	22.7	41.0	62.8	141.8
	Okayama prefecture							
Age	*30-34*	*35-39*	*40-44*	*45-49*	*50-54*	*55-59*	*60-64*	*65-69*
Total death rate	192.3	215.2	396.2	563.7	901.3	1,309.6	2,147.0	3,680.[illegible]
B22	6.2	11.3	35.8	94.3	167.1	337.6	550.4	1,149.1
B25-27	12.3	18.4	33.4	44.8	88.6	134.5	227.7	315.1
B28, 29	—	—	4.8	9.4	12.7	10.2	28.8	49.8

*Classifications as in figure 1

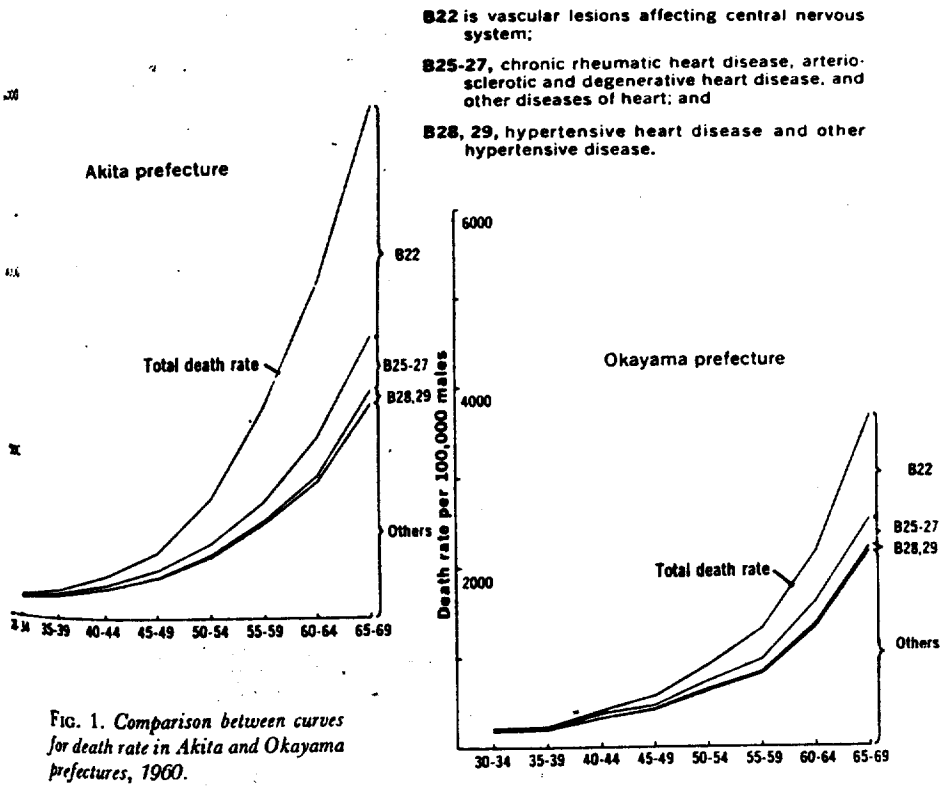

FIG. 1. *Comparison between curves for death rate in Akita and Okayama prefectures, 1960.*

that regional differences in mortality from apoplexy and in the level of blood pressure of the inhabitants are in a parallel relation, investigation of regional differences in the mortality is considered a clue to an elucidation of high blood pressure which is the primary cause for death from apoplexy. It is epidemiologically important to reveal development of a chronic disease in its early stage.

Blood pressures of the Japanese It has been reported that the blood pressures of the applicants for life insurance and the blood pressure of the Japanese from the data of *National Nutrition Survey* in Japan are nearly the same as those of Americans.[3]

However, there seem to be marked regional differences in blood pressures of the Japanese, as well as in the mortalities from apoplexy. The authors previously made comparative investigations on the results of measurement of blood pressures of various districts in Japan and reported on the regional differences.[2] Itahara and associates[4] reported that the frequency of essential hypertension in 4 localities in Japan, taking persons of over 20 years of age in active life in their respective home cities, increased as the north latitude increased. The authors made numerous mass measurements

of blood pressure of the inhabitants of the northeastern district.[5] The results are summarized in table 2. The values of not only middle-aged and elderly adults but also younger persons are much higher than the general values of the Japanese for all Japan obtained from the data of applicants for life insurance and *National Nutrition Survey*. Also, in the northeastern district, the blood pressures measured in the colder season below a mean temperature of 10° C. were higher than those measured in the warmer season above 10° C. In the fifth decade, those whose blood pressures were higher than 150 mm. Hg in the systolic blood pressure and 90 mm. Hg in the diastolic blood pressure were 32 to 38% in the warmer season and 39 to 59% in the colder season. These are the average values of the blood pressures of numerous inhabitants of the northeastern district which the authors measured. Therefore, the levels of blood pressure actually differ with communities, the ratio of hypertensive persons in the fifth decade being less than 10% in one community while it was over 70% in another. The authors also observed that where the level of blood pressure of the inhabitants was high, the mortality from apoplexy was also high.

The relationship of salt intake to hypertension in the Japanese

Factors related to regional differences From the epidemiological viewpoint, the observation that there are regional differences in mortality from apoplexy and levels of blood pressures in Japan is considered to give a good clue to clarification of essential hypertension. Studies have been made from various viewpoints, such as climate, characteristics of water and soil, labors, dietary habits, life stresses, blood groups, and living standards, to elucidate this regional difference. However, the correlation between regional differences in mortality from apoplexy and the foregoing factors are not more definite than the correlation between mortality and the habitual salt intake which we have reported.[6,7] From the data of the *Nutrition Survey of Farmers* made in 1958 by the Ministry of Agriculture and Forestry[8] we calculated and classified by prefecture the correlation between a farmer's average intake of each nutritive element per day and the mortality from apoplexy in the middle age (30 to 59 years). The correlation coefficients between mortality and intake of salt and fat, respectively, were +0.6572 and +0.4549 and were significant at less than the 1% level of significance. The larger the intake of salt or fat, the higher was the mortality. No significant correlation was noted between the mortality and the intake of calories, protein, calcium, vitamin A, vitamin B_1, vitamin B_2, or vitamin C. With regard to the types of food, significant correlations at less than the 1% level of significance were observed between mortality and pulses, seasonings, other vegetables and pickles, and the total food intake. The larger the intake, the higher was the mortality. In order to investigate the relationship between mortality and items of salt intake, the author examined the salt intake from the data of the *Nutrition Survey of Farmers*. The correlation with the mortality was investigated for salt in seasonings, miso, other vegetables and pickles, soy sauce, other foods, and total salt. It was significant at less than the 1% level of significance for salt in miso

Table 2 Systolic blood pressure of inhabitants in northeastern district of Japan (averages and standard deviations)

Age	Males: All Japan*	Males: I**	Males: II***	Females: All Japan*	Females: I**	Females: II***
6-11			109±13 (287)			110±15 (299)
12-14		118±13 (485)	122±15 (291)		123±13 (451)	123±16 (283)
15-19		131±14 (610)	139±13 (72)		127±13 (1176)	133±13 (114)
20-24	126±15	131±15 (998)	142±15 (167)	120±13	128±15 (688)	127±12 (308)
25-29	127±16			121±15		
30-34	127±16	131±17 (706)	142±19 (204)	120±15	127±17 (802)	133±19 (350)
35-39	127±17			122±17		
40-44	129±18	137±23 (752)	148±27 (283)	127±19	137±25 (871)	141±24 (476)
45-49	131±20			132±23		
50-54	137±25	148±27 (657)	160±28 (309)	138±24	147±28 (793)	157±30 (440)
55-59	146±28			142±28		
60-64	150±30	158±31 (359)	171±30 (203)	150±28	158±29 (466)	169±30 (275)
65-69	155±29			156±30		
70-74	158±27	170±32 (98)	185±35 (66)	161±30	174±30 (122)	177±30 (98)
75-79						
80-84		173 (6)	197 (6)		175 (6)	185 (13)
85-89						

Numbers in parentheses refer to numbers of persons in each age-sex group.

* *National Nutrition Survey* in November 1959

** Warmer season (May to Oct.); males (4,671); females (5,375)

*** Colder season (November to April); males (1,891); females (2,656)

(+0.7944), total salt (+0.6572), and salt in vegetables and pickles (+0.5282) and not significant in the others.

Salt intake in Japan The salt intake of the Japanese seems to be much larger than that of other nations; especially in the northeastern district, an extremely excessive amount of salt is considered to be taken. According to the *Nutrition Survey of Farmers*, an adult farmer of the northeastern district takes 27 gm. a day, while one of the Kinki district takes 17 gm.[9] Average salt intake of farmers per capita per day from 1953–58 in 11 farm districts is shown in figure 2. Fukuda[10] measured the excretion of chloride per day in the twenty-four-hour urine specimens of 293 farmers in the Akita prefecture and found 26.3 gm. of salt per day on the average, with a maximum of 50 gm. Takamatsu[11] reported that the salt excretion examined among 65 farmers in the Akita prefecture was 27.3 gm. on the average, with a maximum of 61.4 gm.; measured among 59 farmers of the Okayama prefecture, it was only 15.4 gm. on the average, with a maximum of 25.9 gm. Suzuki and associates[12] observed salt excretion of 27.3 gm. on the average, with a maximum of 41.4 gm., in examination of 36 male farmers in the Yamagata prefecture, and 21.5 gm. on the average, with a maximum of 30.6 gm., for 40 female farmers in the same prefecture. Dahl[13] observed 14 gm. of salt excretion among the inhabitants in Hiroshima. We[14] collected urine of 38 men and women of middle age at an agricultural village in the Akita prefecture for a minimum of ten days and maximum of twenty-two days and measured sodium by flame photometry; we found 18.1 gm. of salt a day on the average, with a maximum of 30.4 gm. and a minimum of 12 gm.

The relationship of salt intake to hypertension in the Japanese

In the districts where salt intake is high, mortality from apoplexy and hypertension is often noted. Fukuda[10] reported that the prevalence of hypertension was 30 to 40% in the inhabitants of about 50 years in Akita farm villages, and Takamatsu[11] and we, too, observed the same findings. Besides, as stated above, definite correlation is observed between regional differences in salt intake and in mortality from apoplexy. Therefore, it is necessary to investigate why the salt intake of the Japanese is so high and why there should be such differences in salt intake.

The salt intake is related to long-standing eating habits. Japanese people take salt mainly in the form of (1) soy sauce, (2) seasonings or table salt, (3) miso, (4) pickles, and (5) other foods. According to the survey, Japanese farmers take 6.5 gm. of salt from soy sauce, 5.83 gm. from seasonings, 3.14 gm. from miso, 2.53 gm. from other vegetables and pickles, and 1.21 gm. from other foods per capita per day, respectively, and the total salt intake is 19.21 gm. on the average.

According to the Standard Tables of Food Composition in Japan, salt contained in soy sauce was 18 gm. per 100 ml., but the results of our analysis of the materials sold in the northeastern district[5] showed 16.8 to 25.2 gm., with an average of 21.4 gm. However, no regional differences were observed in the salt content of soy sauce among various districts of Japan.

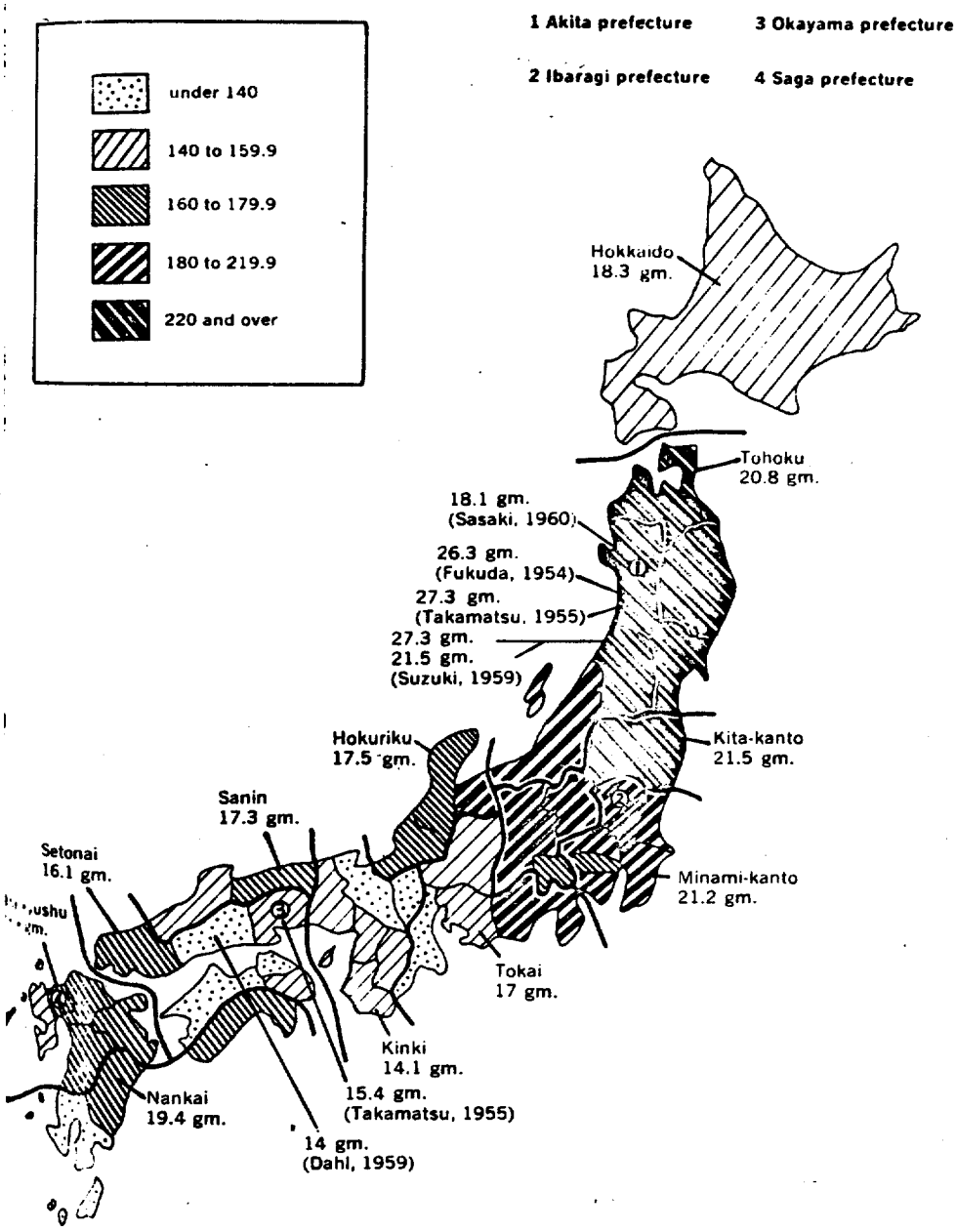

Fig. 2. *Distribution of age-adjusted death rate per 100,000 males from cerebral apoplexy (B22) by 46 prefectures (1960). Average daily salt intake in grams per day of farmers in 11 farm districts (1953-58)*

Miso is made from soybeans, yeast, and salt; it is classified as commercial miso made in factories and homemade miso. According to the analysis table, "sweet miso" contains 7% salt and "salty miso" 12%, but miso actually sold in various districts contained a maximum of 15%, a minimum of 4%, and an average 12.1% salt; regional differences were marked. Generally, miso in the northeastern district contained more salt, while that in the southwestern district contained less, and a significant correlation was found between regional differences in the amount of salt in miso and that in the mortality from apoplexy.[16] As to homemade miso, the ratio of salt to soybeans and yeast differed with districts and homes, and generally the ratio was higher in the northeastern district than in the southwestern district.

The relationship of salt intake to hypertension in the Japanese

In the actual daily life, Japanese people take miso in miso soup at meals, and regional differences were noted in the salt concentration of miso soup.[17] Samples of 3,758 kinds of miso soup were collected and analyzed from 4 districts: the Akita prefecture in the northeastern district, the Ibaragi prefecture where the mortality from apoplexy was high, the Okayama prefecture where the mortality was low, and the Saga prefecture where it was relatively high. Miso soup contained 1.5% of salt in the Akita prefecture, 1.63% in Ibaragi, 1.44% in Okayama, and 1.47% in Saga. The average was 1.51%. As to a frequency of miso soup consumption, it was quite different with districts (Fig. 3, Table 3). In the Akita prefecture it was served 2.4 times a day; in Ibaragi, 2.4 times; in Okayama, 1 time; and in Saga, 1.2 times. Naturally there were great individual varia-

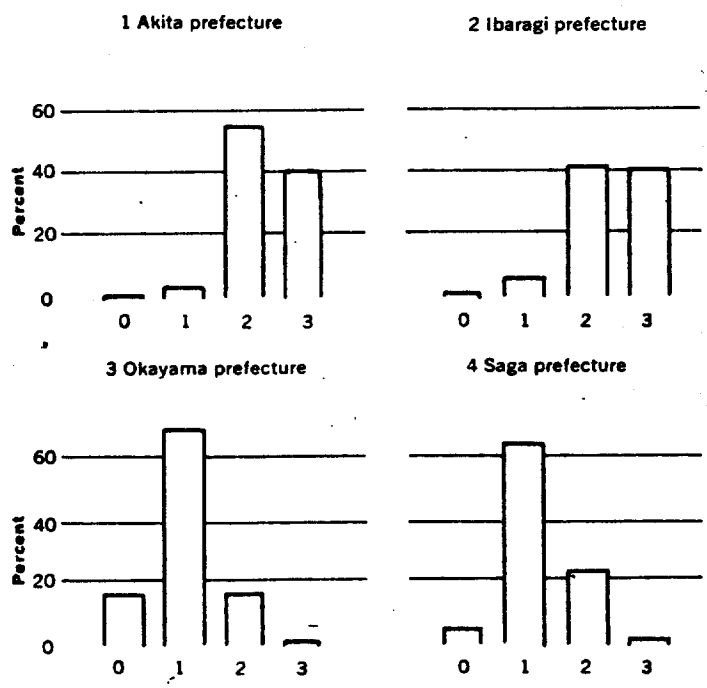

Fig. 3. *Times miso soup eaten a day among 4 prefectures*

tions in salt intake depending upon how many cups were taken at a time. A cup usually contains 150 to 200 ml.

Differences in salt content of pickles were not definitely shown in the analysis table. According to the values used for calculation in the *Nutrition Survey of Farmers*, "takuan" (pickled radish) contained 11.9 gm. of salt per 100 gm., and the other pickles contained 4.12 gm. However, fairly marked regional differences were believed to prevail in the salt content of pickles, since the pickling methods differed with each district. Pickles of the northeastern district seemed to contain more salt. In the Akita prefecture, a habit was noted of eating many pickles with tea between meals.

In addition to miso and pickles, salted fish and preserved food contained much salt. The factors determining the salt intake seem to be the habitual eating habits and the pattern of dietary life of each district. In Japan, the daily salt intake is regulated by the habit of taking miso soup, the number of times a day the soup is taken, the number of cups taken, the habit of eating pickles, and the habit of using soy sauce as a seasoning. Such habits have been produced by the history of Japanese life which is related to the Japanese climate.

On the other hand, when the nutritional requirements were determined in Japan, 13 gm. of salt a day per person was established. The reason given for this amount was that the Japanese take much vegetable food and so consume a large amount of potassium; therefore, the necessary amount of salt was considered larger than that for Europeans or Americans. The Japanese preference and taste also were taken into consideration. This opinion was considered to be supported by the experiment and reasoning presented by Bunge[18] in 1873. There is a question, however, because recent observations of metabolism of sodium and potassium are different from

Table 3 **Comparison of times miso soup eaten a day among 4 prefectures**

Times miso soup eaten a day	*Akita*		*Ibaragi*		*Okayama*		*Saga*	
	*	%		%		%		%
3	751	41.6	670	45.5	8	0.5	29	2.3
2	945	52.3	686	46.5	239	13.7	330	26.0
1	99	5.5	106	7.2	1,224	70.0	806	63.4
0	10	0.6	12	0.8	276	15.8	106	8.3
	1,805	100	1,474	100	1,747	100	1,271	100

what Bunge reasoned. The authors also were unable to bring out any evidence to prove that the Japanese salt intake was high because the potassium intake was large. As stated above, the salt intake seemed to be determined by the eating habits of the district, and there was an observation to suggest that potassium rather prevents chronic salt intoxication. Meneely and associates[19] demonstrated this phenomenon in animal experiments. The authors found that the mortality from apoplexy and the blood pressure levels were lower in the Aomori prefecture where the inhabitants ate more apples containing abundant potassium daily than in the neighboring Akita prefecture; they have been making various investigations on this point.[7] Perhaps attention should be paid to the balance between sodium and potassium in food and drink.

The relationship of salt intake to hypertension in the Japanese

It seems necessary to consider the relation of salt intake in daily life to pathogenesis of diseases of the cardiovascular system which is commanding increasing attention with changes in problems of human health. Comparative investigations between salt intake and cardiovascular disease in all the races are necessary, and the relation between excessive salt intake and the high ratios of apoplexy and hypertension in Japan, especially in the northeastern district, is worth noticing.

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SOME EFFECTS OF CONSTANT INTRAVENOUS INJECTION OF POTASSIUM CHLORIDE SOLUTIONS INTO NORMAL CONSCIOUS DOGS

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THE MAJORITY of workers are in disagreement as to the nature of the fundamental cause or causes of adrenal insufficiency. The most widely held theory is that the syndrome is due to some defect in salt and water metabolism. Some have suggested that defective sodium metabolism is the primary cause (1, 2, 3); others, that it is a failure of the blood-diluting mechanism (4, 5, 6, but see also 7); and still others, that the syndrome is due to hyperpotassemia (8, 9, 10).

Various workers have induced some of the symptoms of adrenal insufficiency by KCl administration, but the majority of the authors have not examined their animals or experimental subjects on more than a few of the facets of the problem. Zwemer and Truszkowski (11) present the most comprehensive report to date, but their report lacks details of experimental technic and treats only subjectively of the symptoms of adrenal insufficiency. In their experiments, after intraperitoneal injections of KCl solutions, the blood potassium level of otherwise normal cats rose and was maintained at high levels by subsequent injections. Anorexia was present when the serum potassium was 30 mg.%, severe muscular asthenia appeared when the serum potassium was above 40 mg.%, convulsions were obtained at about 50 mg.%, and death occurred at 60 mg.%. In measuring the rate of disappearance of potassium from the blood stream, Whelan (12) injected 10% KCl intravenously into dogs and found an increase in urine sodium like that of adrenal insufficiency. In normal human beings, intravenous injections of KCl solutions produced hemoconcentration, a symptom prominent in Addison's disease (13). Winkler et al. (14) injected isosmotic KCl solutions (intravenously) into normal dogs and obtained electrocardiographic records of some of the phenomena reported in dogs with adrenal insufficiency (15). In acute experiments, Hastings and Compere (8) injected KCl intravenously into dogs and reported that death occurred when the potassium in the serum reached 80 mg.%, a concentration lethal to the mammalian heart in vitro (16) and in vivo (14). Low blood sugar values after injection of potassium salts have been observed (17). Keith and Binger (18) reported the experimental induction of high levels of serum potassium in normal human beings and the presence of equally high potassium levels in diseased conditions without concomitant clinical symptoms of potassium poisoning. Thus it can be seen that each single report is concerned with only a few of the changes due to hyperpotassemia. In the experiments to be described, therefore, an attempt has been made to record quantitative as well as subjective data on as many different aspects of adrenal insufficiency as possible.

It should be noted that most of the reports in the literature are concerned with acute experiments in which the injections of potassium were made during a few minutes or a few hours. In our preliminary experiments an attempt was made to maintain constant intravenous injections of KCl solutions in such amount and concentrations that the individual dogs would live as long as untreated adrenalectomized dogs. It was found that 6 to 8% KCl injected at a rate of 6 cc./hr. was lethal after 2 to 12 days.

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The injection of 6 cc./hr. produced a minimum of blood dilution. Despite the fact that the injected material has an osmotic strength 4 to 6 times as great as that of blood, no hemoglobinuria was observed at any time.

METHODS

A dog was lightly anesthetized with pentobarbital sodium (Nembutal), 32 mg./kg., and a small longitudinal incision was made in the neck near the jugular vein, rigid asepsis being observed. A white rubber tube of standard make (3/16 in. diameter with 1/16 in. bore)[1] was put into the jugular vein proximal to a tight ligature and was pushed cardiad for 6 or 8 inches. This tube, serving as an elongate cannula, was tied in place by two tight ligatures around the vein wall, leaving 5 or 6 inches of tube outside the vein. A piece of ordinary tire-patching was cemented on the tube about 3 inches from the external end. A needle was threaded, using a foot or so of linen thread which was tied to the end of the tube. This needle was pushed through the subcutaneous spaces and out through the skin about an inch caudad to the inion. Thus, when the thread was pulled, the tube followed it to a subcutaneous position near the middle of the back of the neck. The incision near the jugular vein was closed, and the dog was bandaged and allowed to recover.

On the third postoperative day, the bandage and stitches were removed. After local anesthesia had been induced in the skin at the back of the neck, the thread was pulled until the tube was brought out of the dog's body to the point at which the tire-patching was directly beneath the skin. In this position, the tire-patching prevented the tube from pulling out of the animal. Another piece of tire-patching was cemented to the tube outside of the skin to prevent the tube from slipping back beneath the surface. Through this tube, intravascular injections of any amount of fluid could be made at any desired rate. Blood samples could be withdrawn through the tube rapidly and efficiently. When a sample was to be drawn, about 1 cc. of Ringer's solution was injected through the tube and then 1 cc. or more of blood slowly withdrawn before the sample was collected. The whole process of injection and sample collection was painless, simple and convenient.

The tube was held in one position on the dog's neck by a heavy leather collar. By means of a constant injection pump, fluids were injected into a system of rubber tubes which led into the intravenous tube. The rate of injection could be varied from 1 to 40 cc. per hour. In all the experiments here described, a rate of about 6 cc./hr. was used, thus preventing undue blood dilution. The dogs were kept in metabolism cages. A chain from the side of the cage to the dog's collar allowed free but not excessive movement.

Determinations were made of the serum and urine content: (*a*) of potassium, by the microphotoelectric method of Hoffman (19); (*b*) of sodium, by the method of Hoffman and Osgood (20); (*c*) of chloride, by the method of Patterson (21); (*d*) of urea, by the method of Van Slyke (22) and (*e*) of glucose, by the method of Miller and Van Slyke (23). Rectal temperatures were recorded in some instances, and hematocrit determinations were made by centrifuging citrated or heparinized blood in capillary tubes.

RESULTS

The data on 6 dogs are presented in tables 1 through 6. The urine volumes reported below are those collected as the dog voided them. Each sample was analyzed as a unit.

[1] Manufactured by Salisbury Rubber Company.

TABLE 1. PROTOCOL ON *dog 1*, INJECTED WITH 6% KCl AT A RATE OF 6 CC./HOUR

Hr. Inj.	Hematocrit	Serum			Urine							
		K^+	Na^+	Cl^-	Vol.	Per hr.	K^+	K^+	Na^+	Na^+	Cl^-	Cl^-
	% cells	mg.%	m.eq./l.	m.eq./l.	cc.	cc.	mg.%	gm.	m.eq./l.	m.eq.	m.eq./l.	m.eq.
0	38.8	17.5	156	102.6								
4½	43.5	13.8	171	85.9								
17½	42.7	19.0	168	103.7								
29½		20.5										
42½	36.1	19.0	165	102.8	250	4.7	2580	6.45	64	16.0	233	58.3
52½	27.8	17.8	168	99.1								
72					200	7.0	3640	7.28	34	6.8	166	33.2
78½	29.9	28.7	168	98.8								
90½		23.8	166	96.4	215	11.3	3450	5.27	12	2.5	406	87.3
101½	29.9		170	102.3								
111½	Found Dead				188	17.9	2260	4.25	11	2.0	526	98.9

The last serum sample from *dog 2* was drawn at the very moment of death. The injection pump had been disconnected 5 to 7 minutes before the heart ceased to beat, so blood from the auricle (for autopsy showed this to be the location of the end of the tube) was a well mixed sample.

None of the gross symptoms of adrenal insufficiency was seen. At no time did the animals exhibit the asthenia reported in cats (11) and in dogs (8). It should be emphasized that the dogs were visited at least once each 5 hours so that there could have been no severe or prolonged collapse or asthenia without its having been noticed and recorded. The interesting dog in this connection was *dog 2*. This dog remained in good spirits until after urine *sample 8* was collected. A severe lassitude, but no weakness, appeared during the following 2 hours. The tube was disconnected from the injection pump and several minutes passed before the last blood sample was drawn. And in the next 5 minutes the dog collapsed and died. The reactions of the dogs to the manipulations necessary to obtain rectal temperatures were normal. Although they did become accustomed to the handling, muscular tension was always present and the dogs promptly jumped to their feet when the thermometer was withdrawn. There was no sign of the convulsions described by Zwemer and Truszkowski (11).

The dogs were offered a large bowl of cooked beef lung and bread at the same time each day, and in no case, except within a few hours of death did the food go untouched. The daily food consumption toward the end of the experiment was the same as during the control period, and the daily ration was more than enough to maintain body weight. In contrast to the findings of Zwemer and Truszkowski (11) in cats, these dogs had no indication of anorexia. Although no quantitative tests were

TABLE 2. PROTOCOL ON *dog 2*, INJECTED WITH 8% KCl AT A RATE OF 6 CC./HOUR

Hr. Inj.	Serum			Urine				
	K^+	Na^+	Urea N	Vol.	Per hr.	Na^+	Na^+	Urea N
	mg. %	m. eq./l.	mg. %	cc.	cc.	m. eq./l.	m. eq.	mg. %
0	17.5	161	19.5	295	6.1	77	22.7	749
2				55	27.5	40	2.2	874
6	31.2	154	14.6	85	21.2	177	15.0	297
12½	24.1	148	12.2	100	14.3	72	7.2	228
18½	30.6	175		45	7.5	43	1.9	689
33½				143	9.5	65	9.3	802
40½	32.6	156		100	14.3	73	7.3	925
43½				78	26.0	80	6.2	955
45	58.1	150	27.5	45	22.5	90	4.1	817
46	Died							

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TABLE 3. PROTOCOL ON *dog* 3, INJECTED WITH 6% KCl AT A RATE OF 6 CC./HOUR

Hr. Inj.	Serum		Urine					
	K^+	Na^+	Vol.	Per hr.	K^+	K^+	Na^+	Na^+
	mg.%	*m.eq./l.*	*cc.*	*cc.*	*mg.%*	*gm.*	*m.eq./l.*	*m.eq.*
0	17.8	166	185	7.7	250	0.46	13	2.4
8	15.5	153	55	9.2	760	0.7	94	8.7
21¼	19.0	150	300	23.0	440	1.3	141	42.3
33¼	27.5	152						
81	20.1	160	380	6.3	1700	6.46	93	35.3
105	25.3	186						
119¼	24.0	176	230	6.0	1560	3.58	75	17.3
142¼	20.0	148	280	11.3	3800	10.64	59	15.3
154¼	30.0	140	185	15.4	1850	3.32	29	5.4
165	21.2	132	46	4.2	3110	1.43	19	0.9
175	34.8	134	217	21.7	4040	8.77	46	10.0
190¼	50.5	142	243	16.2	2090	5.08	67	16.3
217¼	42.0	148	690	25.5	1090	7.49	65	44.8
238			375	17.9	2750	10.3	25	9.4
266	39.3	152	375	13.4	3725	13.97	33	12.4
269¼	Found Dead							

performed, as far as one could see, the dogs were perfectly normal with regard to appetite, posture, and general behavior.

DISCUSSION

The theory that the symptoms of adrenal insufficiency are due to potassium poisoning is not upheld by the data. Objectively, the only symptom indicated by the

TABLE 4. PROTOCOL ON *dog* 4, INJECTED WITH 6% KCl AT A RATE OF 6 CC./HOUR

Hr. Inj.	Rectal temp.	Serum				Urine					
		K^+	Na^+	Cl^+	Urea N	Vol.	Per hr.	K^+	Na^+	Cl^-	Urea N
	°C.	*mg.%*	*m.eq./l.*	*m.eq./l.*	*mg.%*	*cc.*	*cc.*	*mg.%*	*meq./l.*	*meq./l.*	*mg.%*
0		9.2	162	91.0	18.8						
0	40.0					235	14.2	460	22	42.6	124
12	40.5	23.0	174	91.5	27.5	230	11.3	1930	154	80	865
16½	40.3					230	51.1	3480	140	244	820
20½	39.8										
24	40.2	20.8	161	93.0	26.9						
33¾	40.0					280	16.4	4600	60	396	859
37½	40.0	33.6	170	93.1	22.1	83	20.8	2625	61	384	598
48	40.0	22.5	153	95.4	24.0	70	6.6	5400	57	378	688
57½						66	6.9	4745	82	416	1107
60		20.0									
63½	39.0					120	15.0	2315	91	455	765
71½	39.3	21.1	156	104.0	28.8	165	26.0	2170	84	426	434
81½						110	11.0	6080	89	369	1039
84		24.9	169	109.0	23.4						
94½	39.6					150	11.5	2800	43	345	623
96	39.0	11.2	156	103.0	27.7						
106½	39.5					130	10.8	3380	43	165	763
108½	39.6	28.2	190	123.0	23.7	110	55.0	4800	70	400	651
117						70	8.2	4200	40	275	778
120	40.0	16.3	154	107.0	29.2						
131½	39.5	34.3	174	121.0	21.2						
135						205	11.3	5220	71	330	1400
144		28.0	180	115.0	21.0	125	14.0	3600	100	410	739
156	Found Dead					110	9.2	5350	191	390	1300

serum, as we were measuring it, occurred. It was observed that there was no consistent relationship between serum sodium and potassium changes.

It can be seen that it was very difficult to increase the serum potassium by a very great margin. Even the injection of KCl in such amounts as were lethal in 48 hours failed to maintain the serum potassium much above 30 mg. % until near death.

In 1883, Bochefontaine (28) found that the injection of 2 gm. of potassium salts would kill a dog. A more recent study (14) showed that the injection of 10 cc. of 1.12% KCl solution per minute would kill their dogs in about 50 minutes. With the constant injection pump employed in our experiments, a much larger amount of potassium had to be injected before the animals succumbed. The data on *dog 3* shows that about 400 mg. KCl were injected each hour. Since the dog survived 265 hours of injection, this would make the lethal amount of KCl 90 gm. The difference between our results and those of previous workers probably can be explained on the basis of rate of injection. At the moderately slow rate of injection used the animals excreted enough

TABLE 6. PROTOCOL ON *dog 6*, INJECTED WITH 6% KCl AT A RATE OF 6 CC./HOUR

Hr. Inj.	Rectal temp.	Hematocrit	Serum					Urine						Ate food
			K^+	Na^+	Cl^-	Urea N	Glucose	Vol.	Per hr.	K^+	Na^+	Cl^-	Urea N	
	°C.	% cells	mg.%	m.eq./l.	m.eq./l.	mg.%	mg.%	cc.	cc.	mg.%	m.eq./l.	m.eq./l.	mg.%	
0		38.8	13.6	162	95.3	25.9								
0		36.8	13.8	153	85.6	26.0								•
0	39.6	34.6	10.8	164	81.8	26.2	96							
0	40.1	33.4	12.2	163	91.8	26.2	79	135	9.0	16	3	18.7	1600	
0	40.3							110	24.4	117	3	4.0	2354	•
0	40.1	31.3	11.0	162	92.7	26.4	91	135	19.4	36	1	19.5	1468	
12	40.0	33.1	15.1	162	94.1	13.9	67							
15½								85	5.5	480	8	180.8	1440	
21½	40.9							200	33.3	635	9	102.2	296	
23½		33.7	17.3	163	91.7	12.3	72							
34	40.2							150	12.5	920	9	304.7	1419	
36½		34.2	15.5	145	91.1	18.8	69							•
48	40.2	37.2	19.0	137	91.1	19.3	85							
54		Died						170	8.5	805	6	360.5	1236	

potassium to survive much longer periods. Undoubtedly much smaller amounts would have killed the dogs had the injections been made suddenly. As an example of the importance of the rate of injection, Winkler et al. (14) report a dog which survived 4.29 gm. of KCl (in isosmotic solution) injected in 54 minutes, but which succumbed at a later date to a much smaller amount, 0.89 gm. (as 9% KCl) when the injection was made in 3 minutes.

The hematocrit values as reported in the tables obviously indicate no hemoconcentration, on the contrary, a slight dilution is evident. This result fails to confirm the work of Levy (13) on normal human beings after potassium injections.

The values for serum glucose show fairly large, but not unusual variations. As can be seen from the tables, the serum glucose rose following the consumption of meals all of which contained bread. Most significant is the fact that at no time did the blood sugar reach dangerous or convulsive levels (25–35 mg.%).

There was no great impairment of the ability of the kidneys to concentrate urea, nor did the excretory rate vary significantly from the normal. Despite the great load of potassium thrust upon the kidneys, the serum urea levels remained reasonably constant. This may be taken as definite evidence that the kidneys were not injured by the potassium.

An attempt has been made to correlate urine volume with the other data collected, but no definite relations could be found. From the data on *dog 2*, it appeared as if the urine volume could be correlated with sodium concentration in the urine, but the records of the other dogs showed that no such correlation could be made. Not even by

data is a high urine sodium. The serum sodium was not consistently altered. The sodium level in the serum of *dog 1* was above the normal level during most of the period of injection, that of *dog 3* was below the normal level; and those of *dog 2, 4, 5* and *6* varied, being sometimes above and sometimes below the normal value. These data do not confirm the contention of Blum et al. (24) that serum sodium falls after the administration of potassium, but are in accord with the work of Whelan (12) who found that intravenous injections of KCl reduced the serum sodium of only one dog in six. The high urine sodium following administration of potassium salts confirms the work of many authors (12, 25). The data show that although there were rises in urine sodium concentrations and in sodium excretion rates (milli-equivalents per hour) dur-

TABLE 5. PROTOCOL ON *dog 5*, INJECTED WITH 6% KCl AT A RATE OF 6 CC./HOUR

Hr. Inj.	Rectal temp.	Hematocrit	Serum					Urine						Ate food
			K^+	Na^+	Cl^-	Urea N	Glucose	Vol.	Per hr.	K^+	Na^+	Cl^-	Urea N	
	°C.	% cells	mg.%	m.eq./l.	m.eq./l.	mg.%	mg.%	cc.	cc.	mg.%	m.eq./l.	m.eq./l.	mg.%	
0	39.0							160	11.0	100	101	91	1318	
0	39.3							230	21.0	35	83	122	934	
0		37.6	15.0	135	103.1		84							•
0	39.2	39.8	14.3	140	94.9	17.4	82							
0	40.0	38.7	14.6	134	102.7	23.8	71	260	20.8	40	65	116	470	
0	39.0	38.9	15.0	140	97.3	22.3	92	250	17.9	17	62	93	847	•
0	39.1	35.9	14.3	138		10.2	91							
0	38.9	40.5	10.7	136	101.4	27.8	101							
12½	40.9	37.9	19.3	139	77.9		83	185	8.2	980	122	399	1130	•
20½	40.9							80	10.0	850	113	364	305	
22½	40.9							40	20.0	400	37	208	197	
24½	41.0	35.3	14.4	129	91.6	13.5	97							
35½	40.1	33.2	17.0	135	94.7	11.1	69	125	9.7	1035	56	940	573	•
43	40.9							115	25.2	1375	15	1035	372	
48		34.5	15.9	129	93.1	11.9	85							
54½	40.5							105	9.0	1520	17	795	1048	
60½		35.5	16.9	131	93.0	8.2	91							
62½	40.1							225	29.0	51	2	437	23	•
70½	41.0							110	13.6	1405	42	119	390	
72½		32.3	25.8	143	111.7	12.6	82							
81½		31.8	15.2	143		12.6	109							
86½								120	7.5	1500	35	595	1290	
92½	40.0	35.2	19.7	141	100.6	15.6	55							
98	40.4							157	13.6	1335	34	610	1185	
101½	40.9	33.5	18.9	132	90.7	11.8	45							
103½								100	18.2	1250	32	800	709	
111½		38.0	33.1	119	93.3	22.5	86							
114	37.9							50	4.8	1700	9	405	760	
117								38	12.2	1480	8	202	940	
121½	36.4	30.6	39.0	145	98.2	32.4	137							
127½								110	10.0	1550	12	367	725	
131	37.0	26.7	38.8	147	104.5	40.1	143							
135½			Died					40	4.5	355	17	182	245	

ing the early hours of injection, both these values later decreased to the normal range or below. The animals continued to eat their daily rations of cooked beef lung and bread, so that the incoming sodium was normal in amount. Thus, although there was a tendency toward a negative sodium balance like that observed in adrenal insufficiency, the animal itself corrected the condition before any of the symptoms of sodium deficiency appeared. The dogs did not show the asthenia described in human sodium depletion (3).

The serum sodium values ran somewhat higher than those reported by most workers (26, 27), although they are in the range reported by Whelan (12). We are at a loss to account for these high values as: the same range of values were obtained in completely normal dogs, so evidently the technic itself was not responsible; nor could any sodium be found in the mineral oil under which the blood was collected; nor does it appear to be the chemical method, for rat serum, using this method, gave values of about 145 milli-equivalents per liter which level is normal for this species. It is clear, at any rate, that no consistent drop in the sodium concentration in the

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plotting the observed urine volumes as percentages of the normal could the urine volume, as recorded, be related to the serum or urine content of potassium, sodium, chloride, or urea. The data certainly do not indicate anuria with high serum potassium (29) nor was there a marked diuresis (12, 30). The small increase in urine volume was of the same order of magnitude as that found in man by Keith and Binger (18) after oral administration of KCl.

The analyses for serum and urine chloride led to no definite conclusions. The urinary chloride accounted for less than one-half of the urinary potassium, thus leading to the assumption that much of the base in the urine was balanced by other anions, probably by bicarbonate. *Dog 4*, for instance, excreted about 1600 m.eq. of potassium and only about 750 m.eq. of chloride during the period of observation. These data not only fail to account for the anion accompanying the urinary base, but fail to account for one-quarter of the thousand or so milli-equivalents of chloride that were injected. The 250 m.eq. of chloride injected but not recovered in the urine might have: (*a*) remained in the blood stream, although the data do not indicate this; or (*b*) been excreted by way of the feces, although Harrop (25) thinks this unlikely; or (*c*) been stored in the tissue cells, but Bourdillon (31) and Hastings and Eichelberger (27) have reported data that render this unlikely. More complete analyses might have shown the location of the last one-fourth of the injected chloride.

Our work lends no support to any existing theory of adrenal insufficiency, but it does throw serious doubt on the theory that potassium poisoning is the responsible factor. In confirmation of this, several instances of crisis, shock, or collapse with normal or low potassium levels in the serum, both in experimental animals with adrenal insufficiency and in Addison's disease, were mentioned in the discussions at Cold Spring Harbor. Without directly confirming any theory, our work indicates that the rôle of potassium in adrenal insufficiency is probably a secondary one, perhaps like that suggested by Britton, Silvette and Kline (26). These authors propose a theory that regards the elevated serum potassium as secondary to a change in permeability of tissue cells (as a source of the potassium), concomitant with changed renal function (accounting for the prevention of potassium loss). They ascribe all the symptoms of adrenal insufficiency to the fundamental change in membrane permeability, while our work merely indicates that the symptoms cannot be considered to be the result of the elevated serum potassium.

Now let us consider the results presented in this paper in the light of those reported by Richter and Eckert (32), Rubin and Krick (33), and Sandberg, Perla and Holly (34). Richter and Eckert found that the adrenalectomized rats, given their choice of solutions of several salts of sodium, KCl and distilled water, 'chose' to drink more KCl solution than did normal rats. That these rats chose well is shown by the fact that their choice maintained their lives. It would appear that the salt hunger of adrenal insufficiency extends not only to NaCl and Na lactate, but also to KCl in measurable amounts. Rubin and Krick showed that normal rats, eating only as much as adrenalectomized rats, had negative balances of sodium, potassium and chloride, which facts are the basis of their explanation of the high serum potassium as a secondary phenomenon. Sandberg, Perla, and Holly demonstrated that, of the food they ate, adrenalectomized rats retained less potassium than did normals. In other words, the excretion of exogenous potassium is no problem to the adrenalectomized rat. The 'hunger' of adrenalectomized rats for potassium salts, the explanation of their negative salt balance by comparison with normal rats, and our inability to produce the significant symptoms of adrenal insufficiency in dogs by injection of potassium salt, minimize the importance of the rôle of potassium as a primary factor in adrenal insufficiency.

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SUMMARY

A technic for constant intravenous injections into normal conscious dogs is briefly described. Solutions of KCl were injected by this technic into 6 dogs, leading eventually to their deaths in from 2 to 12 days. Determinations of blood concentration (hematocrit), body temperature, and serum and urine content of potassium, sodium, chloride, glucose and urea nitrogen were made at intervals throughout the period of the experiment. The dogs showed only one of the symptoms of adrenal insufficiency, namely, an increase in urine sodium. But this change cannot be regarded as of much significance as it was only temporary. No anorexia, asthenia, or convulsions were observed in any of the animals. From these data, it seems doubtful that potassium poisoning is the primary cause of the symptoms of adrenal insufficiency in the dog.

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Papers and Addresses

TREATMENT BASED ON PHYSICAL PRINCIPLES FOLLOWED BY RECOVERY IN SODIUM CHLORIDE POISONING*

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The study of the action of water and of sodium chloride solutions of different concentrations when introduced into the living organism began to assume a scientific aspect approximately a century ago, having been extensively and intensively pursued since this early period. As a result of the investigations stimulated by the work of the Dutch botanist de Vries, the conception became dominant a few decades ago, and remains dominant, that sodium chloride possesses practically no specific action, that "its action is limited to alterations in the physical properties of the fluids which its presence in excess or limited amount induces and that the action of water is due to diluting the body fluids and lessening their osmotic pressures". A slight specific effect of the sodium ion has been demonstrated, however, upon the ova of fish, on nerves, and on the heart. But the presence of calcium and potassium ions, and possibly a few other ions, counteract this effect which, according to Loeb, explains why tissues live longer in Ringer's solution than in the physiological sodium chloride solution. Accordingly, the effects due to saline solutions differing in concentration from the blood and those due to water are respectively predominantly and solely of anosmotic origin, i. e., of physical origin, and, to the extent to which these effects may be prevented or removed osmotically, these agents are reciprocally antidotal. By the nature of things concerned, therefore, it would seem that, above all others engaged in therapeutic endeavor, the treatment of poisoning produced by sodium chloride or by water should claim the interest of the physical therapist.

In view of the early point at which investigation began to take a scientific turn in this field, the paucity of cases of sodium chloride poisoning mentioned in the literature and the outcome in these cases cannot fail to prove arresting. For, on painstaking search, the original reports were yielded of only four such cases and mention encountered in other communications of only four others[1], and fatal termination was encountered in each of these cases cited. Campbell (1912) reports the case of a five year old child in which death occurred after an enema of strong salt water was given as a cure for worms. The mother had been advised to use a tablespoonful of salt to a quart of water but misunderstood and used a pound. Combs (1905) and Brooks (1910) each report a case in which death occurred after the administration of an almost saturated solution of sodium chloride given in mistake for the physiological saline. In Brooks' case a rectal enema composed of one pint of the latter had been ordered repeated three times at half hour intervals. About 270 grams of sodium chloride were administered. In Comb's case 500 c. c. of isotonic sodium chloride solution were ordered injected under each breast. The patient received 124.4 grams sodium chloride. The typical symptoms of sodium chloride poisoning were observed in each case.

Robertson (1878) reports the case of a man of seventy who "had been eating chloride of sodium largely and regularly for several years, and who, when first seen was pale, bleeding from the gums, had ecchymotic spots on his skin, suffered from palpitation and diarrhea, had two attacks of syncope, was unable to speak, became comatose, presenting symptoms of cranial extravasation, and expired". Heinz and Haas (1923) who produced sodium chloride poisoning in chickens, assert that poisoning from this agent, although not frequent in man, has been observed usually from attempts to control bleeding or during the aura of an epileptic attack for which salt in large amounts is a popular remedy. They state that v. Jaksch, in *Die Virgiftung*, reports such a case and that Kobert reports the case of a hysterical woman who ingested 250 grams of salt and thereupon collapsed, had severe gastrointestinal disturbance, numerous evacuations, dehydration of the tissues and generalized muscular cramps, followed by prostration and death. Kobert (1906) states that Lersch reports the case of a woman who, after the ingestion of half a pound of cooking salt, succumbed with symptoms of general paralysis and that Taylor relates a similar case[2].

Other features that cannot fail to prove arresting are, first, that in the cases cited, treatment is mentioned in only one instance[3], and secondly, that in spite of the early point at which a study of the effects of water and of sodium chloride was undertaken, interest in combating the effects of these agents seems to have been manifested at no time, an examination of the important works on pharmacology, therapeutics, and toxicology disclosing only one instance in which treatment is mentioned, (the item here consists of only

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[1] The original reports could not be located either because no reference was given or the references were too incomplete.

[2] Because of the lack of adequate references, it could not be determined whether the Kobert case, mentioned by Heinz and Haas, and the Lersch case, mentioned by Kobert, may be one and the same.

[3] Combs states that his patient "was kept in a continuous hot pack and allowed and even urged to drink water freely" and that he was "satisfied that the combined excretions (urine, sweat, feces, vomiting, tears) removed nearly half of the salt,—"

a few lines and is lacking in exact information) and a search of the literature revealing nothing indicating that even up to the beginning of the present decade the possibilities and limitations of treatment based upon osmotic principles had been systematically investigated.

The case to be reported here was encountered in the course of the writer's studies reported in 1931 and 1933 in which, among other things, these possibilities and limitations were the subject of study. The patient, who had ingested a lethal amount of sodium chloride, recovered following treatment based on data already accumulated here. The latter is discussed under the heading of Experimental Basis.

Case Report

M. S., female, seventeen years of age, weighing 102 pounds. The patient had taken no food for a period of nearly twenty-four hours during which she suffered severe menstrual cramps. She became suddenly profoundly ill subsequent to drinking strong salt water, used, on the advice of a friend, for the relief of the cramps. Two heaping dessertspoonfuls of salt to a glass of water were advised taken at about twenty minute intervals until vomiting was produced. Four such doses were taken, hence approximately 150 grams of sodium chloride. The certainly fatal dose of this salt being three grams per kgm. and much smaller amounts have been known to kill, a fatal quantity was doubtless ingested.

Within a few minutes after drinking the last dose, i. e., at 3:00 p. m., the patient developed dyspnea, choking, vertigo, nausea, and great thirst, and became extremely nervous and weak. She retched repeatedly, but did not vomit, and had several loose bowel evacuations. When first seen, which was about an hour after the onset of these symptoms, she was voiding involuntarily, was extremely prostrated, suffered muscular twitchings, and repeatedly lapsed into unconsciousness. Her pupils were contracted and failed to react to light. There was constant lachrymation, the pulse was 120, temperature 101° F. and respiration 40.

Gastric lavage, employing distilled water, was immediately carried out. Then 1,500 c. c. of this agent were at once introduced intraperitoneally by the gravity method. This was followed by the first of three gastric administrations of 2,000 c. c. each of distilled water, given at half hour intervals. After the first gastric administration was completed, subcutaneous administration of 2,000 c. c. of the physiological saline solution was begun. The gravity apparatus was used for continuous administration and a 50 c. c. Luer piston syringe for injection during the intervals between the gastric administrations. The solution was thrown into the abdominal and the axillary regions.

Retching ceased immediately after the first gastric administration and within approximately twenty minutes copious voiding of urine was begun and was repeated at intervals for the next ten hours. During the first hour here muscular twitchings were observed several times and there was a slight rise in the respiratory and pulse rates and of the temperature. Subsequently the twitchings were observed no further and the respiratory and pulse rates and the temperature gradually declined, and lapses of unconsciousness became less frequent. Water by mouth, urged during lucid and semilucid intervals, was taken only in sips. Prostration persisted for about three hours. (Here it should be stated that the patient had been rushed into my laboratory, where, because of the urgency of the case and very opportunely the entire "set-up" for treatment was immediately available, the procedure already mentioned was carried out, and that now, her condition having improved to a point at which safe transfer might be undertaken, she was taken to her home, hospitalization having been refused.)

Prostration was followed by weakness and by periods of extreme nervousness that reappeared throughout the night, which at first were relieved by one-fourth grain of morphine hypodermically and later by phenobarbital in one-half grain doses by mouth. The next day the temperature as well as the pulse rate was slightly above normal. The patient was very weak and nervous but took nourishment in the form of gruels. After a second night's rest, she asked for food and insisted she was quite normal. She was given soft diet. She rested well the third night and helped herself to a full diet and "took a walk" the fourth day, and went about her work as usual on the fifth day. She has been seen frequently since this time, now over four years, and appears to have suffered no ill effects.

Specimens of the blood and catheterized urine for study were not obtained during the confusion attending treatment. Subsequent specimens of urine proved negative. There is no history of similar attacks encountered either previously or since.

Experimental Basis

The study of the effects produced by the administration of water and of the aqueous hypertonic solutions has engaged my attention now for a number of years. In researches included here the extent was investigated to which the effects produced by these agents, by different routes, might be combated osmotically. Treatment in the present case was based upon what was disclosed, a brief review of which follows.

Sodium chloride poisoning was produced by means of a ten percent solution of that agent intravenously, subcutaneously, intraperitoneally and orally, respectively. The effects were observed (a) of attempts to offset the osmotic influence of the sodium chloride involved by administering water in such amount that together with the latter the components of an isotonic solution were delivered to the organism (i. e., water in ten times the quantity of the hypertonic saline solution employed), and (b) of attempts to bring within harmless limits the osmotic influence of the quantity of sodium chloride involved, by administering isotonic saline solution in such amount (i. e., on the average about 400 c. c. per kgm.) that, together with the latter, the components of approximately a 1.5 percent sodium chloride solution were delivered to the organism. By the nature of things, each of these administrations required the introduction into the organism of an enormous bulk of fluid and, in view of the rapidity with which salt poisoning develops and of the intensity of

the process, that it be introduced within as short a period as possible.

That the bulk factor may be safely overlooked appeared from the results obtained in the experiments in which the effects of increasing amounts of isotonic saline solution were observed intravenously, subcutaneously, intraperitoneally and orally, the maximum amount of the agent employed being 600 c. c. per kgm. Here intravenous injection was carried out continuously at a rate not exceeding 4 c. c. per kgm. per minute, whereas, administration was carried out by the other routes in 50 c. c. per kgm. amounts at half hour intervals. Irrespective of the route employed, nothing unusual was observed in the behavior of the experimental rabbit until the dose reached 450 c. c. per kgm. From this point, coincident with a progressive enlargement of the body, as a result of which seemingly twice the normal size was attained, the animals showed less and less tendency to move about. However, since the animals unquestionably maintained the usual interest in their surroundings, this was doubtless due to being fatigued from supporting the added weight involved rather than to any degree of intoxication. Throughout, irrespective of the mode of administration employed, the animals regained their former proportions in about twelve hours, then moved about as actively as before the administrations.

That a means is afforded of introducing the same volume of fluid into the organism within a much shorter period of time, and without immediate or remote untoward effect, appears from the fact that the identical result was obtained by successively administering 50 c. c. per kgm. amounts of this agent intraperitoneally, orally, and subcutaneously, i e., 150 c. c. per kgm. at one sitting, and repeating this process at half hour intervals until 600 c. c. per kgm. were administered. The practical significance of this will be seen on noting that the administration of 600 c. c. per kgm. by this method requires approximately 100 minutes whereas on intravenous injection, at a rate not exceeding 4 c. c. per kgm. per minute, requires 150 minutes, and on either the intraperitoneal, oral or subcutaneous use, in 50 c. c. per kgm. amounts at half hour intervals, 340 minutes, and also that the omission of intravenous injection here naturally avoids the possibility of producing untoward effects due to a too rapid infusion rate, the latter all too likely to be employed because of undue haste.

Observing the effects upon sodium chloride poisoning of antidotal attempts in which water[4] was employed in the amounts stated above (i. e., in ten times the quantity of the ten percent saline solution employed to produce the poisoning), the following results were obtained: (a) This effect, irrespective of the route by which the ten percent saline solution used to produce it was employed, was rapidly and completely removed (and prevented) by the nonhemolytic administration of water, namely, by tap water[5] orally and by distilled water orally and intraperitoneally. (b) The subcutaneous administration of distilled water as well as combined intraperitoneal, oral, and subcutaneous administrations of water, both hemolytic administrations, generally seemed to be of doubtful value, although in some cases the animals recovered very much more rapidly than the controls. (c) Distilled water intravenously, the most hemolytic of the water administrations, in no case seemed to exert any appreciable antidotal influence.

Unusual interest, therefore, attaches to these findings. For, since hemolysis, as occasioned by water, is an effect that can be neither prevented nor removed by sodium chloride administration, the interpretation obviously suggesting itself is that the integrity of the erythrocytes is essential in order to obtain the full antidotal influence of water.

Observing the antidotal influence exerted upon sodium chloride poisoning, of the isotonic solution, the following results were obtained: (a) If the administration of the isotonic solution preceded the use of the ten percent solution, poisoning failed to develop. (b) If its use immediately followed the development of poisoning due to the ten percent solution, that effect disappeared within a few hours although the animals remained manifestly nervous for another twenty-four hours. That is, it appeared that if the two administrations involved deliver to the organism the components of a solution that closely approximate isotonicity the animal will survive[7].

Summarized, the important findings are: (a) That salt poisoning may be successfully combated by means of adequate amounts of isotonic saline solution and may be rapidly and completely removed by means of the nonhemolytic water administrations. (b) That, in the interest of throwing the antidote into the organism within a very short period of time, administrations combining, at one sitting, its intraperitoneal, oral, and subcutaneous use, may be undertaken without fear of either immediate or remote untoward effect.

Discussion

The enormous increase in the bulk of the normal experimental animal observed when the isotonic saline solution is administered in amount equivalent to 600 c. c. per kgm., already described, naturally suggests that the administration of the large amounts of fluid antidotally employed in this case should have produced a corresponding increase in bulk. It must be borne in mind, however, first, that the total amount of fluid administered was only about one-third of the amount of isotonic saline solution mentioned: secondly, that in sodium chloride poisoning the administration of fluid is undertaken under conditions differing vastly from those in the normal subject: that as a result of one or more processes—lachrymation, salivation, vomiting, and purging and an almost continuous voluminous urinary output—an enormous amount of fluid has already been lost to the body; therefore the normal fluid relations cannot be approached until a correspondingly large amount of fluid has been replaced; and thirdly, that during replacement, as well as hours afterward, the voluminous output from the kidney continues unabated. In the untreated experi-

[4] Orally, either tap water or distilled water was employed, by the other routes, distilled water.

[5] The osmotic pressures, based on the freezing point as determined by the Hortvet Cryoscope, were ascertained of distilled water and of eight tap waters obtained from widely separated sources. The result was almost identical throughout, hence, for all practical purposes, the osmotic influence exerted by any one of these tap waters may be regarded as identical with that exerted by distilled water.

[7] The related work, although completed in 1932 is reported here for the first time.

mental animal, for instance, one sees, coincident with the constant loss of fluid, a decrease in bulk of the soft tissues that is so rapid that within a few hours the skin hangs loosely over the body. On postmortem examination here one finds also ample evidence that an intense abstraction of fluid from the soft and circulating tissues has been in progress. Thus, Brooks found "the blood to be scanty throughout, the skeletal muscles dry, the encephalon anemic, with areas here, as in the heart, in which the cells appear as though hardened too rapidly in too strong a fixing agent."

In the present case, the individual doses given by the different routes, as well as the total dosage for these routes, although seemingly large, were nevertheless relatively smaller than those regularly given the experimental animal. The oral and intraperitoneal doses, for instance, were smaller by one-fifth and the number of times administered was also less.

At first sight, the use of the isotonic sodium chloride solution for antidotal purposes seems unsound since its use implies an actual increase in the amount of this salt already present in the organism. It must be borne in mind, however, that the object of treatment is osmotically to offset the action of sodium chloride taken by the patient and that, since the subcutaneous use of distilled water involves a hemolytic administration of doubtful value, the use of the isotonic sodium chloride solution affords a means giving promise of achieving this end. Thus, the sodium chloride content of the salt water taken by the patient together with that of the isotonic solution administered was approximately 152 grams. This, together with the amount of water involved in the various administrations and taken by mouth by the patient, would yield approximately a 1.5 percent solution which, given in equivalent amount to the experimental animal, proves nontoxic.

The intravenous injection of isotonic saline solution as well as the rectal use of water in combination with the administrations employed, naturally suggesting itself, was not undertaken for the reason that an experimental basis was lacking assuring the safety of superimposing the added bulk of fluid involved upon the organism. In the light of the results already discussed, it naturally should be expected that the isotonic or a nonhemolytic hypotonic saline solution intravenously, or the latter subcutaneously, should prove antidotal.

Summary and Conclusion

The patient here ingested a lethal amount of sodium chloride, developed the symptoms seen when the experimental animal is given a poisonous dose of this salt, and recovered following treatment based upon physical principles.

It is fully appreciated that ordinarily the result obtained in a single case can give little indication of the survival value of the treatment employed. It seems, however, that if all the facts are taken into account, reasons appear why it should be considered as deserving of further trial:

Cases of salt poisoning are so rare that the possibility of reporting results in a series could hardly be hoped or asked for.

Treatment in this case was based upon data accumulated in studies in which its use in poisoned animals was followed time and time again by recovery.

A search of the literature has failed to yield another equally promising basis or another case that has recovered in which a lethal amount of sodium chloride was concerned.

Treatment is neglected in the text-books and literature.

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1022 WALNUT STREET.

Discussion by Allen S. Johnson

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The disastrous effects of drinking strong salt water have been known to seafaring people since time immemorial and the ingestion of a pint of saturated salt solution is said to be a common method of suicide among the Chinese. The specific action of individual ions such as sodium, potassium, calcium, etc., has been studied in heart and skeletal muscle perfusion experiments in the laboratory; but the results of variations in the body's total sodium chloride content can apparently be interpreted in terms of altered osmotic relationships between the exterior and interior of the cells. About seventy percent of the body weight is due to water, of which about fifty percent is intracellular and about twenty percent extracellular. The normal habitat of sodium and chlorine ions is almost exclusively extracellular. A constant concentration of these extracellular electrolytes must be maintained if the normal osmotic relationships are to be preserved between the exterior and interior of the cell. A loss of sodium and chlorine ions as in Addison's disease or profuse sweating therefore necessitates a reduction in the total extracellular fluid, part of which accordingly moves into the cell or is lost from the body. The result is a decrease in the total effective extracellular fluid with the consequent clinical phenomenon of dehydration.

The reverse phenomena result from an abnormal increase in the sodium and chlorine content of the extracellular fluid. To restablish osmotic equilibrium between the interior and exterior of the cells fluid now moves from the cells into the extracellular spaces. The clinical manifestations of such a shift appear to be thirst rather than dehydration. If this shift of intra-

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Sodium Chloride Preference in Essential Hypertension

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Sodium chloride preference was determined in ten patients with essential hypertension and in 12 normal volunteers. All exhibited normal detection and recognition thresholds for the taste of sodium chloride. All were placed on a constant dry diet containing 9 mEq of Na^+ and given, as their only source of fluids, a choice of drinking either distilled water or 0.15 M sodium chloride. Patients with essential hypertension consumed a markedly greater proportion of their total fluid intake as saline (38.2% vs 10.6%, average daily preference over one week) and also showed a greater total fluid intake (1,269 ml vs 668 ml, average daily intake over one week). The hypertensive patients consumed more than four times as much salt as did the normal volunteers.

Among the many possible factors that contribute to the cause of essential hypertension, excessive intake of sodium chloride in genetically susceptible individuals has been considered to be prominent.[1-4] This factor was considered by early investigators,[5,6] and restriction of salt and water intake resulted as one of the first forms of therapy for this condition.[7-8] Other studies suggested that a high sodium intake itself was associated with an increased incidence of hypertension and, conversely, that a low sodium intake was associated with a decreased incidence of hypertension.[1-4] Indeed, some investigators have claimed that excessive intake of sodium chloride in laboratory animals will induce hypertension.[9-11]

Since excessive intake of sodium chloride in patients with essential hypertension could be related to alterations of their taste acuity, this aspect of the problem was evaluated by several investigators, and some abnormality of salt taste acuity was suggested.[12,13] However, studies in our laboratory, in which detection and recognition thresholds were measured in patients with hypertension of several different origins with or without treatment, did not disclose any consistent differences between patients with hypertension or normal volunteers. Thus, if some aspect of taste were to play a role in essential hypertension, then this role had to involve some abnormality of taste, per se, other than that related to thresholds. Recent studies in our laboratory gave us some insight into this problem, for rats with a genetic type of hypertension exhibited a marked increase in their preference for sodium chloride.[14] With this in mind, we wondered whether patients with essential hypertension might exhibit a different pattern of salt preference than individuals without essential hypertension.

Methods

The subjects were ten patients with a history of sustained essential

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Comparative Data

	Subject No.	Sex/Age/Race	Body Weight, kg	Admission Blood Pressure, mm Hg	Sodium Chloride Thresholds, mM*†	Average Daily Na+ Intake, mEq‡	Average Daily Na+ Intake/ Body Weight, mEq/100 kg
Hypertensives	1.	F/43/W	81.00	160/100	30/30	92.8	114.6
	2.	M/39/N	89.02	150/100	12/12	54.8	64.6
	3.	F/25/W	73.32	170/130	30/60	51.6	70.4
	4.	M/51/N	100.75	190/110	6/6	61.7	61.2
	5.	F/44/W	84.44	150/108	60/60	250.8	297.0
	6.	M/56/W	76.78	180/120	30/60	84.5	110.0
	7.	M/50/N	56.92	170/102	60/60	34.7	60.9
	8.	M/42/N	84.40	140/100	30/30	62.2	73.7
	9.	M/47/N	70.42	156/112	30/30	11.0	15.7
	10.	M/54/N	73.40	162/108	60/60	39.1	53.3
Mean			79.05		30/45‖	74.3±21.0§¶	92.1±24.4§¶
Volunteers	1.	M/19/W	72.20	128/72	12/60	10.2	14.2
	2.	M/19/W	69.60	120/72	30/60	2.2	3.2
	3.	F/43/W	54.58	130/78	12/12	0	0
	4.	M/19/W	71.02	100/60	60/60	2.6	3.7
	5.	M/19/W	72.04	132/70	60/60	0	0
	6.	M/21/W	61.72	136/84	60/60	43.1	73.6
	7.	M/23/W	80.82	124/80	30/30	45.8	56.6
	8.	M/21/W	65.40	110/70	12/12	63.6	97.2
	9.	M/19/W	73.46	120/80	30/30	1.8	2.4
	10.	M/19/W	58.68	120/70	30/30	0	0
	11.	M/21/W	78.66	118/80	30/30	3.7	4.8
	12.	F/19/W	61.10	118/70	6/6	0	0
Mean			68.27		30/30‖	14.4±6.5§	34.2±9.9§

*Numerator of fraction is detection threshold, denominator of fraction is recognition threshold.
†Normal values according to Henkin et al[16] 12/30 mM (range, 8 to 60/6 to 60 mM).
‡Excluding daily 9 mEq of Na+ intake in constant diet.
§Mean ± SE.
‖Median values (not significant, Wilcoxson rank sum statistic).
¶$P<.02$ (two-sided t test) compared to normal volunteers.

hypertension of a mild-to-moderate degree, and 12 nonhypertensive volunteers. The patients were ten consecutive inpatients admitted to the Experimental Therapeutics Branch, National Heart and Lung Institute, for study of their hypertension. The 12 volunteers were consecutively admitted to the normal volunteer study program[15] designated to the Experimental Therapeutics Branch. Assignment to the hypertensive or nonhypertensive group was based on diastolic blood pressure at admission. Blood pressure was always measured by the arm cuff method after the subject had remained supine at rest for 15 minutes. If diastolic blood pressure was greater than or equal to 100 mm Hg, the subject was assigned to the hypertensive patient group; if less than or equal to 90 mm Hg, the subject was assigned to the normotensive volunteer group.

Hypertensive Patients.—The patients were four whites and six Negroes, seven men and three women, and had a mean age of 45 years (range, 25 to 56 years). On admission, the mean blood pressure, measured while supine, was 163/109 mm Hg (range, 140 to 190/100 to 130). Each patient underwent diagnostic studies, which included intravenous pyelography, urinalyses, urine culture, creatinine clearance, and determination of serum electrolyte values. Additional tests were performed for selected patients as follows: renogram with iodohippurate sodium I 131 or chlormerodrin Hg 203 (six patients), transfemoral renal arteriogram (three patients), determination of 24-hour urinary excretion of vanilmandelic acid or metanephrine (eight patients). Results of these tests failed to reveal any specific cause for the elevation in blood pressure in any patient. Three of the ten patients had never been treated for their hypertension. The remaining seven had been taking no antihypertensive agents for ten days to two months prior to the study. Six patients had received relatively short-acting antihypertensive agents (methyldopa in two patients, spironolactone and hydrochlorothiazide in three, chlorthalidone in one, and trichlormethiazide in one). One patient received pargyline hydrochloride therapy, which was discontinued one month prior to the study. None of the patients had dietary salt restriction prior to study. None of the patients exhibited any clinical signs of congestive heart failure, and none were being treated with any digitalis preparations. All patients had serum creatinine levels below 1.5 mg/100 ml, normal liver function, and no evidence of glycosuria. The present study began 1 to 24 days after each patient was hospitalized. At the time of study, the mean blood pressure of the patients was 135/93 mm Hg (range, 112 to 170/74 to 120 mm Hg).

Detection and recognition thresholds for the taste of sodium chloride were determined in each patient by a forced-choice three-stimulus drop technique[16] by at least one investigator and found to be normal in each patient (range for detection and recognition thresholds, 6 to 60 millimols [Table]). These thresholds were measured in two patients at least twice.

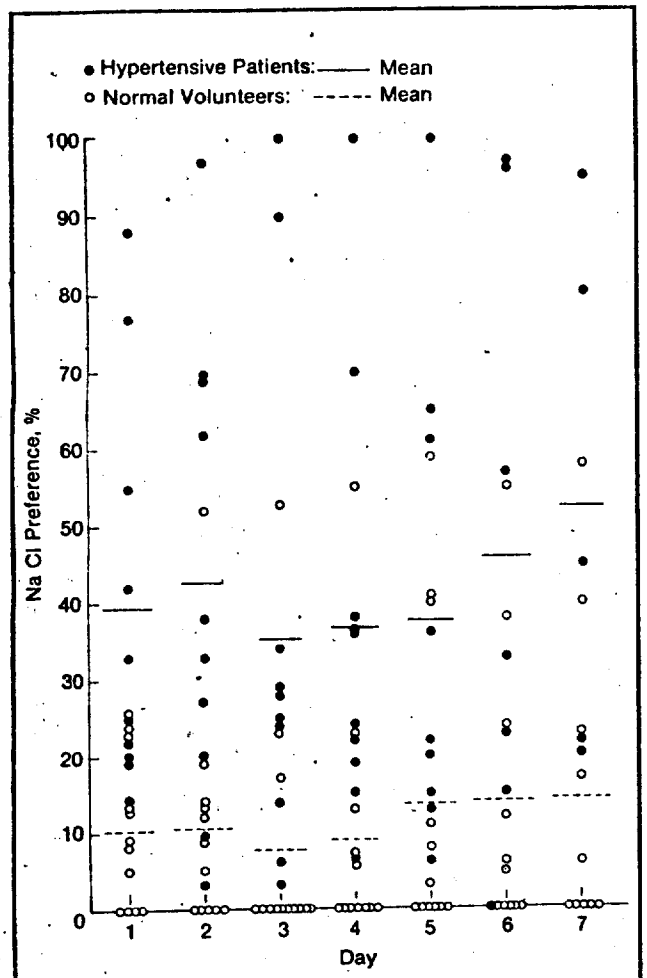

Fig 1.—Sodium chloride preference in hypertensive patients (solid circles) and normal volunteers (open circles) for each day of study. Mean value of hypertensive patients (solid line) was consistently greater than mean of normal volunteers (hatched line).

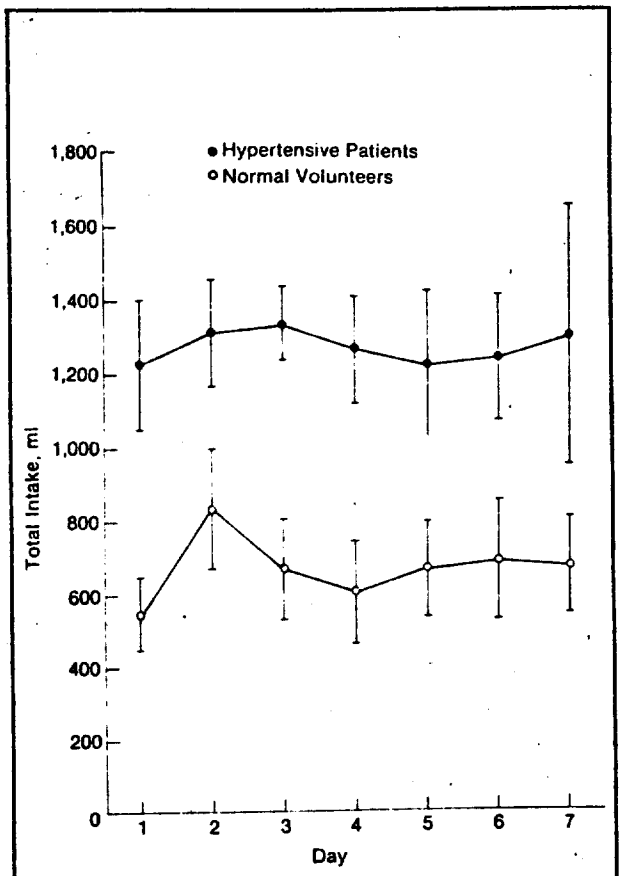

Fig 2.—Daily total fluid intake of hypertensive patients (solid circles) and normal volunteers (open circles). Total fluid intake (0.15 M NaCl with distilled water) is plotted on ordinate, days of study on abscissa. Each point and vertical bars represent the group mean ±1 SE.

Only the lowest thresholds are reported here.

Normotensive Volunteers.—The volunteers were 12 whites, nine men and three women, mean age 22 years (range, 19 to 43 years). At the time of admission, their mean blood pressure, measured while supine, was 121/74 mm Hg (range 100 to 136/60 to 84 mm Hg). Volunteers began the present study two days to three months after their hospitalization began. At this time, their mean blood pressure was 107/65 mm Hg (range, 90 to 120/60 to 84 mm Hg). Each volunteer had serum creatinine levels below 1.5 mg/100 ml, normal liver function, and no evidence of glycosuria. Each demonstrated detection and recognition thresholds that were within the normal range for the taste of sodium chloride (range for detection and recognition thresholds, 6 to 60 millimolars [Table]). Thresholds were measured by at least one investigator. Thresholds in three volunteers were measured at least twice, and only the lowest thresholds are reported.

Study Protocol.—All subjects were housed in semiprivate rooms, restricted to an air-conditioned environment for the entire course of the study, and their activity was limited to normal walking without strenuous exertion. For this study, each patient or volunteer was placed on a constant diet containing 9 mEq of Na for four to seven days. The diet contained no fluids other than the natural fluid content of the foods chosen; fruit juice, milk, coffee, tea, soft drinks, and soups were excluded. The diet was served at normal mealtimes. The only source of fluids available to either the patients or volunteers was the contents of two 2-liter Erlenmeyer flasks, one filled with distilled water, the other filled with a solution of 0.15 M NaCl made with reagent grade sodium chloride and distilled water. The flasks were kept at room temperature, covered at all times with plastic laboratory film, and opened only for decantation of fluid into a 150-ml

beaker for immoderate drinking. Fluids were aerated prior to presenting them to the subjects.

Each flask was coded and the code changed daily, so that the subjects were visually unaware of which one contained salt. Flasks were changed daily at 8 AM. Each subject was required to sample the contents of each flask daily.

Each subject was instructed to drink as little or as much of each fluid as he desired. The subject was encouraged to mix different amounts of each test fluid, if he so desired, until a preferred mixture was obtained. Fluids could be added to foods but had to be consumed in their entirety if this were done.

The amount of fluid taken from each flask was calculated daily from the difference between initial and final volumes. Urine was collected for 24 hours each day, and total volume, and sodium, potassium, and creatinine contents were measured. Body weight of each subject was measured daily; this allowed an external control as to salt or fluid intake from sources other than the diet and prescribed fluids. Data from a single additional hypertensive patient were rejected when it was found that her urinary output exceeded her ostensible total fluid intake by an average of 450% without any appreciable change in body weight.

Several aspects of the data were analyzed. Preference for sodium chloride was calculated as follows:

$$\% \text{ pref} = \frac{\text{ml } 0.15 \text{ M NaCl}}{\text{ml total volume}} \times 100.$$

In addition, median detection and recognition thresholds for the taste of sodium chloride, the average daily sodium intake (in milliequivalents), the average daily sodium intake per kilogram of body weight, and the daily total fluid intake (salt and water) were compared in each patient in the two groups, and between the two groups. Statistical significance of differences for Na^+ and total fluid intake was determined by the Student t test. Significance of differences between median detection and recognition thresholds was determined by the Wilcoxson rank sum statistic as previously described.[12]

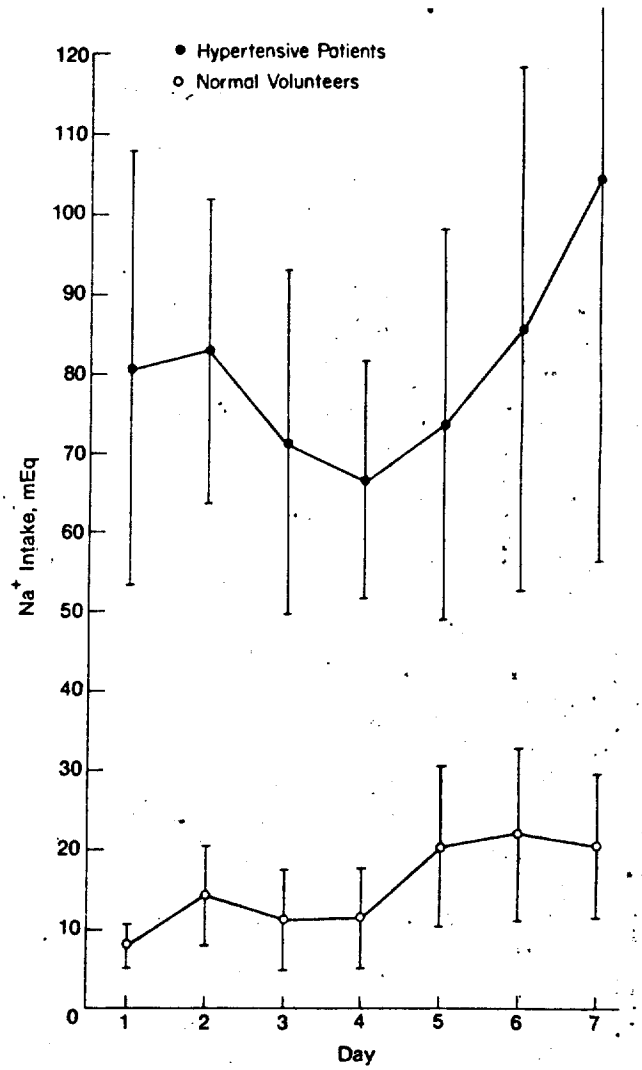

Fig 3.—Daily intake of Na^+ of hypertensive patients (solid circles) and normal volunteers (open circles). Na^+ intake is plotted on ordinate, days of study on abscissa. Each point and vertical bars represent group mean ±1 SE.

Results

The mean daily preference of sodium chloride for the hypertensive patients was significantly greater than that of the normal volunteers for each of the first four days of the study ($P<.05$, two-sided t test) (Fig 1). On days 5 through 7, mean sodium chloride preference for the patients was consistently greater than for the normal volunteers but was not statistically significant ($P>.05$). (This may be due, in part, to the decreased number of subjects participating in the study after day 4. Some subjects terminated the study prior to day 7 because of their unwillingness to persist in eating the low sodium diet or because of personal reasons which necessitated their leaving the hospital.) The daily mean total fluid intake of the patients and the volunteers is shown in Fig 2. The average total fluid consumption by the patients was significantly greater than that of the volunteers ($P<.05$, two-sided t test) for the first six days of the study.

The daily mean total intake of Na^+ (in milliequivalents) of the patients and the volunteers is shown in Fig 3. The average daily intake of Na^+ by the patients was significantly greater than that of the volunteers ($P<0.05$, two-sided t test) for each of the first four days of the study. On days 5 to 7, mean total intake of Na^+ for the patients was consistently greater than for the normal volunteers but was not statistically significant ($P>.05$). (This, too may have been the result of the decreased number of subjects participating in the study.)

The Table shows the mean daily intake of Na^+ (excluding the 9 mEq of constant dietary intake) in each subject. On the average, patients with hypertension consumed over four times as much Na^+ per day as did the volunteers. This was true whether the absolute Na^+ intake (patients, 74.3±21.0 mEq/day, mean±1 standard error of the mean; volunteers, 14.4±6.5, $P<.02$) or the Na^+ intake per 100 kg body weight (patients, 92.1±24.4 mEq/100 kg/day; volunteers, 21.3±9.9 mEq/100 kg/day, $P<.02$) were compared.

The increased salt preference, total fluid intake, and Na^+ intake observed in the hypertensive patients occurred even though the median detection and recognition thresholds for the taste of sodium chloride were similar in the two groups.

No correlations could be made between the amount of Na^+ consumed by the patients and whether or not they had had prior antihypertensive drug therapy or prior diuretic therapy.

Comment

We have demonstrated that patients with essential hypertension show a greater preference for saline than do normal volunteers when given a choice between 0.15 M NaCl and water. In addition, given a free choice, the patients with essential hypertension drank increased volumes of fluid. These differences were demonstrable within the first 24 hours of the study.

The increased preference of the patients for saline cannot be explained on the basis of elevated thresholds for sodium chloride. Although Fallis et al[12] suggested that patients with hypertension consumed more salt because of a decreased salt taste acuity, the patients in the present study exhibited increased preference for sodium chloride without any alteration from normal sodium chloride taste thresholds. We have also studied one hypertensive patient, not reported here, who demonstrated an elevated recognition threshold for the taste of sodium chloride. His sodium chloride preference and total fluid intake were as increased above that of the volunteers as that of the hypertensive patients with normal thresholds for sodium chloride.

The average amount of salt consumed by both the hypertensive patients and the volunteers during this study was well below the estimated average daily intake of salt consumed by adults in the United States (ie, 4 to 24 gm as determined by urinary Na^+ and chlorine excretion[1,18]). This may reflect the restraints inherent in the present study, although the average Na^+ intake of both the volunteers and the patients was within the range estimated to maintain a normal sodium balance.[1,18] It is recognized that the two study groups were not matched for factors other than blood pressure and, in particular, differed in age and racial makeup. When the four white patients were compared with the six Negro patients, no significant differences in sodium chloride preference, total volume intake, total Na^+ intake, or Na^+ intake per 100 kg of body weight were found.

Whereas salt preference of other animals may reflect an attempt to maintain sodium homeostasis,[19,20] it has been suggested that, in man, salt appetite is acquired, at least in part, as a result of social and dietary customs.[1,2,18] It is possible that the increased salt intake observed in patients with essential hypertension reflects an acquired preference which predated any cardiovascular changes. In genetically susceptible individuals, this increased salt intake may act as one causal factor in the genesis of the hypertension. However, the possibility that an increased salt appetite is a manifestation of some, as yet unidentified, aspect of the disease cannot, as yet, be excluded.

Diane A. Bronzert and C. Joan Franklin provided technical assistance throughout the study.

Nonproprietary Names and Trademarks of Drugs

Methyldopa—*Aldomet.*
Chlorthalidone—*Hygroton.*

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Mortality Associated with Hypertonic Saline Abortion

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In New York City from July 1, 1970, through June 30, 1972, there were 49,474 intraamniotic instillations of hypertonic saline for midtrimester abortion. Ten of the patients died. In this presentation the causes of death are documented and some consideration is given to the prevention of such complications in the performance of these procedures. The causes of death were not the expected hypernatremia except in one instance. The main factors which led to death were the complications secondary to incomplete abortion with bleeding and sepsis, complications which arose from the use of other agents to shorten the instillation–abortion time, and complications secondary to preexisting medical conditions which were not compatible with this method of abortion.

IN THE PERIOD July 1, 1970, through June 30, 1972, there were 401,584 abortions performed in New York City; 49,474 of these procedures were intraamniotic instillations of saline for midtrimester abortions. In this latter group there was a relatively high incidence of complications, mainly bleeding and infection, as compared with terminations in the first trimester by suction or curettage.[1] This report will be confined to an analysis of the fatalities associated with the saline instillations. In the 2-year period during which the 49,474 saline abortions were performed, there were 10 deaths associated with the procedure of which 8 occurred in New York City and 2 followed return of the patient to other states. The mortality rate for the New York City deaths following saline abortions was 16.2 per 100,000 abortions. If the 2 additionally known deaths which occurred in other states are included, the rate is 20.2 per 100,000 abortions.

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CASE REPORTS

The 10 cases to be described here (Table 1) each had an amnio-infusion of hypertonic saline in New York City. Five were done in City Hospitals, 3 in proprietary hospitals and 2 in voluntary hospitals. The case studies were reviewed and summarized by the Obstetric Consultants of the Bureau of Maternal Health and Family Planning in the New York City Department of Health. The autopsies performed by the medical examiner were not all completed at the time this communication was prepared. The causes of death were not sharply defined in all of the cases.

Four of the patients died of sepsis. Of these 1 was readmitted 24 hours after the instillation of saline with a temperature elevation of 104°F. She continued to bleed after expulsion of the products of conception. The uterus was vacuum aspirated following which the patient was treated for septic shock and oliguria; she died 3 days after the instillation. The cultures taken from the blood stream showed no growth, and mixed organisms were cultured from the cervix. The autopsy described hydropneumothorax with no gross evidence of infection of the uterus; the microscopic preparations were not available. The description of the uterus must be

TABLE 1. MORTALITY ASSOCIATED WITH HYPERTONIC SALINE ABORTION IN NEW YORK CITY—JULY 1, 1970 TO JUNE 30, 1972.

Case No.	*Gestational size (wk)*	*Amniotic fluid (ml)*	*Saline (ml)*	*Significant past History*	*Cause of death*	*Autopsy findings*
1	12	120	120	Rheumatic cardiac	Cardiac failure, pulmonary edema	Acute pulmonary edema, chronic rheumatic heart disease
2	15-16	150	120	None	Water intoxication	Bronchopneumonia, cerebral necrosis
3	18	150	180	None	Septic shock, disseminated intravascular coagulation	Hydropneumothorax, no gross evidence of infection in uterus?
4	20	150	300	Epilepsy, BP 150/100 mmHg	Water intoxication ?? Amniotic fluid embolus?	Marked edema of lungs, narrowing of lateral ventricles
5	14	50	150	None	Sepsis, *S aureus* septicemia	No autopsy
6	22	Not noted	180	None	Hypernatremia, cerebral edema	None described
7	23	150	250	Asthma	Acute pulmonary edema, asthma? Water intoxication?	Pulmonary edema and cardiac arrest
8	19	Not Stated	Not Stated	None	Peritonitis	Perforation uterus and bowel with diffuse suppurative peritonitis with septicemia
9	16	60	230	None	Postabortal sepsis	Necrotizing endometritis with septicemia, bilateral pleural effusion, ascites
10	Not known	Not known	Not known	Not known	Hemorrhage	Unknown

considered as questionable. Generalized tissue edema was stated as being suggestive of fluid overloading.

The second patient to die of sepsis delivered 36 hours after the saline instillation. Her temperature was recorded as 99.6°F 8 hours prior to delivery, none was recorded after the abortion. Following the abortion she was given an antibiotic intramuscularly, a prescription for oral antibiotics, and discharged to her home in another state. Two and one-half days after the abortion she developed chills and fever with a temperature elevation to 106°F. The blood cultures were positive for *Staphylococcus aureus*. This patient developed hypotension several days prior to her death on the ninth postabortal day. There was no autopsy performed.

The third septic patient returned to the hospital 1 day after instillation and aborted 6 hours later. Three attempts were made to empty the uterus because of bleeding; the first attempt was 10 hours after abortion, the next two attempts after 48 hours. None of the three procedures to empty the uterus was performed with the assistance of general anesthesia. It was noted following the final attempt at evacuation of the uterus that the patient had peritonitis. Laparotomy was performed 7 days later and the patient was found to have acute suppurative peritonitis with bowel perforations which were later confirmed at autopsy.

The fourth patient in this series of deaths due to sepsis delivered spontaneously 12 hours after the saline instillation and was discharged 14 hours after the delivery. The patient was given antibiotics by mouth as a routine in management. Nineteen days later she was readmitted with the chief complaint of bleeding for 2 days. At the time of readmission the uterus was the size of a 12 weeks' gestation with drainage of purulent material from the cervical os. A curettage yielded large amounts of what was described as placental tissue. A temperature elevation to 104°F on the second day was treated with oral antibiotics and changed later to parenteral methods when the temperature rose to 106°F followed by the development of shock. An anaerobic streptococcus was cultured from the blood stream. The patient was treated medically with all available methods. Her uterus showed necrotizing endometritis at postmortem examination.

As for other complications leading to death, 1 patient was known to have cardiac problems with a history of moderate functional disability

and a recent history of expectorating bloody sputum, as well as being a known heroin addict. She developed pulmonary edema and cardiac arrest 8 hours after instillation. The postmortem examination revealed pulmonary edema and chronic rheumatic heart disease.

The syndrome of water intoxication apparently accounted for another fatality. An intravenous infusion of 1000 ml of 5% glucose and water with 40 units of oxytocin was administered starting 18 hours after the saline instillation. Twenty-two hours later, after 3000 ml of fluid and 120 units of oxytocin the patient convulsed. She aborted 3 days later and died on the tenth day. The pertinent postmortem findings were bronchopneumonia and cerebral necrosis.

A patient who was gravida 6 and a known epileptic was also considered to have suffered from water intoxication; however, the possibility of amniotic fluid embolism is not excluded. She was given 80 units of oxytocin in 1000 ml of 5% glucose in water 10 hours after the instillation, the fetus was expelled 26 hours later. The membranes had ruptured spontaneously just prior to delivery at which time the patient was having severe pains. Manual removal of the placenta was followed by convulsions and cardiac arrest. The patient was noted in the record as presenting a clinical picture of amniotic fluid embolus. The autopsy findings were that of marked edema of the lungs and narrowing of the lateral cerebral ventricles. There was no microscopic description of the lungs.

Only 1 of the 10 patients apparently died as a result of hypernatremia. One and one-half hours after instillation this patient became comatose. She convulsed 8½ hours later and died 14 hours after instillation. Autopsy findings were not available for this patient.

A known asthmatic was given 50 units of oxytocin in 1000 ml of Ringer's solution after the saline instillation. She delivered 29 hours later and was noted to have some wheezing, with a temperature elevation of 101.8°F. This patient's total intake involved 2450 ml of Ringer's solution with 110 units of oxytocin; her urine output was recorded at 2500 ml. The placenta was removed with a sponge stick. A small dose of Demerol® and Valium® sedation were used for this procedure. Adrenalin was given for the wheezing 50 minutes later, as the patient had respiratory distress, cynanosis, and cardiac arrest. She was intubated but never recovered. The autopsy finding was pulmonary edema. The diagnosis in this case is not certain; this patient may have died from a severe asthmatic episode or she may possibly have had water intoxication.

Hemorrhage at the time of delivery of the fetus was a cause of death in a patient who had an intraamniotic saline instillation in New York City and was discharged from the hospital with instructions to return when labor began. However, she returned to her home in another state and was found in extremis in the bathroom of her home, hemorrhaging and with partial extrusion of the fetus. The patient was dead on arrival at a nearby hospital in her home state.

DISCUSSION

Intraamniotic instillation of hypertonic saline has become the accepted procedure for inducing abortions in New York City for a gestation of more than 16 weeks. Most of the writers[2-12] in this field have stressed the technic and proper selection of patients. As experience in the method developed it became very apparent, as our predecessors with this method in other countries[8,9,13] had previously informed us, that there were serious complications associated with the saline method. The literature[3,4,14-16] revealed many complications, some of which had not been anticipated. Hypernatremia, which was of greatest concern, presented as a serious and fatal complication in very few cases. Hemolysis[13,17] especially in patients with sickle cell disease was described. Amniotic fluid embolism in association with high parity and Pitocin® was recognized. Pulmonary edema, cardiovascular shock, and other complications became known in association with saline instillation.

Finally, and most commonly, retained placentas or partially retained secundines, serious infection, and hemorrhage of various degrees became factors of serious proportion to the groups who were committed to the use of hypertonic saline for the induction of midtrimester abortion. Consumptive coagulopathy was recognized as a complication in this method of termination of pregnancy. Various workers[4,16,18,19] in the field of

clotting mechanisms presented their findings and conducted research in the area. It was concluded by one group[18] that the defibrination phenomenon occurs with saline instillation but only occasionally has serious clinical sequelae.

Technics were varied[3,5-7] and surgical technic was occasionally violated in the interest of rapidly handling the demanding number of abortions. The economics of costly hospitalization as well as complications secondary to a prolonged period before completion of abortion gave impetus to efforts for expediting delivery by using oxytocin. Reinstillation of saline and instrumentation at inopportune times when expected labor did not occur led to a further incidence of complications. Another serious aspect of saline instillation is the seeming ease with which it is accomplished, leading relatively inexperienced personnel who may not be knowledgeable regarding the potential problems, pitfalls, and means of avoiding them[17] to become involved in carrying out these procedures. In addition, some institutions are not fully equipped or staffed to handle all the complications which may arise. The technic of instillation must be meticulous as has been described in many prior dissertations on this subject.[3,5-8] The selection of patients should be made with a critical clinical attitude; the contraindications must be well observed and heeded. Manabe[12] stated that the risks of this procedure could have been minimized if information from other countries had been noted earlier.

The complications of infection should be handled in the same manner as any other septic threatened or incomplete abortion. Antibiotics when indicated must be given parenterally and with due regard to the organisms and sensitivities of the bacteria to specific antibiotics when this information is available. Septic shock must be managed in a vigorous manner with resort to intensive care units, curettage, hysterectomy, and renal dialysis where indicated. Perforation of the uterus in the presence of sepsis must be managed aggressively.

Bleeding should be controlled promptly and with full regard to all the factors involved; retained secundines, consumptive coagulopathy, lacerations, and also rupture of the uterus. There is no place for procrastination in the presence of bleeding. Exploration of a uterus cannot be performed without proper anesthetic assistance. An anesthetist should be available at all times for emergencies requiring intubation or artificial respiration and other situations where proper gas exchange is interfered with.

In a recent paper reporting 3579 saline amnioinfusions Ballard and Ballard[2] introduced the subject by saying that, "With the use of 20 per cent saline, proper patient selection and strict adherence to proper technic, hypertonic saline has been found an effective means of termination of pregnancy." The authors then proceeded to describe the complictions.

Stewart and Goldstein[11] discussed the medical and surgical complications of induced abortion. They stated that the major complication of this method was the need for a second procedure. Eight percent of all their saline abortions required uterine evacuation under general anesthesia and 25% of that group became morbid as compared to 7% of those without retained products of conception. The febrility increased with retained products of conception, increased abortion time, and increasing time from delivery of fetus to expulsion of the placenta.

The fatal cases we have reported here, as well as a number of other serious, albeit not fatal, complications would suggest that patients be kept in a hospital qualified and equipped with resident staff, blood banks, intensive care units, 24-hour biochemical and blood gas services, as well as an on-the-premise anesthesiologist or anesthetist.

The use of Pitocin to shorten the time interval to delivery may eliminate or reduce

the number of retained placentas and subsequent infection and bleeding;[2,4,7,12] however, this too adds other problems such as water intoxication[3,20] and ruptured uteri.[4] In view of the additional complications seen with the prolonged use of oxytocin and fluids it may be necessary to carefully study such methods of shortening the period between instillation and delivery. To this date there has not been a detailed report of the incidence of ruptured uteri with the use of large doses of oxytocin, but there have been occasional cases reported and possibly there are unreported cases. Failed abortion after saline instillation approximates 5% or slightly more of the total cases.[21] It may be possible to eliminate some of the serious complications by a different approach to these cases. If a nonaggressive approach to a prolonged instillation delivery interval is accepted, it may be safe to send home the afebrile patient with intact membranes who fails to abort within 72 hours, if the fetal heart tones have disappeared.[21] However, it is imperative that such patients are closely followed and maintained as missed abortions. They must be warned to return with the onset of labor, bleeding, rupture of the membranes, or other significant clinical symptoms and signs. A number of patients handled in this manner[21] were without serious sequelae. There are well-known risks associated with the prolonged instillation to delivery time which would also have to be accounted for. The addition of vaginal surgical procedures, including the use of sponge forceps or dilatation and curettage, for the termination of pregnancy following failed saline inductions with uteri of 16 to 20 weeks' gestational size has been suggested.[12] These procedures are formidable and are subject to serious sequelae although the authors who suggest this approach apparently have not had any difficulty in the cases they handled in this manner.

The management of saline abortions following the successful instillation of the hypertonic solution should follow rather well-accepted principles of management in regard to selection of patients, incomplete abortions, septic incomplete abortions, missed abortions, consumptive coagulopathy, perforations, endotoxin shock, hemorrhage and other complications. If measures to shorten the instillation–delivery time are taken, the methods must be monitored meticulously.

Despite the risks and complications, it would seem that at this time the hypertonic saline abortion remains the most satisfactory method for inducing midtrimester abortions. In continuing this method of abortion, meticulous technic, careful selection of patients, and intimate knowledge of possible complications and the means of avoiding them should be stressed at all times. Research and study must be continued in the interest of finding and developing a shorter and safer method for inducing late abortions.

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Arterial pressure and hypertensive disease in a West Indian Negro population
Report of a survey in St. Kitts, West Indies

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Negroes in the United States seem to have a greater tendency to hypertensive disease than do whites.[1,2] This characteristic has been interpreted by some as evidence of racial factors, presumably genetic, and by others as a consequence of environmental stress, notably psychologic. These alternatives cannot be evaluated readily in the complex environment of the United States. The present report describes a study of a Negro population whose remote origin is similar to that of the North American Negro, but whose socioeconomic environment, culture, and ancestry are more uniform than those of most United States Negroes.

Such groups occur in the Lesser Antilles, of which St. Kitts in the Leeward Islands is one. During a visit there in 1957, Dr. R. L. Turnbull, of the Cleveland Clinic, gained the impression from physicians that hypertensive disease was common, and that the high degree of organization of the Government Health Service would facilitate a survey of this or other problems. A plan was developed for a cooperative field study by the staff members of the University College of the West Indies and the Cleveland Clinic Foundation. Subsequently, arrangements for coding and tabulation were made in consultation with one of us (F.E.M.). Major aims were, first, to determine distributions of arterial pressure in adults, and, second, to evaluate relative severity of hypertensive disease in subjects with diastolic hypertension. These data form the substance of this report. Concurrent observations on aspects of nutrition and ischemic heart disease are reported elsewhere.[3]

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Locale

St. Kitts (St. Christopher) was named by Columbus, colonized by the British and French in 1623, and became part of the (British) Leeward Islands Colony in 1713. James Grainger, M.D., hailed it in his poem "The Sugar Cane" (London, 1766), lines 60 to 67:

"Such green St. Christopher, thy happy soil.
Not Grecian Tempe, where Arcadian Pan
Knit with the Graces, tun'd his sylvan Pipe . . .
Not purple Enna . . .
Can vie, blest isle, with thee."

At that time it was a flourishing economic and cultural center; its rich sugar plantations were worked by African slaves until slavery was abolished in 1833. Descendants of former slaves now form some 98 per cent of the population.

Land area is 68 square miles. The birth rate in 1957 was 52, and the crude death rate 15, per 1,000; 40 per cent of the population was less than 15 years of age; population density was about 800 per arable square mile. Living standards and income were well below North American levels; wages of laborers in 1954 were about $7.00 (U.S.) weekly during full employment and have not increased greatly since. Total population is about 35,000,* of whom some four fifths live in villages and work on neighboring plantations, whereas most non-Negroes, who are sedentary, clerical and factory workers, live in Basseterre, the capital. The villages are linked, at intervals of a few miles, by a perimetric coastal road.

Medical and health services of the villagers are provided almost entirely by the Government Health Service, which is administered by the Senior Medical Officer. The several Health Districts of the island are based on village Health Centers; each is administered by a District Medical Officer and staffed by a Sanitary Inspector, a Public Health Nurse, a midwife, and a pharmacist.

Some 74 per cent of live births are classed as illegitimate.[4] This arises from the custom of "keeper unions"; in these, a woman, termed a "home domestic," and her children are provided for but do not enter into a paternal family group.

*Data based on official estimates.

Procedures

Study group. The field work was carried out in early 1958. Since essential hypertension manifests itself in early or middle adult life, the study was done on subjects who were 20 to 49 years of age.* Lenore Harney** suggested that representative samples of the total rural and village population of some 28,000 of all ages could be found in three villages, namely, Sandy Point (2,700), Old Road (700), and Cayon (1,000).† The former two villages are on the southwest, and the latter one is on the northwest coast. Although they are not far apart, social communication between villages seems limited.

With the cooperation of the Government Health Service, and in order to induce adults of desired ages to come to the Health Center for examination at appropriate times, the village Sanitary Inspector and a member of the survey staff visited dwellings in turn until a responsible adult in each had been interviewed and given appointment cards for relevant occupants.

In the age group of 20 to 49 years, 566 of 686 men (83 per cent) and 881 of 992 women (89 per cent) who had been given cards came for examination. The local Public Health Nurse was unable to relate nonresponse to current health status, of which she was usually well aware. Failure to respond affected households and seemed attributable to suspicion or to fear of venipuncture; even when these people were reassured, new appointments made, and venipuncture omitted unless requested, those who had not responded at first still avoided examination.

Far from not responding, 241 subjects came to the clinics without appointment cards, either young men in small groups or persons in the company of friends for whom appointments had been made. Their motives seemed to be casual interest, impatience or congeniality. Half of the men in this subgroup were 20 to 29 years

*In Cayon, and, as the survey ended, in other villages, cards were left for occupants who were 50 to 59 years old. Response of this group was poor and those who came to clinics were often uncertain of their exact age. Accordingly, data from these are omitted from, or only incidentally listed in, the tabulations of this report.

**Lenore Harney, M.D., D.P.H., a District Medical Officer.

†Numbers in parentheses are rough estimates of inhabitants.

old. Together, these "walk-ins" constituted 18 per cent of the men and 12 per cent of the women examined.

Table I indicates distribution of the island's estimated population by age and sex, and of the total group examined and its subgroups. Correspondences in proportions between the estimates and examined group suggest that the sample was representative.

Table II compares the means of systolic pressures between subgroups, viz., respondents and "walk-ins," people of different villages, and those of apparent Negro and mixed race. Since there are no indications of systematic differences in this characteristic, data from subgroups were combined in the tabulations that follow.

The method of selection of population—purposive selection of villages with an attempt to secure 100 per cent coverage of the age group 20 to 49—does not permit the estimation of sampling errors. However, the closeness of agreement of the results for the three villages, as shown in Table II, increases our confidence that the subjects examined are reasonably representative of the rural population of the island with respect to the variables studied.

Documentary corroboration of age was not obtained. Detailed distributions of terminal digits of stated ages showed a uniform spread of digits from 0 through 9 among men; among women, 15.3 per cent reported ages in even multiples of 10, and 8.7 to 10.6 per cent in digits 1 through 9. Furthermore, most of the 1,447 respondents seemed certain of their ages within a year or two; they often revised relatives' estimates listed on appointment cards.

Table 1. Estimated population of St. Kitts, 1951, and population included in the survey of blood pressure made in three villages, 1958, by sex and age

Sex and age	Population of St. Kitts, 1951*	Three-village survey, 1958			
		Cards issued	Card-holders examined	Walk-ins	Total examined
Number of persons					
Males, total	10,980	686	566	123	689
20–29	4,080	246	210	67	277
30–39	3,400	196	163	25	188
40–49	2,270	189	163	25	188
50–59	1,230	37	30	6	36
Age unknown	—	18	—	—	—
Females, total	12,090	992	881	118	999
20–29	4,000	344	308	35	343
30–39	3,300	287	262	32	294
40–49	2,840	264	250	35	285
50–59	1,950	83	61	16	77
Age unknown	—	14	—	—	—
Per cent distribution, persons 20 to 49 yr.					
Males, 20–49 yr.	100	100	100	100	100
20–29	42	39	39	57	42
30–39	35	31	30	21	29
40–49	23	30	30	21	29
Females, 20–49 yr.	100	100	100	100	100
20–29	39	38	38	31	37
30–39	33	32	32	28	32
40–49	28	30	30	31	31

*Official estimates; last census 1946.

Table II. Mean levels of systolic blood pressure, by sample status, race, village, and sex and age

	Males			Females		
	20-29	30-39	40-49	20-29	30-39	40-[illegible]
Mean systolic blood pressure (mm. Hg)						
By sample status						
Card-holders	135.8	140.4	142.8	126.0	139.0	155.8
Walk-ins	135.0	132.0	148.3	130.4	135.5	148.5
By race						
Negro	136.2	139.2	144.0	126.5	138.8	155.3
Mixed	129.7	140.4	139.4	126.0	136.6	150.5
By village						
Sandy Point	136.4	140.9	140.4	127.1	137.1	154.3
Old Road	139.2	139.7	148.6	124.9	139.7	156.5
Cayon	133.6	136.4	143.7	126.5	140.1	154.5
Number of persons examined						
Total	277	188	188	343	294	285
By sample status						
Card-holders	210	163	163	308	262	250
Walk-ins	67	25	25	35	32	35
By race						
Negro	254	172	167	313	268	258
Mixed	23	16	21	30	26	27
By village						
Sandy Point	123	92	85	145	143	128
Old Road	40	38	50	54	66	66
Cayon	114	58	53	144	85	91

Table III. Zero as terminal digit of recorded blood pressure

Study	Total number recorded	Systolic		Diastolic	
		Number ending in zero	Per cent ending in zero	Number ending in zero	Per cent ending in zero
Comstock[7] (1957)	1,162	363	31.2	424	36.5
Master, et al.[23] (1952)	15,711	7,210	45.9	8,036	51.1
St. Kitts	1,688	1,041	61.7	898	53.2

Examination. "Blood pressure" clinics were established in village Health Centers. Each center consisted of a waiting room, two offices, and a pharmacy. During the survey clinics the offices were used as examining rooms, and the pharmacy as a laboratory. A nurse who was familiar with idiom and customs received subjects in the waiting room, and verified information on the card, noting name, sex, age (to last birthday), and her estimates of descent (Negro or "mixed") and of physical activity in work (sedentary, medium, or heavy). Pregnancies and the complications thereof were also recorded. Height (sole to vertex, shoes removed) and weight were

measured; garments were light and standard. Samples of urine were obtained from 1,650 subjects who represented 96 per cent of the women and 98 per cent of the men; the urine was tested with Uristix for protein and glucose. Abnormal proteinuria was frequently confirmed by sulfasalicylic turbidimetry, with good agreement of estimates between methods. The subject was then ushered to a cot in an examining room.

Here a physician inquired as to past or present illness. If this enquiry elicited a recital of symptoms suggestive of cardiovascular or renal disease, more detailed information was sought. Arterial pressure was measured after a rest of 5 to 10 minutes. Then, at the beginning of the survey, venous blood was sampled in most subjects; late in the survey, venipuncture was done only in subjects who seemed to be ill or who had diastolic hypertension. The technique for the measurement of pressure followed the recommendations made by the American Heart Association in its Special Report of 1951[5]; diastolic pressure was noted as the point of cessation of sound or, if sounds persisted to very low levels, as the point of muffling. Observations were made an hour or more after the subjects had eaten and exercised, during rest in recumbency, indifferently

Table IV. *Levels of blood pressure (mm. Hg) by sex and age**

Sex and age	*Number of persons*	*Systolic*		*Diastolic*		
		Mean	*Standard deviation*	*Mean*	*Standard deviation*	*100 mm. or more (%)*
Males						
20–29	277	135.6	16.14	79.2	10.12	4.3
20–24	151	134.7	15.96	78.0	9.62	2.0
25–29	126	136.8	16.29	80.7	10.49	7.1
30–39	188	139.3	18.17	83.0	11.98	12.2
30–34	105	138.2	17.34	82.0	11.69	9.5
35–39	83	140.7	19.08	84.2	12.22	15.7
40–49	188	143.5	22.75	86.0	13.78	16.0
40–44	93	138.5	18.50	84.1	11.62	14.0
45–49	95	148.4	25.32	87.8	15.40	17.9
50–59	36	152.3	25.78	89.2	14.37	22.2
50–54	29	150.3	24.58	88.0	12.49	20.7
55–59	7	(160.6)	(32.68)	(94.3)	(21.78)	(28.6)
Females						
20–29	343	126.5	15.85	76.4	11.66	5.0
20–24	191	125.5	14.67	74.6	10.26	2.1
25–29	152	127.8	17.13	78.6	12.88	8.6
30–39	294	138.6	22.67	84.7	13.96	15.6
30–34	168	134.7	18.68	82.8	13.75	12.5
35–39	126	143.7	26.23	87.2	13.82	19.8
40–49	285	154.9	30.44	90.6	15.33	27.7
40–44	142	151.0	28.49	88.8	15.33	22.5
45–49	143	158.7	31.79	92.3	15.13	32.9
50–59	77	168.5	34.82	93.8	16.00	40.3
50–54	60	167.5	33.85	93.6	15.76	40.0
55–59	17	172.2	38.98	94.6	17.28	41.2

*In this and subsequent tables, statistics based on fewer than 10 cases are shown in parentheses.

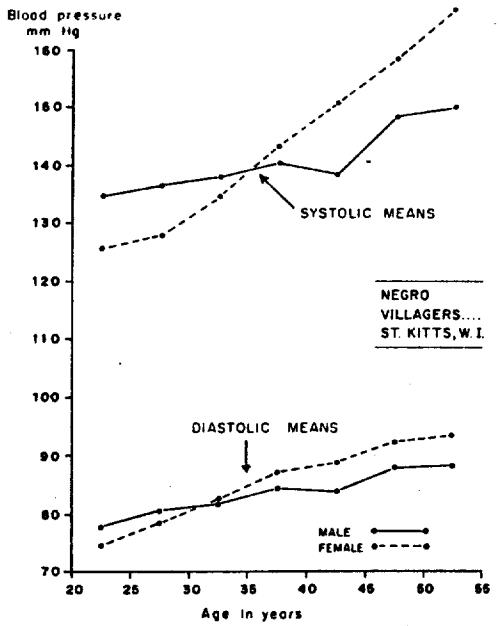

Fig. *1*. Means of systolic and diastolic blood pressures.

from the right or left arm; a 12-cm. hook-on cuff and a standardized aneroid manometer were used. Although pressures were to be recorded in multiples of 2 or 5, the data (Table III) show a distinct preference for zero as the terminal digit.

The basic datum of this report is the examiner's average of the first two or three determinations of pressure made in sequence over 1 or 2 minutes. Subjects (190 men and 265 women) who seemed anxious, and/or whose blood pressure or pulse rate seemed high at this observation, were reassured and kept at rest for second determinations 10 to 15 minutes later; determinations were repeated in a few after an additional hour of rest in the waiting room and at a second visit a day or two later. These determinations of pressure and pulse usually gave lower values than did first readings. These data are omitted from this report.

Observations

1. Means of blood pressures. Data are presented as means (Fig. 1), and means and standard deviations (Table IV) of systolic and diastolic blood pressures. Means and, to a somewhat greater degree, variability of the distributions increased with age. Mean systolic pressures in women exceeded those in men at about age 35 to 39, and diastolic pressures at age 30 to 34 years; thereafter, both pressures rose more steeply in women than in men. Table V enables comparison of the means of the data obtained at St. Kitts with the home-recorded blood pressures of urban Negroes and whites of Nassau, Bahamas,[6] and the clinic records of rural Negroes and whites of Muscogee County, Georgia,[7] and of a large, substantially white, urban group in Framingham, Massachusetts.[8] The systolic pressures of the three Negro groups are alike and are higher than those of white groups. The means of systolic pressure of Negro men and of white women begin to rise steeply at about age 45. The means of diastolic pressures of Negro women, but not of Negro men, are higher than those of white groups.

2. Frequency distribution of blood pressure. Basic tables of frequency distribution of blood pressures for each sex by 5-year age groups were prepared.* These data are summarized in Appendix Tables I through VI (see pp. 624-627), as histograms (Fig. 2), and as cumulative frequency curves (Fig. 3,*A-C*). Proportions of individuals with high arterial pressures (over 160 mm. Hg systolic or 100 mm. Hg diastolic) increased with age; accordingly, curves projected along the histograms flatten and skew toward high pressures with increasing age. The cumulative curves facilitate estimates of proportionate distributions; they show that the blood pressures of women were distributed in lower ranges than were those of men at ages 20 to 29, were nearly the same at ages 30 to 39, and exceeded those of men at ages 40 to 49.

3. Prevalence of "hypertension." The arbitrary character of segregation used in classifying high arterial pressures as "hypertension" is implied in the use of quotation marks and of multiple criteria. Those of Table VI include the criteria suggested by a committee of the World Health Organization (WHO) on recommendation of the Brookline Conference,[9] together

*It is proposed to deposit these (A.D.I. Tables I to IV, inclusive) with the American Documentation Institute.

with others in common use.* The prevalence of "hypertension" by any of the criteria listed increases with age. When groups of like age are compared, systolic hypertension is more common in men, but diastolic hypertension is equally distributed between the sexes at ages 20 to 39; prevalences by the various criteria are similar at ages 30 to 39 and those of systolic and/or diastolic "hypertension" as compared at ages 40 to 49 are about twice as great in women as in men.

*These are estimates prepared from Appendix Tables I-VI.

Reference to Table 9 of the Framingham data,[8] even when allowance is made for the use of two rather than one estimate of blood pressure, with resultant regression toward the mean, indicates a much lower prevalence of "definite hypertension" than in Kittitians, especially among women.

4. Ponderal (height-weight) index and blood pressure. Of the several expressions of body weight in relation to stature, that selected was Sheldon's ponderal index[10]; this is height to the nearest inch divided by the cube root of weight to the nearest pound. The values ranged from less than

Table V. Comparison of findings in surveys of blood pressure in Negro populations in St. Kitts, Bahamas, and Georgia, and in white populations in Bahamas, Georgia, and Massachusetts

Sex and age	Negro			White		
	St. Kitts	Bahamas[6]	Georgia[7]	Bahamas[6]	Georgia[7]	Mass.[8]
Mean systolic blood pressure (mm. Hg)						
Male						
25–34	137.5	134.4	132.9	127.7	121.5	*
35–44	139.6	142.5	142.8	130.0	123.8	134.1
45–54	149.3	150.2	150.6	133.0	132.6	139.9
Female						
25–34	131.1	129.5	124.9	120.2	112.6	*
35–44	147.2	144.7	146.3	132.3	120.9	128.4
45–54	162.8	154.3	158.2	145.0	135.5	147.0
Mean diastolic blood pressure (mm. Hg)						
Male						
25–34	81.3	85.2	81.1	75.6	78.5	*
35–44	84.2	91.5	91.5	82.0	82.5	85.8
45–54	87.9	93.8	96.2	83.2	86.1	88.4
Female						
25–34	80.6	82.4	80.1	72.7	72.3	*
35–44	88.0	89.4	90.5	81.9	76.9	81.6
45–54	92.9	91.9	93.6	86.0	82.5	89.4
Number of persons studied						
Male						
25–34	231	156	21	68	40	*
35–44	176	122	34	44	65	748
45–54	124	104	25	34	59	632
Female						
25–34	320	214	44	72	87	*
35–44	268	196	47	52	96	928
45–54	203	121	32	35	62	744

*Comparable data not available.

Note: Data for St. Kitts, Bahamas, and Massachusetts were originally reported for 5-year age groupings. Means reported in this table are weighted means of the 5-year age group components, with weights proportional to the total U. S. population in these age groups in 1940. Means for Georgia are unweighted means as originally reported.

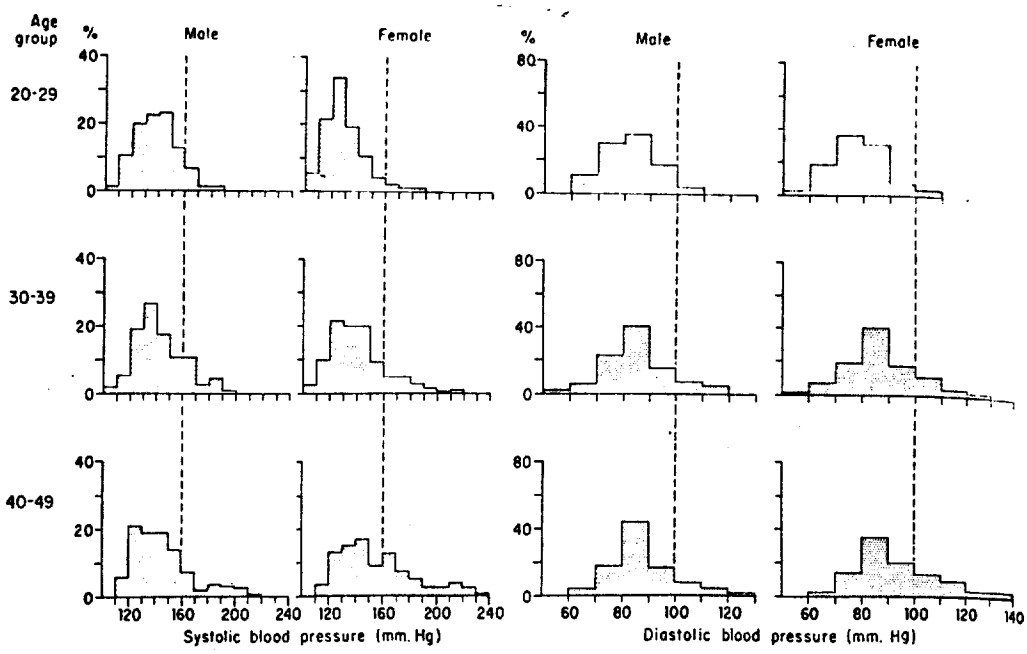

Fig. 2. Frequency distributions of systolic and diastolic blood pressure by age and sex.

12 (overweight) to 13.5 or more (underweight). These interpretations may not apply precisely to heavily muscled Kittitians; however, indices of about four fifths of the subjects lay between the extremes. Men showed some tendency to gain weight with age, but were infrequently (1 in 20) overweight; proportions of underweight men (about 1 in 10) were nearly constant among age classes. Among women, the proportion who were overweight increased from 13 per cent at ages 20 to 29 to 31 per cent at 30 to 39 and 35 per cent at 40 to 49, with concurrent decreases in the proportion who were underweight.

Five ranges of ponderal index are listed with age and sex groupings of means of systolic and diastolic pressures in Table VII. Differences in pressures between categories were small and irregular, but systolic pressures in women and, to a lesser degree, diastolic pressures in both sexes tended to increase with weight and age. The sharp increases observed in overweight women who were 40 to 49 years of age were not corrected for artifacts of arm size[11]; these may have contributed to the apparent increments in pressure.

Hypertension and hypertensive disease

A second aspect of the survey was characterization of the status of subjects with diastolic hypertension (100 mm. Hg or more) so as to gain some insight into the nature of the hypertensive state and the severity of concomitant hypertensive vascular disease. Techniques were limited by conditions of the field survey in a medically unsophisticated population. The 245 subjects presenting diastolic hypertension were closely questioned as to family history, work status, and symptoms referable to cerebral, cardiac, or renal dysfunction, and the women were questioned with regard to parity and toxemia of pregnancy. Signs of cerebrovascular disease were sought by brief neurologic tests and funduscopy, signs of cardiac disease by symptoms and electrocardiographic evidence of left ventricular hypertrophy, and signs of renal disease by estimates of proteinuria and, in many subjects, of creatininemia.

1. Work status. Diastolic hypertension could not be associated with work status, perhaps because so few (less than 4 per cent of the subjects) engaged in light or sedentary work, and because of indefinite

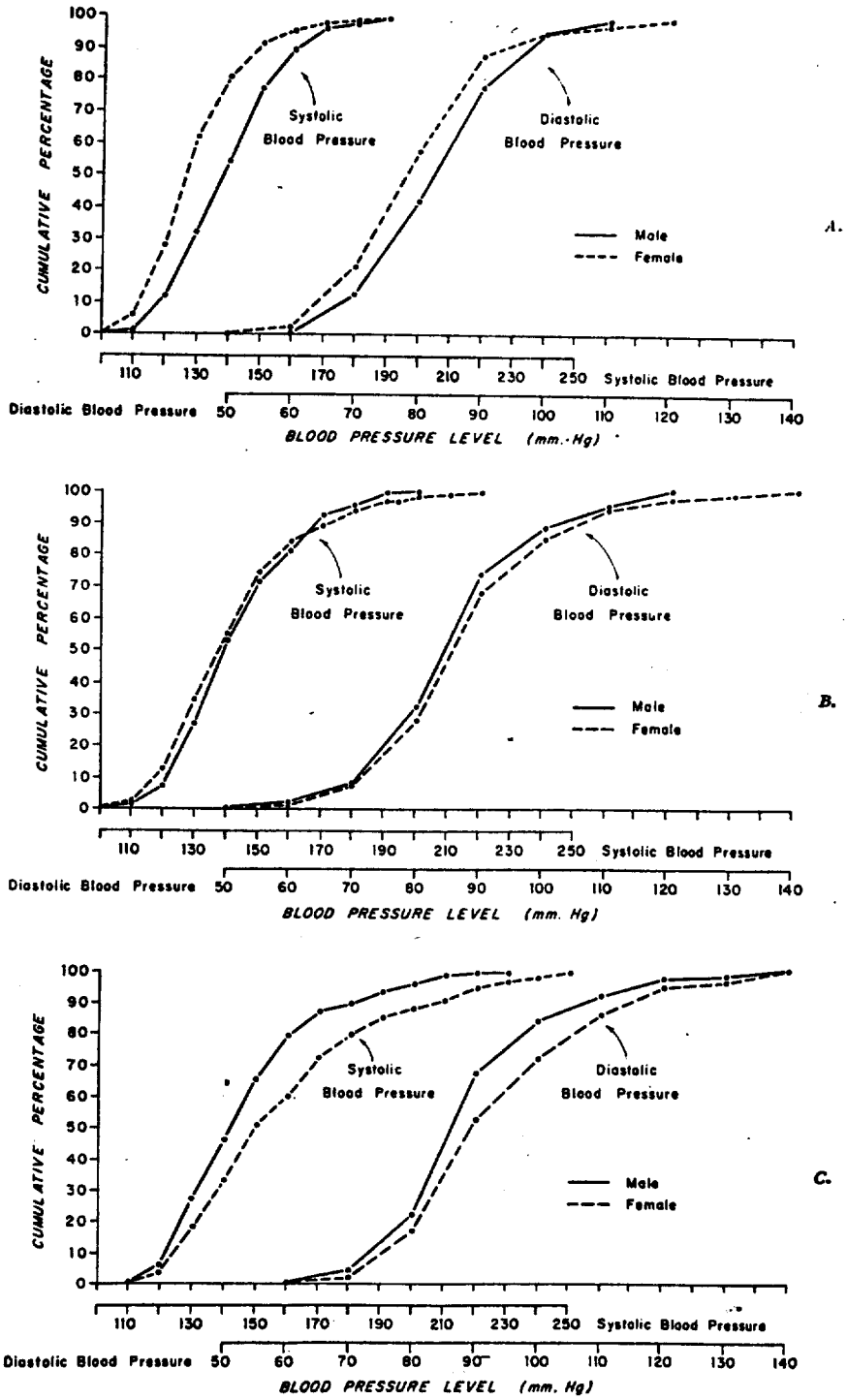

Fig. 3. Cumulative frequency distributions of levels of systolic and diastolic blood pressure. *A*, Males and females, aged 20-29. *B*, Males and females, aged 30-39. *C*, Males and females, aged 40-49.

Table VI. Prevalence of "hypertension" by sex and age in St. Kitts villagers

Sex and age	Per cent of persons					
	WHO criteria*					
	Normotension	Borderline	Hypertension	160/100† mm. Hg	Systolic 160 or more	Diast. 100 or more
Males						
20–29	50.5	35.4	14.1	12.6	9.7	4.3
30–39	49.5	26.1	24.5	22.9	18.6	12.2
40–49	44.1	28.2	27.7	24.5	20.7	16.0
Females						
20–29	79.0	14.0	7.0	5.0	4.4	5.0
30–39	51.7	24.1	24.1	20.7	16.3	15.6
40–49	31.6	23.9	44.6	42.5	40.7	27.7

*Criteria for population studies proposed by WHO Expert Committee on Cardiovascular Diseases and Hypertension (WHO/CVD & H/14, 27 October, 1958). *Normotension:* Systolic B.P. below 140 *and* diastolic B.P. below 90 mm. Hg. *Hypertension:* Systolic B.P. 160 or more *and/or* diastolic B.P. 95 or more. *Borderline:* All other.
†Systolic B.P. 160 or more *and/or* diastolic B.P. 100 or more.

distinction between "moderate" and "heavy" work.

2. *Family history.* The character of many family unions and lack of understanding of the nature of illness made this inquiry fruitless.

3. *Pregnancy, parity, and toxemia.* Pregnancy was listed as such in the first or, at least, in the second and third trimesters. Association of current pregnancy and parity with blood pressure are summarized in Table VIII. Diastolic pressures in the pregnant women were lower than those in the nonpregnant woman at ages 25 to 39, and systolic pressures were lower at ages 30 to 39; means of pressures were not affected by pregnancy at ages 20 to 29.

Hypertension was no more common in those women (the majority) who had four or more pregnancies than in those who had had only one to three (Table IX). However, subjects who had experienced toxemia of pregnancy (characterized by edema, proteinuria, and hypertension which were discovered when the women were attending prenatal clinics) showed a greatly increased prevalence of hypertension when compared with women whose pregnancies had progressed normally. Nulliparity seemed to be associated with increased prevalence of hypertension at ages 30 to 39.

4. *Symptoms.* The symptoms were not usually informative. Possibly as an expression of presbyopia, most older people had "dim eyes." Few complaints were elicited from men; women readily complained of "headache," "backache," and "pain in the belly bottom." Nocturia seemed to be a domestic ritual among both sexes and at all ages.

5. *Cerebrovascular disease.* Only two of the entire sample who were examined showed signs of previous strokes. Both were among the 245 persons with diastolic hypertension.

Funduscopic examinations were made on 176 hypertensive subjects after pupillary dilation with cyclopentolate hydrochloride. Factors evaluated were arteriolar constriction and sclerosis,[12] and hemorrhages, exudates, and papilledema[13]; results of the examinations were summarized by Keith-Wagener-Barker grades.[14]

Fundal abnormalities (Table X) were found in 85 per cent of the hypertensive subjects; prevalence and grades of retinopathy increased with rising diastolic pressures; fundi were abnormal in all with diastolic pressures of 120 mm. Hg or more. Papilledema was found in one subject; diastolic pressure in this person was only 104 mm. Hg, and proteinuria was greater than 100 mg. per 100 ml.

6. Cardiac symptoms. Symptoms were graded[15] in 112 hypertensive subjects. Of these, 98 were classed in functional Grades 0 or 1, 12 were in Class 2, and 1 each was in Classes 3 and 4. Rheumatic heart disease, constrictive pericarditis, and aneurysmal dilatation of the aorta were each diagnosed once.

Electrocardiograms (subject supine; 3 standard and 3 unipolar limb leads and 6 precordial leads) were recorded in 243 of 271 men who were 35 to 49 years old and in 131 of 143 women who were 45 to 49 years old. These records included 42 of 43 men and 45 of 47 women with diastolic hypertension. Criteria used in the diagnosis of left ventricular hypertrophy were modifications[16] of those of Sokolow and Lyon.[17] Results are listed in Table XI. Of 2 men who had left ventricular hypertrophy, one had diastolic and the other systolic hypertension with aortic insufficiency. Diastolic hypertension was present in all 8 women who had left ventricular hypertrophy. Other abnormalities, such as nonspecific myocardial changes, remote myocardial infarction, and right bundle branch block, were almost as common (about 1 in 8) in the electrocardiograms of those men without as in those with diastolic hypertension (1 in 5).

7. Renal symptoms. Proteinuria was estimated in 643 of 653 men and 909 of 922 women who were 20 to 49 years of age.

Table VII. *Mean levels of blood pressure by ponderal index (height/$\sqrt[3]{\text{weight}}$), sex and age*

Sex and age	Ponderal index				
	Less than 12.0	*12.0–12.4*	*12.5–12.9*	*13.0–13.4*	*13.5 and over*
	(Heavy ←			→	*Light)*
Mean systolic blood pressure (mm. Hg)					
Males					
20–29	(150.3)	139.4	135.7	133.7	131.3
30–39	(133.0)	143.1	137.8	138.6	139.8
40–49	145.1	144.2	144.7	144.6	126.1
Females*					
20–29	128.9	126.6	126.1	126.0	125.6
30–39	146.0	140.9	134.7	136.6	139.4
40–49	164.7	150.0	156.7	145.9	136.7
Mean diastolic blood pressure (mm. Hg)					
Males					
20–29	82.9	82.9	79.1	78.7	74.9
30–39	80.0	85.0	83.2	81.9	81.4
40–49	90.0	86.5	86.9	83.9	78.0
Females*					
20–29	79.6	76.1	77.2	75.7	72.9
30–39	88.4	85.7	84.0	84.7	81.7
40–49	93.8	89.3	91.6	86.2	83.6
Number of persons					
Males					
20–29	7	38	130	78	23
30–39	8	36	64	62	16
40–49	16	33	81	40	14
Females*					
20–29	41	63	106	70	24
30–39	81	67	58	45	12
40–49	97	84	46	44	10

*Excludes pregnant women.

Table VIII. *Mean levels of blood pressure in women by age and current pregnancy status*

Pregnancy status	Age			
	20–24	25–29	30–34	35–39
Mean systolic blood pressure (mm. Hg)				
Not pregnant	125.7	127.6	136.4	145.3
Pregnant	123.7	128.7	121.0	126.9
Mean diastolic blood pressure (mm. Hg)				
Not pregnant	74.8	79.1	83.9	88.2
Pregnant	73.2	74.8	73.3	77.0
Number of persons				
Not pregnant	172	135	150	115
Pregnant	19	17	18	11

Table IX. *Per cent of women with diastolic blood pressure of 100 mm. Hg or more, by age, toxemia, and history of pregnancy*

Parity and toxemia status	Age		
	20–29	30–39	40–49
Per cent of women with diastolic blood pressure of 100 mm. Hg or more			
Never pregnant	1.5	25.6	32.4
No history of toxemia			
1 to 3 pregnancies	5.8	8.6	23.6
4 or more pregnancies	4.9	13.4	23.2
History of toxemia	19.0	36.7	61.5
Number of women*			
Never pregnant	65	39	37
No history of toxemia			
1 to 3 pregnancies	137	81	72
4 or more pregnancies	81	112	142
History of toxemia	21	30	26

*Excludes women who were pregnant at the time of survey.

Among those in whom the diastolic pressures were less than 100 mm. Hg the prevalence of abnormal proteinuria (20 mg. per 100 ml. or more) ranged from 5 to 7 per cent and was 12 per cent in men who were 40 to 49 years old (Table XII). Among those with diastolic hypertension the prevalence was not increased at ages 20 to 29, but increased with age thereafter. Associations of proteinuria with hypertension are shown in Table XIII. The prevalence increased with systolic pressure in excess of 140 mm. Hg at ages 20 to 39 years, and with pressures of 160 or more at ages 40 to 49 years; it increased regularly with diastolic pressures over 80 mm. Hg in both age groups.

Concentrations of serum creatinine were measured in 272 subjects, of whom 73 had diastolic hypertension (Table XIV). In normotensive men and women, creatinemia corresponded with normal levels in North American subjects[18]; the average increased in hypertensive individuals.

8. Relative severity of hypertensive disease. The unreliability of symptoms, limited facilities, lack of repeated measurements of blood pressure over long periods, and loss of a number of samples of serum and urine, limited this analysis to 87 subjects with diastolic hypertension, and rendered inapplicable a "severity index" used in the trials of therapeutic drugs.[19] The estimate used was based on selected criteria, viz., retinopathy of Grade 2 or more, left ventricular hypertrophy by electrocardiogram, and abnormal proteinuria of 20 mg. per 100 ml. or more, any of which was classed as a "stigma" of hypertensive disease. Among 35 men and 52 women, 39 showed no stigma of hypertensive disease, 41 showed one and 22 showed two or more (Table XV). The most common stigma was retinopathy, and the next most frequent was proteinuria.

Table X. *Distribution of persons with levels of diastolic blood pressure of 100 mm. Hg or more, by level of blood pressure and grade of retinopathy*

	Diastolic blood pressure (mm. Hg)		
	100–109	110–119	120 or more
Per cent of persons			
All grades	100	100	100
Grade 0	25	10	0
Grade 1	57	49	33
Grade 2	14	27	33
Grade 3 or 4	4	14	33
Number of persons*	77	63	36

*Includes only persons whose optic fundi were examined.

9. Association with sodium chloride.

A. SERUM ELECTROLYTES. Means of concentrations (Table XVI), in agreement with the observations of others,[20] were alike as between normotensive and hypertensive individuals, and correspond to North American normal values.[18]

B. CONSUMPTION AND EXCRETION. Intake of brackish water was considered to be a possible explanation for the high prevalence of hypertension among Bahamian Negroes, although subsequent studies[21] have suggested that other factors are of more importance. In contrast, the water on St. Kitts is collected in mountain catchments, and our analyses showed a content of Na of less than 15 mg. per liter. However, meat and fish are commonly preserved by salting. The possible associations of the intake of salt were studied in two ways.

First, frozen homogenates of samples which represented three daily adult servings were collected from four families in each of the three villages and analyzed.* Moisture averaged 76.6 per cent; Na and Cl per volume of moisture yielded averages per day of 1.85 Gm. of Na (range of 1.0 to 2.6) and 6.27 Gm. of Cl (range 5.4 to 8.0). These levels correspond to average United States estimates (see Table 31 in Reference 18). Since the local custom is not to salt food after cooking nor to take salt between meals, the analyses probably represent average daily intake.†

Second, casual specimens of urine were analyzed for Na, Cl, and creatinine. The considerations in mind were that the creatinine content would represent daily output in a range of ±30 per cent,[22] and that daily output in this group of 128 men and 208 women would average about 1 Gm., so that the milliequivalents of Na or Cl per milligram of creatinine would represent a little less than the electrolyte output for 1 minute. Data summarized in Table XVII show that the Na and Cl contents were alike in corresponding age, sex and blood-pressure groups at ages 20 to 39 years. The output of electrolytes in hypertensive subjects seemed greater than in normotensive subjects at ages 40 to 49, but wide individual variability detracts from the possible significance of this difference. In the group as a whole the mean output corresponds to about 10 Gm. of NaCl daily. This estimate corresponds well with that from analysis of food. Furthermore, the data on urine do not indicate any regular association between the output of salt, and, by inference, the intake of salt, and the occurrence of hypertension.

*By the Army Medical Nutrition Laboratory, Fitzsimmons Army Hospital, Denver, Colo., through the courtesy of Lt. Col. L. M. Hursch, MC, USA, and Mr. C. F. Conzolazio.

†For completeness, it is noted that the Ca content of the mixture by weight averaged 1.12 Gm. daily.

Comment

1. Procedures. The bases for assuming that the sample is representative of the island's rural population, and justification for combining data from subgroups were noted above. The nurses' estimate that only 10 per cent of the subjects were of "mixed" race indicates racial homogeneity. Stated ages were apparently not seriously in error; however, when possible, documentary verification of this datum is desirable. The distribution of terminal digits of arterial pressures yields greater zero perference in our data (as in those of Master, Garfield, and Walters[23]) than that in Comstock's data[7]; agreement of our means with those he obtained from Negro laborers in Georgia suggests that zero preference has not significantly distorted our data. Most of his measurements and all of Johnson and Remington's[6] were made in the subjects' homes, whereas ours were made in the privacy of a Health Center with which the subjects were familiar; agreement of means in the three groups may indicate that locale makes little difference.

2. Means, distributions, and prevalence. Means of blood pressures in the various Negro groups are higher than those in white populations. Estimates of prevalence indicate that hypertension is more common among Negroes, especially women, than among whites of like ages. With advancing age, progressive skewing of the histograms and flattening of the curves of cumulative distribution of pressure occur. This can be considered to represent the appearance in age classes of increasing proportions of persons with "hypertension"[24,25] or age-conditioned increments of pressure in most of the population.[26] Like other

Table XI. *Electrocardiographic finding of left ventricular hypertrophy (LVH) by sex, age, and diastolic blood pressure*

Sex, age, and diastolic blood pressure (mm. Hg)	*Number of ECGs recorded*	*Electrocardiographic findings*			[illegible]
		Normal	*LVH*	*Other abnormalities*	[illegible]
Males					
35–39	70	65	—	5	13
Diastolic, less than 100	57	53	—	4	13
Diastolic, 100 or more	13	12	—	1	—
40–44	87	74	—	13	6
Diastolic, less than 100	74	64	—	10	6
Diastolic, 100 or more	13	10	—	3	—
45–49	86	68	2	16	9
Diastolic, less than 100	70	57	1	12	8
Diastolic, 100 or more	16	11	1	4	1
Total 35–49	243	207	2	34	28
Diastolic, less than 100	201	174	1	26	27
Diastolic, 100 or more	42	33	1	8	1
Females					
45–49	131	107	8	16	12
Diastolic, less than 100	86	78	—	8	10
Diastolic, 100 or more	45	29	8	8	2

cross-sectional surveys, our data do not resolve the current epidemiologic controversy as to the nature of essential hypertension. Rather, as McKusick[27] has suggested, a solution may come from a longitudinal study "in a population as homogeneous as possible in racial background and environmental circumstances," conditions which are fulfilled by the population of St. Kitts.

Parenthetically, the fact that the means of pressure and prevalence of hypertension among white women exceed those of men at ages 45 to 50 years has contributed to the belief in a "menopausal hypertension." The fallacy of this association is shown by the fact that means of pressure of Negro women begin to exceed those of men at ages 35 to 39, and also that castration does not provoke hypertension in young women.[28]

3. Ponderal index and blood pressure. Obesity is much less common in Kittitian laboring men than in the urban white groups. Definite obesity, with massive arms, seemed to us to be less common in Kittitians than in middle-aged Negro women in the United States, where, by another estimate, these *average* more than 20 per cent overweight.[29] Furthermore, a gain in weight by mature Kittitian women is due in part to increased muscle mass. Possibly because Kittitians tend to be muscular rather than obese, associations between blood pressure and body weight are less striking in our subjects than in other surveys.

4. Hypertension and hypertensive disease.

A. PREGNANCY, TOXEMIA, AND NULLIPARITY. Current pregnancy did not depress means of pressures in women who were 20 to 24 years old, in whom diastolic hypertension is uncommon, whereas pregnancy decreased means in older women, many of whom were presumably hypertensive. This association accords with the view that pregnancy is an antihypertensive state in man[30] as it is in experimentally hypertensive animals.

Pyelonephritis is a cause of hypertension

Table XII. *Per cent of persons with proteinuria of 20 mg. per 100 ml. or more, by sex, age, and level of diastolic blood pressure*

Sex and diastolic blood pressure (mm. Hg)	*Age*		
	20-29	*30-39*	*40-49*
Per cent of persons with proteinuria (20 mg./100 ml. or more)			
Males			
Diastolic, less than 100	5.0	5.5	12.1
Diastolic, 100 or more	8.3	14.3	26.7
Females			
Diastolic, less than 100	5.0	7.0	5.4
Diastolic, 100 or more	5.9	15.2	23.1
Number of persons*			
Males			
Diastolic, less than 100	260	163	157
Diastolic, 100 or more	12	21	30
Females			
Diastolic, less than 100	322	244	202
Diastolic, 100 or more	17	46	78

*Includes only persons for whom the level of urine protein was measured.

and has been studied epidemiologically from this aspect in Jamaica and in Wales,[31] where the blood pressures of those with bacteriuria increased with parity. The fact that in St. Kitts the means of pressures were not higher in women who had had four or more pregnancies than in those who had had one to three suggests that pyelonephritis does not account for most of the hypertension observed in these women.

The association of hypertension with nulliparity at ages 30 to 49 years is unexplained, except as a like association has been described in childless men and women in two European surveys.[32,33]

Finally, the strong association between previous toxemia of pregnancy and subsequent diastolic hypertension substantiates the view, now widely held, that toxemia is a cause of persistent hypertension.[30,34]

B. SIGNS AND SEVERITY OF HYPERTENSIVE DISEASE.

Cerebral: Signs of cerebrovascular disease were infrequently severe; only 2 of 245 subjects with diastolic hypertension demonstrated stroke residuals, and the proportion who showed severe fundal changes (Grade III or more) was also small (13.5 per cent). If Grade IV retinopathy is accepted as an index of the syndrome of malignant hypertension, this condition seems to be relatively uncommon in Kittitians. In other hypertensive groups it is usually estimated as occurring in 1 or 2 per cent.

Cardiac: The fact that signs of left ventricular hypertrophy were observed in only 9 of the 87 subjects also accords with the view that hypertensive disease is relatively mild; earlier onset of hypertension in women than in men probably accounts for the fact that this finding was made in 8 women and only 1 man.

Table XIII. *Per cent of persons with proteinuria of 20 mg. per 100 ml. or more, by age and level of blood pressure*

Level of blood pressure (mm. Hg)	*Age*	
	20-39	*40-49*
Per cent of persons		
Systolic		
Less than 130	4.5	11.0
130–139	3.4	7.6
140–159	10.4	5.9
160–179	9.5	10.8
180 or more	10.3	29.9
Diastolic		
Less than 80	4.2	9.0
80–89	5.9	8.1
90–99	8.8	9.2
100–109	11.3	13.2
110 or more	14.7	34.5
Number of persons		
Systolic		
Less than 130	441	100
130–139	235	79
140–159	286	135
160–179	84	74
180 or more	39	77
Diastolic		
Less than 80	452	89
80–89	389	173
90–99	148	87
100–109	62	53
110 or more	34	55

*Table XIV. Mean level of serum creatinine, by sex and levels of diastolic blood pressure**

Diastolic blood pressure (mm. Hg)	Sex	
	Male	Female
Mean serum creatinine (mg. %)		
Diastolic, less than 100	1.00	0.85
Diastolic, 100 or more	1.45	1.12
Number of persons†		
Diastolic, less than 100	81	118
Diastolic, 100 or more	17	56

*Data for persons aged 20-49 combined.
†Includes only persons for whom serum creatinine was measured.

Table XV. Occurrence of stigmata of hypertensive disease in villagers with diastolic hypertension (100 mm. Hg or more)

Sex and age	Stigmata of hypertension		
	None	One	Two or more
Per cent of persons			
Males			
35–44	63	31	5
45–59	43	19	37
Females			
45–59	38	37	25
Number of persons			
Males			
35–44	12	6	1
45–59	7	3	6
Females			
45–59	20	19	13

Renal: Prevalence of proteinuria increased at ages 40 to 49 years in subjects with normotension, and among those with hypertension at ages 30 to 39 but not at ages 20 to 29. Among hypertensive subjects this is attributable to the slow development of nephrosclerosis, which also accounts for increased serum creatinine in some with hypertension. However, the highest serum creatinine observed was 4.5, and there were only 4 among 73 individuals with diastolic hypertension whose concentrations were greater than 3.0 mg. per 100 ml. The regular association between diastolic pressure greater than 90 mm. Hg and proteinuria suggests that 30 mg. per 100 ml. is indeed an upper level of "normal" proteinuria by the method used. The age distribution of subjects with excess proteinuria indicates that renal disease is sequential to persistent hypertension rather than causal, and the association with diastolic pressure that proteinuria may be an index of nephrosclerosis in field surveys of hypertension.

Relative severity of hypertensive disease: A unique aspect of this survey was the attempt to study the clinical significance of hypertension as well as its numerical distribution. This objective was not achieved to the extent desired, although we have shown that such evaluation is feasible in a field survey. The data obtained in this respect would be more valuable if there were comparable data on the prevalence of hypertensive disease in a random sample of a population with diastolic hypertension. As it is, the distribution of abnormalities gave us the impression that the hypertensive disease observed in St. Kitts is not severe. However, this estimate is based on our experience in clinics and

*Table XVI. Mean levels of serum sodium and chloride, by sex and levels of diastolic blood pressure**

Diastolic blood pressure (mm. Hg)	Sex	
	Male	Female
Mean serum sodium (mEq./L.)		
Diastolic, less than 100	140.9	139.5
Diastolic, 100 or more	139.8	138.4
Mean serum chloride (mEq./L.)		
Diastolic, less than 100	101.4	102.2
Diastolic, 100 or more	103.0	102.7
Number of persons†		
Diastolic, less than 100	121	175
Diastolic, 100 or more	19	57

*Data for persons aged 20-49 combined.
†Includes only persons for whom serum sodium and chloride were measured.

Table XVII. *Mean ratios of urine chloride/creatinine and sodium/creatinine, by sex, age, and levels of diastolic blood pressure*

Sex and diastolic blood pressure (mm. Hg)	*Age*	
	20-39	*40-49*
Mean chloride/creatinine ratio		
Males		
Diastolic, less than 100	.207	.171
Diastolic, 100 or more	.173	.209
Females		
Diastolic, less than 100	.189	.158
Diastolic, 100 or more	.182	.185
Mean sodium/creatinine ratio		
Males		
Diastolic, less than 100	.204	.167
Diastolic, 100 or more	.179	.215
Females		
Diastolic, less than 100	.183	.156
Diastolic, 100 or more	.181	.186
Number of persons*		
Males		
Diastolic, less than 100	60	37
Diastolic, 100 or more	15	16
Females		
Diastolic, less than 100	99	33
Diastolic, 100 or more	35	41

*Includes only persons for whom urine chloride, sodium, and creatinine were measured.

hospitals where patients with symptomatic hypertensive disease tend to congregate.

5. *Nature of the hypertension.* Although examinations were limited, it seems unlikely that, with the probable exception of hypertension subsequent to toxemia of pregnancy, most of the hypertension observed was primarily renal. Rather, like primary or essential hypertension in other groups, diastolic hypertension in Kittitians increased in prevalence with age, was more common in women than in men, was followed rather than preceded by proteinuria, and, up to age 50, was infrequently severe. Similarities of means of blood pressure of Kittitians to those of other North American Negroes suggest a greater concentration in these groups than in white individuals of the genetic factor or factors which make for essential hypertension. We would anticipate, therefore, that this trait would also be as prevalent in West Africans, the forebears of these people; however, recent studies in Nigeria[35] and Liberia[36] suggest that this is not the case. Unpublished observations by one of us (R.E.S.) demonstrate that means of blood pressure of an urban Jamaican group are lower than those of a rural group. Since the genetic factor is equal, these observations imply existence of significant environmental factors (e.g., nutrition, infection, etc.) that may diminish increased arterial pressures of groups otherwise possibly predisposed to hypertension.

Negatively, in the United States the social environment and diet, particularly intake of salt, do not seem to be determinants of the high prevalence of hypertension in North American Negroes. Speculatively, the greater resistance of American Negroes than of whites to anticholinergic drugs[37,38] suggests the possibility that a difference in autonomic, particularly vasomotor, tone may be a contributory cause and should be evaluated.

Summary and conclusions

The means of systolic and diastolic arterial pressures of 1,575 Negro villagers of St. Kitts, West Indies, aged 20 to 29 years, are higher than those of white groups of like age in the Bahamas and in the United States after about age 30 years: means of blood pressures are higher in Kittitian women than in men at 35 years of age and over.

This population tends to be muscular rather than commonly obese; means of pressures show only moderate associations with increasing body weight (decreasing ponderal index), except among women aged 40 to 49, in whom the recorded pressures may have been increased by the artifact of arm size. The intake of sodium chloride at meals and the output in the urine are not excessive; output shows no definite association with distribution of arterial pressures.

The survey was unique in that it aimed at assessment of severity as well as prevalence of hypertension. Data indicate that the hypertension is not often severe in the age group studied; the syndrome of

malignant hypertension was encountered doubtfully only once in 275 subjects with diastolic pressures of 100 mm. Hg or more. Fundal changes greater than Grade II were uncommon, as were electrocardiographic signs of left ventricular hypertrophy and creatininemia of renal failure. The common manifestations of hypertensive vascular disease were mild retinal changes (Grades I or II) and slight proteinuria. Although this assessment was less complete than we hoped that it would be, the practicality of clinical evaluation in field surveys has been established.

Since the means of blood pressures of Kittitians parallel those of Bahamian and Georgian (United States) Negroes, the predisposition of American Negroes to arterial hypertension seems to be a [illegible] trait which might be advantageously studied in terms of levels of auto[illegible] function.

We deeply appreciate the assistance of [illegible] Mrs. Ralph Lambert, Dr. Kenneth Standard, Patricia Maynard, R.N., and Pearl Walters, R.N., as members of the field group, and the help given by Colonel H. A. C. Howard, Administrator, and Dr. J. M. Semple, Chief Medical Officer, and his staff, which includes District Medical Officers, Chief and District Sanitary Inspectors, and District Public Health Nurses, as well as that of many others.

Appendix Tables I-VI. Frequency distribution of blood pressures for each sex by age groups

Appendix Table I. *Distribution of levels of blood pressure, systolic by diastolic: males, aged 20 to 29 years*

Systolic blood pressure	Total	Diastolic blood pressure													
		Less than 65	65-69	70-74	75-79	80-84	85-89	90-94	95-99	100-104	105-109	110-119	120-129	130-139	140 and over
Total	277	16	18	49	34	68	32	43	5	8	3	1			
Less than 110	4	3		1											
110-119	29	4	6	13	4	2									
120-129	55	3	5	12	11	17	4	3							
130-139	63	1	4	12	8	19	11	8							
140-149	64	5		6	8	19	8	14	3	1					
150-159	35		3	3	2	5	6	8	1	4	2	1			
160-169	18			2	1	4	2	7	1		1				
170-179	4					1	1	1		1					
180-189	3					1		2							
190-199	1									1					
200-209	1									1					
210-219															
220-229															
230-239															
240-249															

Appendix Table II. *Distribution of levels of blood pressure, systolic by diastolic: males, aged 30 to 39 years*

Systolic blood pressure	Total	Diastolic blood pressure													
		Less than 65	65-69	70-74	75-79	80-84	85-89	90-94	95-99	100-104	105-109	110-119	120-129	130-139	140 and over
Total	188	8	8	18	26	46	31	23	5	11	2	9	1		
Less than 110	4	2	1	1											
110–119	10	2	2	2	3	1									
120–129	36	3	2	5	11	11	4								
130–139	50		3	7	8	19	6	6	1						
140–149	33			2	1	9	12	6	1	2					
150–159	20	1		1	1	1	6	3	1	5		1			
160–169	20				2	3	3	5	1	2	1	2	1		
170–179	5							2	1		1	1			
180–189	8					1				2		5			
190–199	1							1							
200–209	1					1									
210–219															
220–229															
230–239															
240–249															

Appendix Table III. *Distribution of levels of blood pressure, systolic by diastolic: males, aged 40 to 49 years*

Systolic blood pressure	Total	Diastolic blood pressure													
		Less than 65	65-69	70-74	75-79	80-84	85-89	90-94	95-99	100-104	105-109	110-119	120-129	130-139	140 and over
Total	188	4	4	19	15	54	30	24	8	12	3	9	3	2	1
Less than 110															
110-119	11	1	2	4	3	1									
120-129	40	1	1	10	5	16	6	1							
130-139	36	1		4	5	14	9	3							
140-149	36			1	1	13	11	6	3	1					
150-159	26		1		1	7	3	5	3	6					
160-169	14					2	1	6		3	1	1			
170-179	4								1	2		1			
180-189	7							1			1	3		1	1
190-199	6					1		2	1			2			
200-209	5	1									1	1	2		
210-219	1											1			
220-229	2												1	1	
230-239															
240-249															

Appendix Table IV. Distribution of levels of blood pressure, systolic by diastolic: females, aged 20 to 29 years

Systolic blood pressure	Total	Diastolic blood pressure													
		Less than 65	65-69	70-74	75-79	80-84	85-89	90-94	95-99	100-104	105-109	110-119	120-129	130-139	140 and over
Total	343	31	42	79	46	75	30	18	5	8	2	4	1	2	
Less than 110	20	12	7	1											
110–119	75	9	21	31	10	3	1								
120–129	117	5	11	34	17	39	9	1				1			
130–139	67	3	3	9	14	21	11	6							
140–149	35	2		4	4	9	5	9	1	1					
150–159	14				1	2	4	1	2	4					
160–169	7					1			2	2	1	1			
170–179	3							1		1			1		
180–189	3										1	2			
190–199															
200–209	1													1	
210–219															
220–229	1													1	
230–239															
240–249															

Appendix Table V. Distribution of levels of blood pressure, systolic by diastolic: females, aged 30 to 39 years

Systolic blood pressure	Total	Diastolic blood pressure													
		Less than 65	65-69	70-74	75-79	80-84	85-89	90-94	95-99	100-104	105-109	110-119	120-129	130-139	140 and over
Total	294	14	10	34	24	74	43	38	11	22	7	8	3	6	
Less than 110	8	4		3	1										
110–119	29	5	3	14	4	3									
120–129	64	1	3	12	13	31	2	2							
130–139	59	4	4	3	3	26	13	5		1					
140–149	59			2	3	9	21	15	5	4					
150–159	27					3	5	6	5	6		2			
160–169	14					2	1	5		4	1	1			
170–179	15						1	4	1	3	3	2	1		
180–189	8							1		1	2	2	2		
190–199	5									2	1			2	
200–209	1											1			
210–219	2									1				1	
220–229	1													1	
230–239															
240–249														2	

Appendix Table VI. *Distribution of levels of blood pressure, systolic by diastolic: females, aged 40 to 49 years*

Systolic blood pressure	Total	Diastolic blood pressure													
		Less than 65	65-69	70-74	75-79	80-84	85-89	90-94	95-99	100-104	105-109	110-119	120-129	130-139	140 and over
Total	285	3	3	18	24	63	38	38	19	27	11	25	5	9	2
Less than 110	1	1													
110–119	11		1	5	3	2									
120–129	38	1	1	6	14	13	3								
130–139	44		1	5	2	23	9	4							
140–149	50			2	4	18	15	8	2	1					
150–159	25	1				1	8	7	4	3	1				
160–169	36				1	4	3	9	8	9	1	1			
170–179	22							4	4	5	3	6			
180–189	15					2		5		4	1	3			
190–199	8							1		2	2	2		1	
200–209	8								1	1	1	4	1		
210–219	11									1	1	4	2	3	
220–229	8									1	1	4	1	1	
230–239	3											1		1	1
240 and over	5												1	3	1

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Low Salt Diets and Arterial Hypertension*

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It is difficult to discuss the effect of diets low in salt upon the blood pressure and subsequent course of patients suffering from arterial hypertension of unknown cause without first examining the condition itself and its fundamental nature. So little is known of its etiology and mechanism that the attitude one adopts becomes a matter of belief based on the scant evidence available. One idea is that etiologic factors in all cases of arterial hypertension are the same; that similar interacting influences, or disturbances, lead to an altered vascular physiology expressed by hypertension. This view is untenable. Four simple mechanical factors act to maintain a normal blood pressure as well as a high one; i.e., the volume of blood, its viscosity, the function of the heart and the resistance to blood flow in the periphery. Many others affecting not only the cardiovascular system, but general and apparently unassociated bodily functions, may express their actions by changes in blood pressure. In fact, the blood pressure can be considered as a resultant of a number of interacting influences, and can become decompensated in a number of ways. Some of these are well understood but many are not. It may be as untrue to state that all cases of arterial hypertension have the same basic disturbance as it is to state that all patients with fever have the same etiologic agent.

Another theory is that patients with pathologic conditions associated with hypertension, and to which hypertension is considered secondary, may have different etiologies and mechanisms depending upon the disease present. All other patients with conditions not associated with these known diseases are considered to have "essential" hypertension. A similar etiology and mechanism is, therefore, postulated for such subjects. This is the prevailing viewpoint at present. With this theory goes a tacit assumption that diagnostic methods are accurate enough to differentiate obscure underlying conditions. The mechanism of the elevation of blood pressure of most patients in this category may well be a uniform one, but the assumption of a common etiology cannot be made at this time.

The third point of view, so aptly expressed by Fishberg,[1] is that so-called "essential" hypertension is a "collective concept for a number of conditions having in common the positive characteristic of arterial hypertension and the negative one of the absence of primary renal disease." On this theory, it becomes possible to consider that a number of different pathologic conditions are being grouped as a syndrome in which various disturbances lead to elevation of the blood pressure with its concomitant cardiovascular and renal changes. Furthermore, when therapeutic results are obtained in some individuals but not in all, one can examine them according to their actions on these different disturbances and not accept or discard them in the light of one mechanism only. In the following discussion, the possibility that we are dealing with several different conditions having a common clinical finding should be borne in mind.

SODIUM CHLORIDE AND ARTERIAL HYPERTENSION

Any relationship which may exist between salt (sodium, chloride or both) and arterial hypertension is a complex one. To break it down into its component parts, one

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must consider the various interacting influences which may be at work. The question of whether or not there is a disturbance of sodium chloride balance in hypertension can only be answered by examining ways in which such a disturbance could act, for the evidence on this point is far from clear.

The kidneys are the first organs to be considered. Is there a specific retention of sodium chloride by hypertensive kidneys? Little is known of the effect on these ions of mild renal vascular disease or constriction. In right-sided heart failure, for example, the clearance and the excretion of salt are reduced.[2,3] In hypertension Ambard and Beaujard[4] postulated a "dry retention" of salt and advocated for the first time a salt-poor diet to counteract it. Later, Ambard abandoned this idea, but many others continued to entertain it. Recently, with the present revival of interest in the subject, Perera[5] showed that hypertensive patients placed on twenty-four hours of rigid salt restriction did not lose weight or water, while normal individuals did. Both groups lost the same amount of salt, but in two pairs of subjects the clearance of sodium chloride was reduced in the hypertensives. Farnsworth,[6] on the other hand, studying the renal excretion of chloride, found that chloride clearances were increased in hypertensive patients and that the renal tubules demonstrated a specific failure to reabsorb this ion. Furthermore, Selye[7] recently reported that some hypertensive patients with high diastolic pressures showed an increase in blood sodium relative to chloride. She believes that this is the result of the action of the adrenal cortex. These findings do not support Ambard's original idea of "retention chlorurée sèche."

If there has not been demonstrated a specific renal diminution of salt excretion, one must examine those factors which can influence the kidney to retain salt and relate their activities to what is known about hypertension. Among them are: (1) the relation of the adrenals to salt balance; (2) the relation of the adrenals to hypertension; (3) the relation of other influences to the adrenals and (4) the relation of salt to hypertension.

Salt and the Adrenal. The relation of sodium chloride balance to the adrenal cortex is well known. Steroid compounds similar to those of the adrenal are specific for counteracting the derangements in sodium balance found in adrenal insufficiency. The loss of sodium chloride in Addison's disease is the predominant, though not the only, disturbance which accounts for the symptoms and findings. Disturbances of electrolytes do occur in some cases of adrenal cortical hyperfunction, with increases in the blood levels of sodium and decreases in potassium.[8a] Rats reared on a low sodium diet excrete radioactive sodium more rapidly than normal rats,[8b] and a diet free of sodium has been said to produce changes in the adrenals.[8c] The action of the adrenal cortex and of desoxycorticosterone on salt balance is thus an established fact, although there are still many important questions to be answered concerning its method of acting and its relation to other organs and systems.

Adrenal Cortical Factor in Hypertension. Any discussion of the rôle of sodium chloride in arterial hypertension must involve the part the adrenal glands play in this condition. About this there is considerable controversy. Evidence for an adrenal factor, and particularly an adrenal cortical factor, in arterial hypertension is suggestive but not thoroughly convincing. It is obvious that the adrenal cortex is necessary for maintenance of an elevated blood pressure. It is also necessary for maintenance of a normal blood pressure. Bilateral adrenalectomy will prevent the development of experimental hypertension and lower the blood pressure in one already established unless substitution therapy is used. But other conditions, sometimes disregarded, are just as necessary as mentioned previously. Even a normal body temperature may be required, for an elevated blood pressure may fall during bouts of fever induced by disease or by the introduction of pyrogens.[9]

A conclusion that the adrenal cortex is necessary for hypertension to be established does not necessarily implicate this organ as being primarily involved. In some cases it may be, however. Hypertension, associated with Cushing's syndrome, can apparently result from tumors or hyperplasia of the adrenal cortex. In this condition one is drawn to the conclusion that an adrenal mechanism plays a primary or initiating rôle. Small adenomas of the adrenal cortex have been described in about 1.45 per cent of all autopsies; patients showing these tumors frequently exhibited hypertension in life.[10] Hyperplasia of the cortex, associated with hypertension, has also been described.[11] These findings have been doubted.[12] It is possible that these cases represent a different type of the condition, although so far no attempt has been made to classify them accurately on clinical grounds. Heinbecker,[13] however, believes that an adrenal-pituitary-renal relationship is disturbed in arterial hypertension, resulting in pituitary changes and renal ischemia.

Measurements of the urinary excretion of cortical-like and other steroids have not offered proof that these substances are abnormal in cases of hypertension even if values are low.[12] Total amounts of 17-ketosteroids or other steroid metabolites may give misleading information concerning the activity of the adrenal cortex, unless the values found are well outside the normal (and fairly wide) range. All cortical steroids and their metabolites are not measured by this method. If there is any suggestion to be gained from such studies, it is that the cortex may be underactive.

More direct and exact measurements of these substances have yielded interesting evidence that some disturbance of the adrenal cortex or of the body's ability to metabolize steroids may be present in some cases of hypertension. Recently, Dobriner[14] reported the presence of a metabolite of adrenal cortical steroids Δ^9, etiocholenalone, in the urine of half of six patients with hypertension. These were all women. It was also present in two normal subjects, one male and one female, out of twenty-eight and in twenty-five of twenty-six patients with carcinoma, excluding that of the breast. It was present in five of seven subjects with Cushing's syndrome as well. Another so far unidentified metabolite occurred in the urine of all of five hypertensive women and in only two of nine normal subjects. These findings suggest that alterations in the metabolism of adrenal cortical steroids occur in some cases of arterial hypertension and Cushing's syndrome. Dobriner's careful analyses, and time-consuming methods of extraction and identification of minute quantities of steroids from large volumes of urine are inapplicable to the usual research laboratory. It is hoped that more rapid and simple methods for the study of these compounds may be developed in order that they be correlated further with special varieties of hypertension and other diseases.

Goldblatt,[15] in his recent excellent review, states, "Of the endocrine organs, the only one that may possibly play a significant, even if only a secondary part (in hypertension) is the adrenal, although this conclusion is contested on basis of inadequate evidence." Newer evidence does not yet controvert this viewpoint. Until the disease with its secondary manifestations can be consistently reproduced by disturbing adrenal function, evidence that the adrenals are implicated as primary etiologic agents in most patients must be considered inadequate. If they are concerned, it is uncertain whether their secretions are increased or diminished.

Relation of Salt and Desoxycorticosterone Acetate to Hypertension. The relation of salt to hypertension is a matter of controversy. Most of the evidence for salt being important comes from studies on patients using low salt diets which will be discussed later. There is accumulating evidence, however, that at least under some conditions sodium chloride, taken in large amounts, may adversely affect the blood pressure of hypertensive patients more than of normal ones. A high salt intake may reverse the hypoten-

sive action of low salt diets.[16] The treatment of Addison's disease by desoxycorticosterone acetate and salt is often accompanied by a rise in blood pressure to levels over 140 mm. Hg systolic and 90 diastolic, sometimes reaching diastolic levels of 100 to 110.[17,18] When desoxycorticosterone acetate and salt were given to normal subjects a small but significant rise in blood pressure occurred after two to three weeks. When the same amounts were given to patients with hypertension, the blood pressure became higher in one to four days.[19]

Experimentally, the blood pressure of rats and dogs can be elevated by the administration of salt and desoxycorticosterone acetate, and renal vascular lesions have been reported to occur.[20] That desoxycorticosterone acetate and salt will elevate blood pressure appears to be an established fact, not only in animals, but in human beings as well. The administration of large amounts of salt alone, however, to normal subjects, does not change blood pressure.[21] Plasma volume and venous pressure may, in some cases, become considerably elevated after salt, with extracellular water, measured by "thiocyanate space" increased and much sodium retained.

Landis[22] recently reported some interesting observations on hypertensive rats. Choices of sodium chloride and other electrolytes were offered to three groups of animals: normal, moderately hypertensive and severely hypertensive. Those exhibiting moderate hypertension ingested significantly less salt than did the other groups. One implication of these results is that there may be some dysfunction of certain functions of the adrenal in these animals.

These experiments do not incontrovertibly support the idea that the adrenal cortex is disturbed in hypertension. They merely show that experimental alterations in salt balance may affect blood pressure when desoxycorticosterone acetate is used. These results have not been duplicated by the administration of whole cortical extract.[8] Furthermore, the adrenalectomized animal and human being seem to be more susceptible to the hypertensive actions of these substances than are normal ones. This has led Soffer[34] to suggest two vascular regulating factors of opposite action in the adrenal cortex which may be disturbed in hypertension and which are present in whole gland extracts. One of these may be similar to desoxycorticosterone acetate. The other may be diminished in hypertension and absent after adrenalectomy. If desoxycorticosterone acetate can act as a pressor or vasoconstrictor substance, this would be important in some cases of hypertension.*

Relation of Other Influences to the Adrenals and Kidneys. It is not within the province of this discussion to consider the complex interrelations between the adrenal cortex and other endocrine organs. There are three influences, however, which should be mentioned. The first concerns the work of Marshall and Kolls[23] who found that in dogs extirpation of one adrenal resulted in markedly increased excretion of chloride and water by the homolateral kidney, while the excretion of creatinine was unaffected. This suggested that the change was independent of renal blood flow. The same effect was observed after unilateral section of the splanchnic nerve and Marshall concluded that the results were due to nervous influences. It is probably impossible to remove a dog's adrenal without severing many of the sympathetic nerves which lie in a plexus about the gland and thus partially denervating the kidney. These experiments suggest the presence of a nervous (or hormonal) influence on the renal excretion of chloride which is not humoral. The converse, i.e., that stimulation of sympathetic nerves leads to salt retention, has not been demonstrated.

The second influence concerns the recent demonstration by Vogt[24] directly and by

* Since this manuscript was prepared, desoxycorticosterone acetate has been found to elevate the blood pressure of hypertensive patients when it is injected intravenously. It does not change the blood pressure of normal individuals. Similar compounds, i.e., progesterone, dehydroisoandrosterone acetate, Δ^5 pregnenolone, testosterone and whole adrenal cortical extract (Upjohn) did not significantly affect blood pressure.[37]

Long[24] indirectly that epinephrine is a potent stimulus to adrenal cortical secretion. Of great interest to this problem is the finding that "stimulation of the sympathetic nervous system and consequent liberation of epinephrine is capable of increasing cortical hormone output."[25] If hyperactivity of the sympathetic nervous system can cause the adrenal cortex to liberate salt-retaining hormone, which has not yet been shown, a mechanism is provided for salt retention in hypertension. There seems to be evidence that overactivity of the sympathetic nervous system may play a part in some cases of "essential" hypertension, although perhaps not in all.

If one were to attempt to diagram these influences in order to clarify them, a scheme such as Figure 1 would result. Of course, these effects have been demonstrated experimentally only and have not been confirmed in human beings.

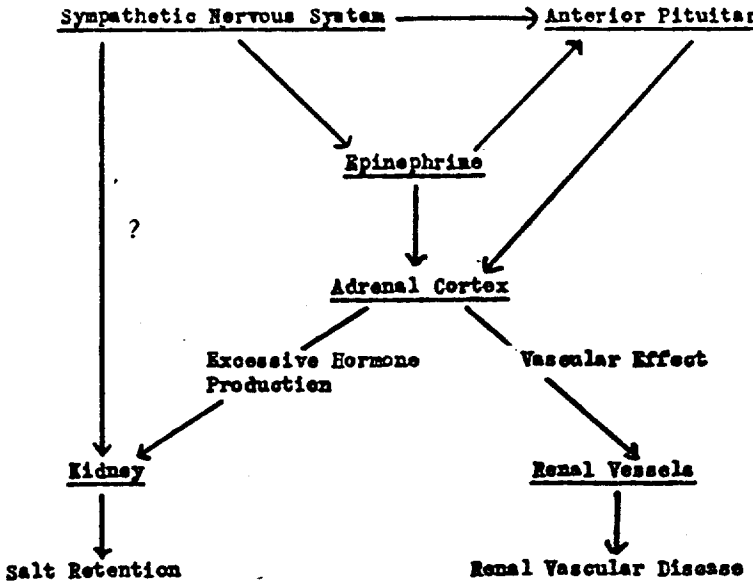

FIG. 1. Possible influences on the renal retention of sodium chloride.

EFFECT OF LOW SALT DIETS ON HYPERTENSION

Ambard and Beaujard[1] were probably the first to recommend a salt-poor diet for the treatment of hypertension. Later Allen and Sherill[26] strongly advocated this measure as did Volhard.[27] Other investigators have obtained effects with this diet and various theories have been proposed to account for them, while many others were not convinced of its efficacy. Diets of this type came into rather wide use in Europe but were not generally applied in this country. Recently, however, a renaissance of this subject has occurred (Table 1), principally because of the work of Kempner.[28] By applying a diet of rice, fruit juices and vitamins to the treatment of hypertension, he found consistent and significant reductions in blood pressure in about 57 per cent of patients. This diet contains less than 200 mg. of sodium. Recently, Grollman et al.[16] studied six patients on a diet containing less than 1 Gm. of sodium chloride. A significant fall in blood pressure occurred in two, with slight reduction in three others.

Probably the most complete work on the subject was done by Viersma.[29] His experiments appear to be the most extensively controlled and deserve attention. He studied eight patients suffering from so-called "essential" hypertension, two from chronic nephritis and four from malignant hypertension. Observations on one patient ex-

tended for more than two and one-half years. His control periods were long and carefully standardized. In seven of the ten patients with "essential" hypertension or that secondary to chronic nephritis, blood pressure became lower under the influence of a diet containing less than 1 Gm. sodium chloride per day, Viersma concluded, however, that the decline was not great, the systolic pressure falling 40 mm. Hg or less and the diastolic 26 mm. Hg at the most. Ingestion of this diet was associated with oliguria but had no demonstrable effect on renal function except in one subject. In this instance, low grade uremia became worse when treatment with ammonium chloride and salyrgan produced a sodium chloride deficiency. The diastolic pressure decreased further when this occurred. In cases of malignant hypertension the salt-poor diet had little effect on blood pressure. In general, but with exceptions, he found that changes of blood pressure during salt retention or loss paralleled changes in blood volume although variations were relatively small. He also noticed that changes in blood pressure lasted for a long period after return to a normal diet.

Viersma found no reasons in his own work and in an extensive review of the literature to assume the existence of any abnormalities of sodium chloride metabolism in "essential" hypertension. In the malignant stage, however, even when renal function was good, he found that salt was lost by the kidneys. He did not believe that the adrenal cortex was involved in the effects noted, but that they occurred because of a decreased cardiac output resulting either from a change in blood volume or from some reduction in peripheral and elastic resistances.

TABLE I

SUMMARY OF RESULTS REPORTED IN THE RECENT LITERATURE

Author	Type of Diet or Sodium Content mg.	No. of Patients Treated	(O) Outpatients; (H) in Hospital	No. Affected Favorably	Change in Diastolic Pressure mm. Hg	Per Cent Afflicted	Remarks
Bryant and Blecha[30]	200	45	O	26	>30	18	
					>20–29	40	
Kempner[28]	Rice	47	H	25	>20	53	Renal disease primary
	(200)	50	H	20	>20	40	Renal disease secondary
		65	H	12	>20	18	Renal disease absent
Grollman et al.[16]	200	6	H	2	>20	33	
Filipse and Filipse[35]	200	32	O	13	>20	41	
Viersma[29]	400	8	H	2	>20	25	"Essential" hypertension
	400	4	H	1	>20	25	Malignant hypertension
	400	2	H	2	>20	100	Nephritis, chronic
Schroeder et al.[32]	Rice (200)	6	H	1	20	17	
Behrendt and Burgess[36]	Rice	9	H	7	....	...	Average pressures reported Diastolic change < 20 mm.

Among others who have recently studied the effects of this diet upon the blood pressure of hypertensive patients, Bryant and Blecha[30] found a significant lowering of diastolic pressure (20 mm. Hg or more) in 57.8 per cent of patients treated with diets containing less than 200 mg. of sodium. These were outpatients and blood pressures were all ambulatory readings. Dock[31] states that about half of the patients in his series were benefited by low salt regimens.

Our own experiences, both with a diet containing 1 Gm. of sodium chloride and with Kempner's rice diet, have been disap-

pointing. Although the series was small, patients were carefully controlled in a hospital. One Gm. salt diets were found to be relatively ineffective in patients with severe arterial hypertension and were not used extensively.[33] After at least a month in the hospital on a normal diet containing salt, the institution of salt restriction was not observed to affect the blood pressure significantly when used for a period of two to eight weeks. On the other hand, decided depression of the blood pressure to normal or near normal was observed in occasional patients. For example, a forty-two year old woman with some of the signs of Cushing's syndrome showed considerable edema and a disturbance of water excretion characterized by a low urine volume which was relatively unaffected by varying the intake of fluids. Her urine contained antidiuretic substance. Coincident with the use of a diet containing 1 Gm. of sodium chloride her blood pressure fell to normal levels from approximately 220 mm. Hg systolic and 120 diastolic. Occasionally, other patients responded similarly. These were in most instances women exhibiting obesity who were often at about the age of the menopause.

Six patients were studied carefully, using the rice diet of Kempner.[32] Control periods in hospital extended for about a month in most instances. The institution of the rice diet did not change the average diastolic pressure of three patients with severe hypertension at all. In two others the fall was slight and in a third the average diastolic pressure fell 20 mm. Hg. The plasma chlorides fell in all patients, but in two in whom plasma sodium levels were followed there was little change. This was also noted by Viersma[29] and no explanation is offered. The one patient in whom the diastolic change occurred exhibited obesity, easy bruising and a reduction in tolerance to glucose. She had at one period of her life taken insulin. The administration of sodium chloride to this diet was not accompanied by consistent changes in the opposite direction as far as blood pressure was concerned. It was concluded that the rice diet had little advantage over a normal diet low in salt and that the change expected was minimal and occurred in those patients with less severe manifestations of their disease. While these studies were not as extensive as those of Viersma, the conclusions were similar, i.e., that the changes expected when the subjects are well controlled are slight. The diet is a rigorous ordeal for most patients and is hardly justified unless it can be shown to have special advantages.

It should be pointed out that adequate control periods are essential in judging the effect of any procedure upon the blood pressure in hypertension. It is an axiom that any therapeutic procedure vigorously pursued and instituted for the relief of "essential" hypertension will lower blood pressure and a corollary that nothing so far discovered is specific for the disease. We believe that the control period must be carefully evaluated and that the blood pressure must be at the lowest stable level for a considerable period of time before any procedure is instituted. Viersma studied some patients as long as three and one-half months without doing anything. The very fact of treating the patient, as well as rest and hospital routine, will often produce decided effects.

In Kempner's studies, when he found declines in blood pressure in twenty-eight of fifty patients with renal disease and in thirty-seven of sixty-five without renal involvement, control periods which we consider adequate were not indicated. In his published charts they varied from seventeen to four days, the average being nine days.[28] In Grollman's series the control periods were somewhat longer, but in no patient were these periods more than two weeks.[16] Controls in other recent studies have not been published, yet it is only by long periods of control, impossible in most general hospitals, that one can evaluate adequately therapeutic procedures of this kind. One recent study was well controlled, but the effects on the diastolic pressure did not appear to be marked.[36]

A small group of female patients has been studied who appeared to respond favorably and sometimes dramatically to a reduction of sodium chloride intake.[33] We have a clinical impression that many of these patients exhibit certain common findings which occur to a more marked degree in Cushing's syndrome. For lack of a better term, they have been called "pseudo-Cushing's."* They are usually obese with the obesity often confined to the trunk. They bruise easily and exhibit a curious mottled cyanosis of the skin especially of the extremities. They sometimes show an abnormal tolerance to glucose of a type resembling diabetes. Rarely has osteoporosis been found. There may be associated menstrual disturbances or the menopausal state. Hirsutism has occasionally been noted. The possibility exists that these subjects represent a separate disease which hypertension is associated with secondarily. If the activity of the adrenal cortex is found to be altered in these patients, their hypertension might be on that basis. On purely clinical grounds that suggestion is possible. The response of their blood pressure levels to salt restriction may indicate that salt is concerned in their hypertension.

If salt-poor diets do lower blood pressure in some cases, when psychotherapeutic influences are excluded, this must be explained. Adequate explanations are lacking. It is difficult to understand how retention of salt *per se* can raise blood pressure unless circulating blood volume is increased. The theories proposed are in the main unconvincing in the light of other and more direct evidence for different mechanisms. Grollman[34] believes that there may be some reduction in extracellular fluid resulting in a diminished plethora of the vascular system. Still, explanations of the mechanism of the lowering of blood pressure by the use of a low salt diet remains as unclear as does the inconsistency of response in different patients.

One of our patients who was put on the diet had exhibited diminution of renal function without nitrogen retention. After two weeks on the rice diet she became drowsy, disoriented and developed nitrogen retention and diminished urine volume. When her plasma chloride and sodium content were finally measured, they were at levels of 118.4 and 69.0 mEq. L., respectively. She died in uremia without significant change in the level of her blood pressure. This case is mentioned for two reasons. First of all, it can not be too strongly stressed that a low salt or rice diet can bring on fatal consequences when renal function is reduced. We have observed the same sequence of events in patients suffering from congestive heart failure after severe salt depletion from the use of mercurial diuretics. Secondly, the fact that this patient's sodium and chloride levels in plasma and presumably in her whole body were reduced markedly without change in blood pressure, suggests that sodium chloride was not concerned in the mechanism of the elevation of her blood pressure. Viersma believes that blood volume changes may account for the results observed but it is difficult to conceive of this mechanism as the principal factor. The explanation is unclear and the mechanism unknown.

It is our present belief, however, that certain patients will respond to a low salt diet with reduction in blood pressure. Some of these patients may be suffering from a disease different from that commonly considered to be "essential" hypertension. Slight changes may be observed in other patients. These are probably due to secondary influences and not primarily concerned in the mechanism of the elevation of blood pressure. On the whole, in our experience those patients with the most severe degree of hypertension respond to salt restriction least. And this is the group which it is necessary to help.

Until more is known about the subject it is therefore advisable to try diets low in salt for the routine treatment of hypertension with the expectation that a majority of patients will not respond. When renal

*Term suggested by Dr. Willard M. Allen.

function is reduced, this should be done under conditions in which the patient can be carefully watched and plasma chloride levels followed. If the response is good, the diet can be continued; if not, stopped. The rigors of the so-called rice diet are hardly justified considering that similar results can probably be obtained by rigid restriction of sodium chloride alone.

The diets used should contain as little salt as possible, depending upon the response of the patient. One Gm. of sodium chloride may be too much in some individuals. Approximately 0.5 Gm. is about as low as can be maintained under the best conditions. Both of these diets require careful selection of foods and fluids and are usually difficult to maintain when patients are ambulatory. It is hoped that methods other than dietary ones for controlling blood pressure will make this difficult and unpredictable form of therapy obsolete for the majority of patients exhibiting so-called "essential" hypertension.

SUMMARY

The dietary management of arterial hypertension by the use of diets poor in sodium chloride is discussed. The possible rôle of the adrenal cortex in arterial hypertension and its relation to disturbances in salt balance is considered in the light of experimental and clinical evidence. While many subjects do respond favorably to rigid restriction of salt by lowering of blood pressure, psychotherapeutic influences may play a large part in this change in some. If subjects are adequately controlled, the change to be expected is not great and may be absent in patients with severe hypertension in whom therapy is most needed. It is possible that patients who do respond favorably to this diet exhibit a different type of hypertension in which the adrenal cortex is overactive. The mechanism of the response is not understood. No special advantage has been demonstrated in the use of a diet exclusively composed of rice, fruit juices and vitamins, as compared with a normal diet with a similar content of salt, and there are several disadvantages to it.

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CASE REPORTS

Hyperkalemia Due to the Rapid Infusion of a Potassium Chloride Solution

Report of a Case in an Infant with Associated Electrocardiographic Changes

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THE occurrence of significant hyperkalemia is usually associated with renal disease, especially those types causing "lower nephron nephrosis." This may be seen in cases of shock, severe trauma, intravascular hemolysis, the absorption of substances that are toxic to the renal tubules, such as carbon tetrachloride,[1,2] and in other conditions causing anuria and oliguria. Also, it may occasionally be seen in the terminal stages of nephritis with uremia.[3,4] Less frequently, it occurs from excessive parenteral administration[5] and rarely by the administration of potassium orally.[6] In the pediatric literature, hyperkalemia due to parenteral administration of potassium and the classic electrocardiographic changes associated with it have been infrequently described. However, this must occur more often than is recognized.

We feel that this case is worthy of note for it demonstrates the potential hazard and the extreme watchfulness necessary when one gives an intravenous solution containing potassium to an infant.

Case Report

A four-month-old American Indian male was admitted to the hospital because of cough, wheezing respirations, and fever of one day duration. There was no history of coryza, draining ears, vomiting, or diarrhea. Prior to the onset of the present illness the infant had been well. He was a full-term, normal spontaneous delivery and weighed 6 pounds, 12 ounces at birth. Growth and development had apparently been normal. Except for occasional episodes of postprandial regurgitation, the dietary history was unremarkable. The child had been on an evaporated milk formula with vitamin supplements, and cereals had been started at three weeks of age. No immunizations had been given.

FAMILY HISTORY.—Two older siblings, ages four and three, were living and well but had a history of wheezing occurring with upper respiratory infections. The sister had had an episode of jaundice six months previously, and the brother had had a febrile convulsion about one year prior to the patient's present illness. There was no family history of tuberculosis, syphilis, diabetes mellitus, or proven allergies, although the mother gave a history of wheezing with upper respiratory infections.

PHYSICAL EXAMINATION.—The patient was a well-nourished, well-developed infant weighing 12 pounds, 14 ounces, in obvious respiratory distress with audible expiratory wheezes and tachypnea at a rate of 40 per minute. The temperature on admission was 102 F. His state of hydration appeared to be good. The anterior fontanelle was flat and admitted two fingers. The eyes, ears, nose, and throat were within normal limits with the exception of moderate injection of the posterior pharyngeal wall. There was no significant adenopathy. Examination of the chest did not reveal any subcostal or supraclavicular retractions, but on auscultation bilateral expiratory wheezes without rales were heard anteriorly and posteriorly. There was no inequality of breath sounds. Percussion of the chest revealed no abnormality. The cardiovascular system was negative except for a regular sinus tachycardia of 14[illegible] per minute heard at the apex of the heart. Aside from a small umbilical hernia the abdominal examination was negative. The remainder of the physical examination was within normal limits. The initial diagnostic impression was that of an upper respiratory infection and associated bronchospasm with an allergic etiology to be ruled out.

The opinions expressed are those of the authors and do not necessarily reflect those of the United States Public Health Service or the Bureau of Indian Affairs.

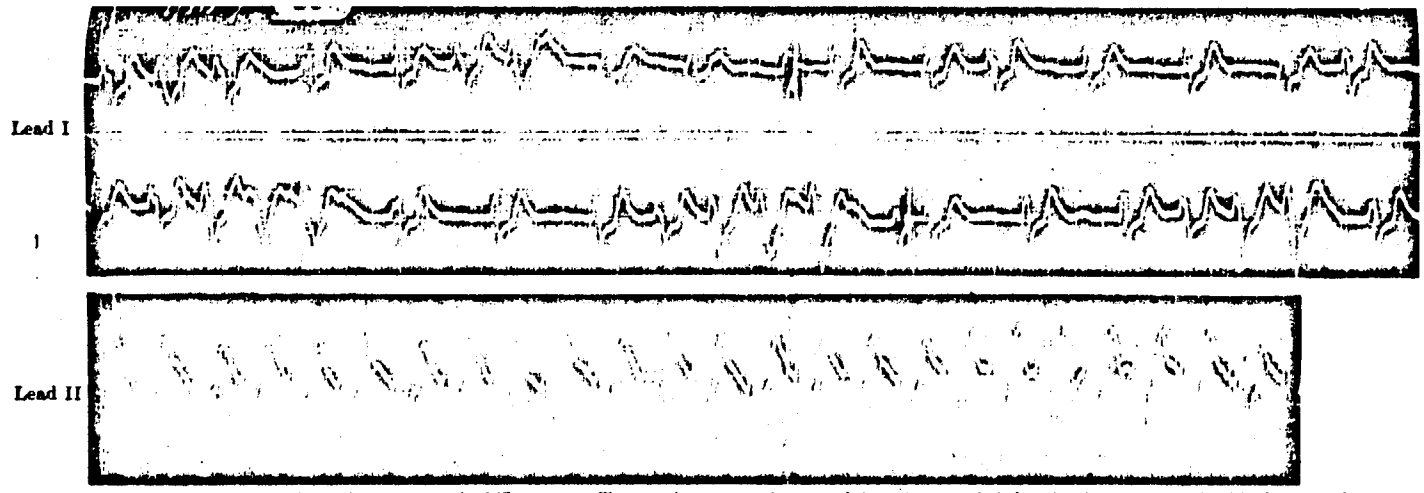

FIG. 1. *Lead I*—Findings of severe hyperkalemia (peaked T waves, S-T wave depression, increased duration of QRS showing intraventricular block, auricular standstill, biphasic curve with progressive delay in ventricular conduction, total arrhythmia, and short runs of ventricular tachycardia). *Lead II*—Ventricular tachycardia.

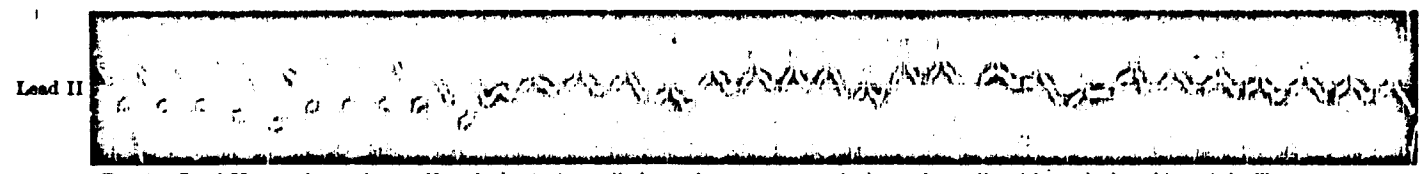

FIG. 2. Lead II ten minutes later—Ventricular tachycardia becoming a supraventricular tachycardia with marked peaking of the T waves.

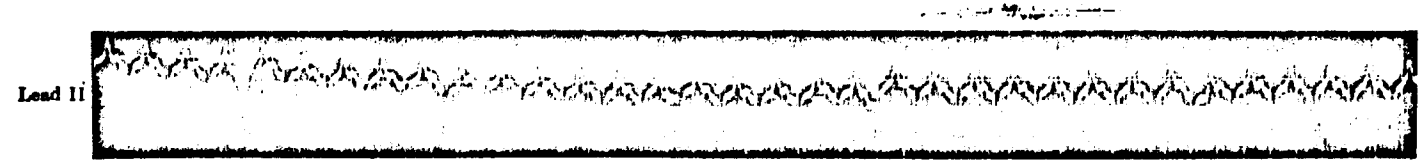

FIG. 3. Lead II two hours after Fig. 2—Supraventricular tachycardia with peaked T waves being less prominent.

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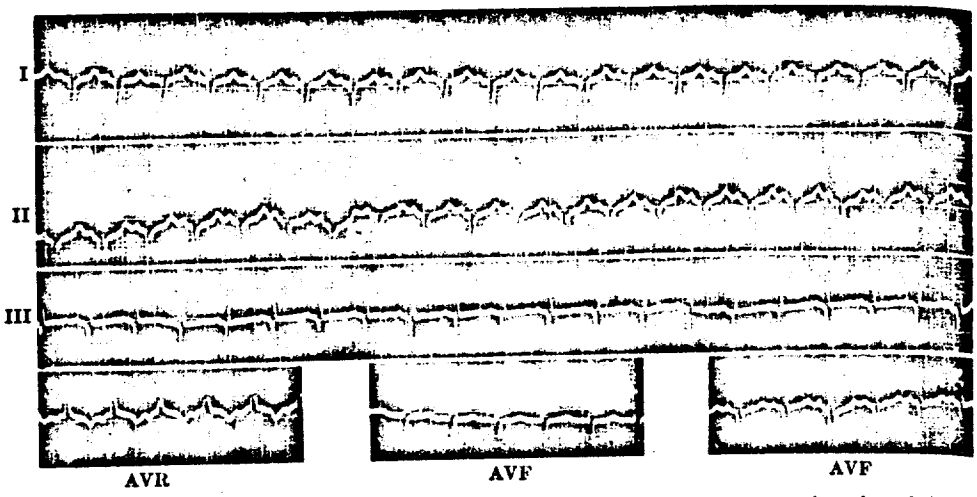

FIG. 4. Three hours and twenty minutes after Fig. 3. Six leads of the electrocardiogram six and one-half hours after the infusion of potassium solution which reveals a normal tracing with a sinus tachycardia.

COURSE IN HOSPITAL.—The patient was started on penicillin, Gantrisin, adrenalin, aminophylline suppositories, and Pyribenzamine. On the second day dyspnea and wheezing had decreased, but vomiting occurred several times. The temperature at this time was 103 F. Although there was no cyanosis, oxygen was begun. On the third hospital day the temperature had risen to 104 F., and the occasional vomiting continued. Examination now revealed evidence of moderate dehydration, but the pulmonary wheezes had decreased. It was felt that dehydration contributed to the fever, and a clysis of 500 cc., one-third normal saline and two-thirds 5 per cent dextrose in water, was given. Gantrisin was discontinued and streptomycin added to the therapeutic regimen. A flat film of the abdomen revealed no evidence of intestinal obstruction.

At 2 A.M. of the fourth hospital day the patient appeared critically ill, was in shock, with gasping respirations, cold extremities, and cyanosis of the lips. He was given adrenalin intramuscularly and 120 cc. of plasma by push transfusion. At this time the upper abdomen was noted to be distended. Bowel sounds were markedly diminished, and a flat plate of the abdomen revealed a generalized ileus. An x-ray of the chest was negative. It was felt these abdominal findings represented a paralytic ileus. Hypokalemia was then considered as a possible cause of the ileus.

A Levine tube was passed, and 500 mg. (6.7 mEq.) of potassium chloride in 100 cc. of 5 per cent dextrose in water were started intravenously. This was to be given over a period of four hours. However, this solution was inadvertently infused in forty minutes. It was then noted that the patient appeared worse, was cyanotic, and had generalized twitchings. The blood pressure was 78/44. An electrocardiogram revealed the findings of severe hyperkalemia as shown in Fig. 1. The potassium solution was immediately discontinued, and a 200-cc. infusion of 5 per cent dextrose in water was given at a rate of 20 drops per minute. Ten minutes later, the electrocardiogram revealed marked improvement but was still abnormal as evidenced by the presence of peaked T waves (Fig. 2). Three hours after this, peaking of the T waves was still present although less prominent (Fig. 3). Blood chemistries now showed a serum potassium 7.7 mEq. per L., sodium 112 mEq., and carbon dioxide combining power 10 mEq. per L. When these results were obtained, 50 cc. of isotonic sodium chloride, 125 cc. of sixth-molar sodium lactate, and 300 cc. of 5 per cent dextrose in water were given to combat the metabolic acidosis and hyponatremia. Six and one-half hours after the rapid infusion of potassium, the electrocardiogram appeared normal (Fig. 4). Four hours after this, the patient was considerably improved, cyanosis was less marked, and the ileus appeared to have diminished.

On the fifth hospital day tachypnea and dyspnea had become more severe, and evidence of bronchospasm had reappeared, although the infant's hydration was good. The urinary output was adequate as it had been throughout hospitalization, but the temperature remained at 103 F. At this time the serum sodium was 126 mEq. per L. and the chloride 82 mEq. per L. The patient's condition became generally worse, and from this point on the course was progressively downhill. He expired on the eighth hospital day. Blood drawn shortly before death revealed a potassium level of 21 mEq. per L. A postmortem examination was refused.

Comment

Potassium is primarily an intracellular cation. Estimates of the concentration within the cells range from 112 to 150 mEq. per L. of cell water,[7,8] whereas the concentration in serum and extracellular fluid

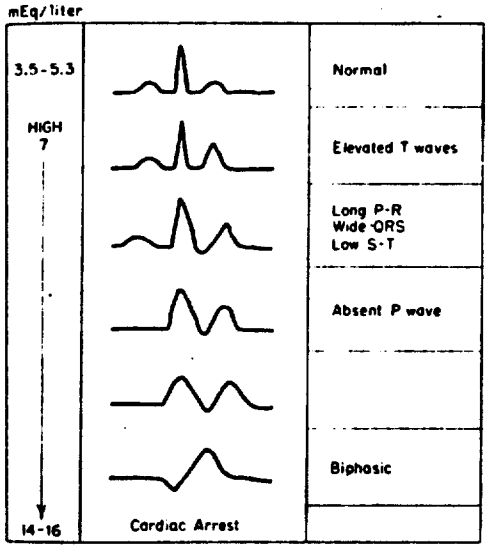

FIG. 5. Schematic diagram of electrocardiographic changes seen in association with increases in the serum concentration of potassium.

is from 3.5 to 5.3 mEq. per L. under normal conditions.[9] The concentration of potassium found in the serum or extracellular fluid at any given time is the resultant value of a state of equilibrium involving cell metabolism, gastrointestinal absorption, and excretion via the urine, bowel, and sweat. The ability of this cation to move either into or out of cells is well known. This may be seen in alkalosis, anoxia, severe muscle contraction, uncontrolled diabetes, diarrhea, starvation, and severe shock.[10-12] Certain hormonal factors enter into the control of the migration of the potassium ion, these being, for example, adrenal corticoids, adrenocorticotropic hormone,[13,14] insulin,[15,16] androgens,[17] and epinephrine.[5] Thus, in certain instances the serum concentration of potassium may be elevated, even though the tissue concentration is normal or low.[10]

The signs and symptoms of hyperkalemia have been well summarized by Darrow.[10] These are (1) listlessness and mental confusion, (2) numbness and tingling of the extremities, (3) cold, gray pallor, (4) bradycardia and occasionally total irregularity of rhythm, (5) peripheral vascular collapse with a fall in blood pressure, (6) in a few patients with uremia, a rapidly ascending flaccid paralysis, and (7) cardiac arrest. When the serum concentration begins to exceed 5.3 mEq. per L., the neuromuscular system in general is involved, but the most serious changes are noted in the conduction system of the heart, as can be demonstrated by the electrocardiogram.

The classic work on dogs by Winkler *et al.*[18] demonstrated the effect of parenteral administration of potassium chloride on the electrocardiogram. Consistent and predictable changes were noted at certain potassium levels. They found alterations in the T waves at a concentration of 5 to 7 mEq. per L., depression of the S-T segments at 8 to 10 mEq., intraventricular block at 10 mEq., P waves disappeared at 11 mEq., and cardiac arrest occurred at 14 to 16 mEq. per L. A schematic representation of these changes is shown in Fig. 5.

In humans similar changes occur but at less predictable levels, as shown by the work of Tarail.[19] He was able to demonstrate that the onset of these changes varied, but they were always found when the serum potassium exceeded 6.8 mEq. per L. Other studies done by Currens and Crawford[20] and Crismon *et al.*[21] have demonstrated the frequently inconsistent correlation between the serum potassium and the electrocardiographic changes. This was attributed to the fact that potassium is mainly an intracellular cation and values of serum potassium were not reflecting that measurement alone. Currens and Crawford feel that the electrocardiogram more closely reflects intracellular content, whereas Darrow[22] has suggested that the gradient of intracellular and extracellular potassium concentration may be the cause of potential differences developing in the cell membranes. This difference cannot be resolved because of the present difficulty in quantitative determination of intracellular potassium. The clinical significance of this is that regardless of whether the electrocardiographic changes are reflecting gradient, intracellular, and/or extracellular concentrations, a serious hazard exists when one gives potassium parenterally at a rapid rate. This may cause an acute rise in the extracellular concentration and assumes prime importance when dealing with a dehydrated infant who has a contracted extracellular volume and resulting impaired renal output.

In this case the child was acutely ill with respiratory distress, fever, and vomiting. The need for parenteral fluids and electrolyte replacement was obvious. Up to the time of the potassium solution infusion there had been no parenteral potassium given, and the intake of this ion was obtained only through the regular diet. This, at best, was poor. The parenteral fluids which were given consisted of dextrose solutions and saline only, and we can assume that the body stores of potassium were low. Since the child became acutely ill shortly after midnight, baseline chemistries could not be obtained. It was felt that potassium was necessary, and inadvertently the amount ordered (0.5 Gm. of potassium chloride or 6.7 mEq. of potassium) was infused rapidly. This quantity could be calculated to give a 3.7 mEq. per L. rise in this child's extracellular fluid (calculated on the basis of 30 per cent of an infant's weight being extracellular fluid). This dose given to an infant with good hydration and normal kidney function could be easily handled, as pointed

out by Darrow[10] who gives 3 mEq. per Kg. per twenty-four hours of potassium as a maintenance requirement. Due to the patient's state of shock, severe hemoconcentration, hyponatremia, and the rapidity of infusion, marked signs of potassium toxicity developed, as seen in Fig. 1. This effect was transient, but the chaotic cardiac disturbances produced might easily have been fatal. Blood drawn three hours later revealed a concentration of 7.7 mEq. per L. of potassium, and one can assume that the concentration at the time of the infusion was considerably higher. In the other instance mentioned in the pediatric literature, Govan and Weiseth[5] reported a case with similar electrocardiographic findings with the potassium concentration being 12 mEq. per L. They treated their patient with intravenous calcium and hypertonic glucose, and within two hours the abnormal electrocardiographic changes had subsided. In our case there was spontaneous reversion to normal six hours after cessation of potassium therapy and the infusion of 200 cc. 5 per cent dextrose in water.

The therapy of hyperkalemia of whatever cause has been varied and extensive. Generally, attempts are made in the following directions: (1) decrease exogenous potassium intake, (2) increase potassium excretion by such means as peritoneal and intestinal dialysis, artificial kidney, and exchange transfusions, (3) deposit potassium intracellularly by administering glucose and insulin simultaneously, (4) attempt to neutralize the toxic effect of the ion on the cardiac function by giving calcium, (5) minimize catabolic processes wherein potassium is liberated, such as by diet and testosterone propionate.

Probably the simplest method of therapy available for the treatment of acute hyperkalemia is the following: (1) avoid all exogenous potassium intake, (2) give a high-carbohydrate diet to supply an adequate number of calories so that tissue catabolism with its subsequent liberation of potassium will be minimized, (3) administer glucose and insulin to cause transient passage of potassium into the cells in the formation of glycogen. A suggested dosage schedule is 1 unit of regular insulin to 2 Gm. of glucose, the latter in a 20 to 25 per cent solution given in a volume that one feels the patient can tolerate.[22] If there is no response to the above regimen, calcium can be added to counteract the effect of the potassium ion on the cardiac musculature.

Summary

1. A case of transient hyperkalemia with intoxication due to inadvertent rapid infusion of a potassium solution is presented.

2. The etiology, clinical and electrocardiographic manifestations, and therapy of hyperkalemia are discussed.

3. The danger of the rapid intravenous infusion of potassium is emphasized.

4. The infrequent description in the pediatric literature of the electrocardiographic changes occurring in iatrogenic hyperkalemia is noted.

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MALIGNANT HYPERTENSION PRODUCED BY TREATMENT WITH DESOXYCORTICOSTERONE ACETATE AND SODIUM CHLORIDE*

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Montreal

THE first observations concerning the production by desoxycorticosterone acetate (D.C.A.) of nephrosclerosis were based upon experiments on chicks.[1] Young birds are particularly sensitive test objects for the demonstration of this hormone action. Indeed, subsequent experimentation showed that most other laboratory animals develop little or no nephrosclerosis if their diet contains normal or low concentrations of NaCl. However, combined treatment with D.C.A. and NaCl results in the production of particularly marked and malignant nephrosclerosis not only in the chick,[2] but also in the dog, rat and monkey.[3]

In the spontaneously occurring nephrosclerosis of man, a rise in blood pressure and morphological changes in extra-renal blood vessels—especially those of the pancreas—are very characteristic. It was therefore decided to take advantage of the particular effectiveness of combined treatment with D.C.A. and NaCl in order

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to produce malignant nephrosclerosis in the rat and to determine whether this experimentally elicited renal disease is accompanied by a rise in blood pressure and structural changes in the systemic blood vessels such as those seen in the renal hypertensive patient.

EXPERIMENTAL

Four groups of young albino rats weighing 30 to 46 grm. were used. Each group consisted of four males and four females and had an average body weight of 35 grm. Throughout the experiment they were fed "Purina fox chow". The animals of the first group received two daily subcutaneous injections of 3 mgm. of D.C.A. in the form of a crystal suspension containing this amount in 0.1 c.c. of water. After one month the dose was raised to two daily injections of 5 mgm. also given in 0.1 c.c. of water. They were given tap water to drink. The second group received the same treatment as the first but was given 1% NaCl solution *ad lib.* instead of ordinary drinking water. The third group received the same NaCl solution and cholesterol in the same amount and manner in which the D.C.A. was given to groups I and II. Since cholesterol has no hormonal properties, but also possesses the sterol nucleus, it was felt that rats treated with this compound would act as injected and NaCl treated controls. The animals of the fourth group received ordinary tap water and no sterol treatment. They acted as entirely untreated controls.

During the course of the experiment one animal in group I and two animals in group II died from hæmorrhagic lung œdema and several rats in group II developed peculiar nervous symptoms similar to those previously seen in various species[3] following combined treatment with NaCl and D.C.A. Most animals in this group developed obvious signs of circulatory failure, as judged by the fact that they became fatigued and collapsed upon the slightest muscular exertion and showed dyspnœa often even during rest. Their skin and mucous membranes became cyanotic and some of them developed some subcutaneous œdema, although this latter sign was rarely prominent. On the 52nd day of the experiment the blood pressure was measured in the animals of all groups using the plethysmographic method of Friedman.[4] The average blood pressure in the animals of all groups except group II was 110/80 and the highest blood pressure observed in any of these groups was 120/90 (if we exclude a single apparently abnormal animal in the cholesterol group whose blood pressure was 140/100). In the D.C.A. and NaCl treated rats of group II, on the other hand, the average blood pressure was 187/130 with a range of 185/130 to 190/130. These observations clearly demonstrate that combined treatment with D.C.A. and NaCl results in a pronounced increase in both the systolic and the diastolic blood pressure and causes clinical signs of cardiac insufficiency, while treatment with NaCl in combination with the hormonally inactive cholesterol exerts no such effect. It is of special interest that even D.C.A. is comparatively inactive when the NaCl intake is not excessive.

It should also be mentioned that the diuretic effect of D.C.A. is greatly enhanced by a high NaCl intake and the animals receiving both D.C.A. and NaCl exhibited a more pronounced diuresis and excreted larger quantities of Na and Cl than those treated only with D.C.A. or exclusively with NaCl. The average daily urine volume, towards the end of the experiment, was 8.8 c.c. in group I, 49 c.c. in group II, 28 c.c. in group III and 6.0 c.c. in group IV. The average daily chlorine excretion (method of Van Slyke[5]) was 0.64, 5.2, 2.8 and 0.61 meq. and the average daily sodium excretion (method of McCance and Shipp[6] slightly modified by Dr. A. Neufeld) 1.5, 5.9, 3.3 and 1.1 meq. in the four groups respectively. It will be recalled that Selye and Dosne[7] and Selye and Bassett[8] observed marked diuresis and increased chlorine excretion in rats treated with D.C.A. Some subsequent investigators confirmed these results, while others noted no change or even an actual diminution in chloride excretion. The present experiments may give a clue to these apparent contradictions inasmuch as they indicate that the actions of the hormone are dependent upon the NaCl intake.

Qualitative tests (biuret reaction) revealed the presence of considerable amounts of protein in the urine of all animals in group II but not in the other groups.

PATHOLOGICAL FINDINGS

On the 53rd day of the experiment all animals were killed and their organs taken for histological examination. Even macroscopical inspection revealed that the kidneys of the animals in group II were definitely abnormal. The surface of the organ was irregular and spotted.

In some animals white, slightly depressed areas prevailed, while in others the surface exhibited the "flea-bitten" appearance so characteristic of small intraglomerular hæmorrhages. There was marked cardiac hypertrophy in all animals of this group and the aorta appeared to be particularly rigid, although definitely sclerotic patches were not detectable. The pancreas was extremely œdematous in many animals of group II, but no such changes were noted in any of the other groups, excepting very slight irregularities in the renal surface of the rats in group I.

It is well known that chronic treatment with D.C.A. causes compensatory atrophy of the adrenal cortex and this was quite evident in the rats of groups I and II in this experiment. It has also been shown by previous work[9] that the adrenal hypertrophy following exposure to stress—which is part of the "alarm reaction"—is inhibited by D.C.A. pretreatment. Hence it is noteworthy that in those animals of group II which were obviously severely ill due to circulatory decompensation, the adrenals were brown and not particularly small. They apparently discharged their lipid granules, a phenomenon which is characteristic of increased activity. We mention this since adrenal stimulation, in spite of treatment with such excessive doses of D.C.A., is of considerable theoretical interest. The present view concerning compensatory atrophy is that an endocrine gland involutes if more than the normal amount of its hormones circulates in the blood. Hence it is believed to be an atrophy of disuse. In our experiment the animals received more D.C.A. than is likely to be produced by their adrenals since our treatment elicited pathological changes which are not seen even in cases of extreme cortical hypertrophy such as that elicited by continuous exposure to severe stress (cold, infections, drugs, etc.). If the treatment still prevented the alarm reaction hypertrophy of the cortex in the damaged rats of our group II, it appears that hormones other than D.C.A. are required for resistance to non-specific damage, a fact which is in agreement with previous observations.[9] It must also be borne in mind that if the cortical hypertrophy caused by damage does not elicit morphological changes such as those produced by D.C.A. the hyperactive cortical cell either produces comparatively small quantities of this type of corticoid compound or secretes them in combination with other hormones which inhibit their toxic effects. Experiments are now under way to determine whether other cortical steroids could inhibit the toxic actions of D.C.A.

In connection with the autopsy findings we should like to point out that in this as in many other experiments we found that unlike most other hormonal steroids, D.C.A. is rather poorly absorbed from the subcutaneous tissue and elicits a considerable local reaction.

A histological study of the internal organs revealed that the kidneys were only slightly affected in group I and remained normal in groups III and IV. In group II, on the other hand, the characteristics of malignant nephrosclerosis were quite evident (see Figs. 1 and 2). The glomeruli were enlarged and showed signs of capsular fibrosis. There was marked hyalinization of the glomerular tuft capillaries and there were particularly pronounced degenerative changes in the afferent glomerular arterioles. The walls of the latter were completely necrotic in many cases. The convoluted tubules and especially their distal sections were dilated and often occluded by hyaline casts. Red corpuscles were but rarely found in the capsular space or the tubular lumina. Wedge-shaped infarct-like areas, with their apex directed towards the pelvis of the kidney, were of common occurrence. They consisted of degenerating nephrons and proliferations of connective tissue stroma.

Outside of the kidney the most severe lesions were found in the pancreas. The vascular changes in this organ were qualitatively similar to those seen in the kidney and equally as pronounced. There was marked proliferation of the perivascular connective tissue which led to sclerosis extending between the acini and surrounding the Langerhans islets. Many of the latter exhibited signs of degeneration while others were greatly enlarged, perhaps in an effort to compensate for the loss of those which ceased to function because of the infiltrative sclerosis. The media and intima of the pancreatic arterioles were likewise seriously affected. In some cases they showed fibromuscular proliferation, while in other instances hyalinization of the connective tissue and sometimes even complete necrosis were observed. Not unfrequently the vascular lumen was completely occluded by a more or less homogeneous necrotic mass (see Figs. 2, 3 and 4).

Vascular changes in other organs were less prominent except for the vessels of the adrenal capsule which were always intensely affected

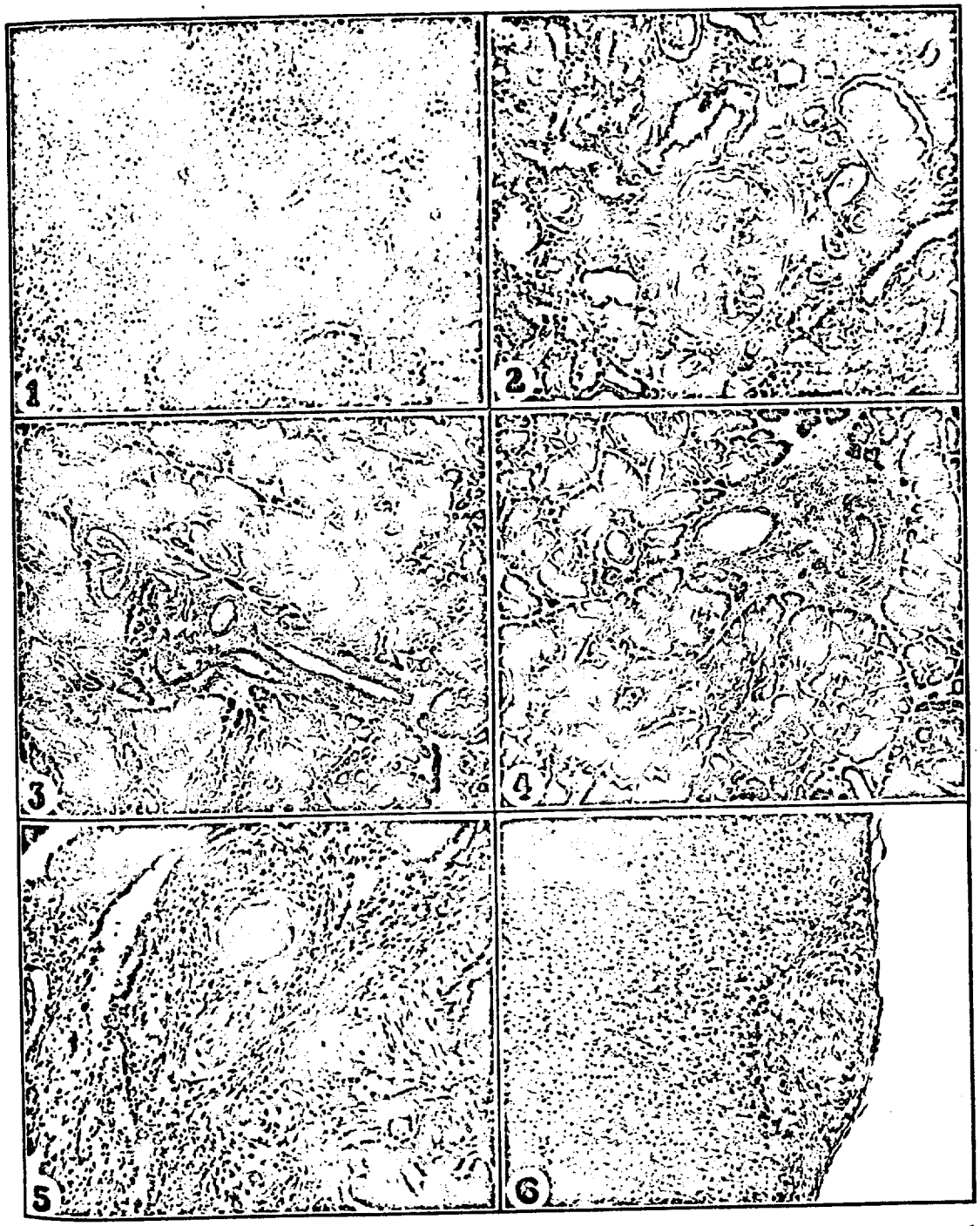

Fig. 1.—Section through the renal cortex of a control rat. **Fig. 2.**—Section through the renal cortex of a rat treated with D.C.A. and NaCl. Note marked enlargement and hyalinization of the glomeruli with capsular fibrosis. The afferent vessel of the central glomerulus is completely necrotic. The convoluted tubules are dilated and some of them contain casts. (Same magnification as Fig. 1.) **Fig. 3.**—Section through the pancreas of a control rat. **Fig. 4.**—Perivascular fibrosis in the pancreas of a rat treated with D.C.A. and NaCl. (Same magnification as Fig. 3.) **Fig. 5.**—Hyalinization and necrosis with complete obliteration of the lumen in two medium-sized arterioles of the pancreas of a rat treated with D.C.A. and NaCl. (Same magnification as Fig. 3.) **Fig. 6.**—Marked perivascular fibrosis and hyalinization of an arteriole in the capsule of the adrenal cortex of a rat treated with NaCl and D.C.A.

and showed fibromuscular proliferation, hyalinization or complete necrosis (see Fig. 6).

SUMMARY AND CONCLUSIONS

The nephrosclerosis produced by D.C.A. overdosage in the rat is particularly pronounced if the animals are kept on a high NaCl intake. Under such conditions D.C.A. causes a pronounced rise in systolic and diastolic blood pressure, marked diuresis with increased excretion of sodium and chloride, proteinuria and clinical signs of severe cardiac decompensation. In some instances death ensues, due to hæmorrhagic lung œdema.

The characteristic pathological findings in rats so treated are hypertrophy and capsular fibrosis of the renal glomeruli with hyalinization of their tuft capillaries. Fibrosis, hyalinization and even actual necrosis of the arteriolar walls is evident not only in the kidney itself—where the vasa afferentia are particularly affected—but also in the pancreas and the adrenal capsule. In the pancreas these vascular changes are frequently accompanied by œdema and pronounced stroma proliferation. The experimental disease thus produced bears a striking resemblance to the hypertensive heart disease of renal origin as seen in man.

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SUBLUXATION OF THE ANKLE

By Major G. F. Pennal, R.C.A.M.C.

THE basis of this article is a series of fourteen cases of subluxation of the ankle encountered in the past year at Camp Borden Military Hospital. The term subluxation is used to define those injuries resulting in a complete tear of the external lateral ligament of the ankle with a subsequent momentary or recurrent outward dislocation of the astragalus, unaccompanied by fracture.

The following case history may indicate that these injuries are disabling and merit further consideration.

CASE 12

A man aged 34. Three and one-half months previous to consultation, he had slipped and severely wrenched the left ankle whilst running. At that time he was removed to hospital. The radiographs were reported negative and he was treated in bed with the ankle embedded in ice packs and elevated on pillows. The subsequent swelling and ecchymosis were quite severe.

In two weeks he was allowed up on crutches, the ankle however remaining moderately swollen and too painful for weight-bearing. At the end of a further two weeks he gradually began weight-bearing with the aid of crutches. These he discarded for a cane about six weeks from the date of the accident, elastoplast bandage having been applied about the ankle.

At the time of consultation he was still having considerable pain and moderate swelling about the joint. He walked with a marked limp, leaning heavily on his cane. There was moderate tenderness over the anterior and middle bands of the external lateral ligament. All movements were markedly restricted and quite painful, especially in forced inversion. Routine antero-posterior and lateral radiographs were negative, except for slight decalcification.

That such a prolonged and severe disability should result from a simple sprain, for that had been the diagnosis, was not reasonable. Further radiographs taken with the foot held in maximum inversion revealed tilting of the astragalus, thus confirming one's suspicion that there had been a complete tear of the external lateral ligament with subluxation of the astragalus.

Following immobilization in a walking plaster for three months he completely recovered and returned to full duty.

Similar incidents of prolonged disability following "sprains" of the ankle are not uncommon and it is suggested that it is often this condition of subluxation of the ankle which forms the basis of the layman's statement, "it may be worse to sprain an ankle than to break it".

Anatomy.—The external lateral ligament of the ankle is distinctly divided into three bands.

The anterior fasciculus extends from the antero-lateral aspect of the external malleolus to the neck of the astragalus. The middle fasciculus, deep to the peroneal tendons, passes from the tip of the external malleolus to the peroneal

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Fatal Drug Reactions Among Medical Inpatients

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In a series of 6,199 consecutively monitored medical patients, deaths due to drugs administered in hospitals was recorded in 27 cases (0.44%). Most of the fatalities were attributed to commonly used drugs. Hyperkalemia and pulmonary edema, attributed, respectively, to potassium supplements and parenterally administered fluid therapy, were particularly prominent. Other drug-attributed mechanisms which resulted in death were depression of the central nervous system, superinfection, hemorrhage, cardiorespiratory complications, dehydration and electrolyte depletion, hypoglycemia, anaphylaxis, liver failure, perforated duodenal ulcer, and hypercalcemia.

It is widely recognized that the administration of drugs may result in serious consequences and even death. The extent and nature of this problem have not in the past been carefully quantitated, since this requires intensive surveillance of large numbers of patients with careful attention given to the apparent effects of each drug administered.

The present communication reports on the drug-attributed death rate in a series of 6,199 consecutive medical inpatients intensively monitored for drug effects. The nature of this monitoring program is such that the potential role of each drug received in the subsequent course of the patient's illness is considered.

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Methods

The methods employed by the comprehensive drug-surveillance program have been described in detail elsewhere.[1-4] For the present study, it is necessary to emphasize pertinent aspects of these methods and to describe some special procedures which were adopted.

Trained nurse-monitors are responsible for recording, in a standardized manner, a large quantity of information on consecutive patients admitted to monitored medical wards. Included for each patient is a record of all drugs ordered, which includes dosage, routes of administration, and starting and stopping dates. Suspected adverse reactions (defined as any undesired or unintended effect of a drug) are detected in two ways. First, whenever a drug is discontinued, the attending physician is promptly interviewed by the nurse-monitor and asked why he stopped the drug. An adverse reaction is one of the reasons which he may give. Second, even if he gives some reason other than a drug reaction, he is, nevertheless, asked whether any suspected reaction occurred while the drug was being administered.

All suspected reactions are promptly reported to the Clinical Pharmacology Unit and then investigated. On the basis of the juvenile evidence, the investigating clinical pharmacologist makes a judgment on whether any drug, or group of drugs, in fact caused the alleged reaction.

In those cases in which death follows shortly after a drug-attributed adverse event, an additonal detailed investigation is made to evaluate the relationship of drug therapy to death. The attending physician is interviewed again, and the patient's record is reviewed to obtain the best available clinical information. No case is included as a "drug death" unless both the attending physician and the Clinical Pharmacology Unit agree that the drug or drugs implicated are the most important or sole factors responsible for death.

For editorial comment see page 512.

This survey has been confined to those drugs that were adminstered while the patients were under surveillance. Deaths due to drugs taken prior to admission have been excluded, unless the same drugs were also taken while the patients were monitored.

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For the most part, a decision on whether to accept or reject a case has not proved to be difficult, but there have been occasions when a final decision was difficult, even after careful review of all the available data. The decision to include or exclude a case was then a matter of judgment. The following reports of cases illustrate a straightforward, an intermediate, and a difficult decision.

Report of Cases

CASE 24.—A 48-year-old man with carcinoma of the bronchus received asparaginase intravenously, 80,000 units in 150 ml of normal saline solution. A second treatment with the same dose of asparaginase was commenced one week later. After a few milliliters had been given, the patient became agitated. Asparaginase was immediately discontinued. (Subsequently, the reason given to the nurse-monitor was adverse reaction— "anaphylaxis.") The patient rapidly lapsed into deep coma, developed irreversible shock, and died a short time later, despite resuscitative measures.

Death was attributed to anaphylaxis following the intravenous administration of asparaginase.

CASE 4.—A 55-year-old man with cirrhosis of the liver was transferred for further treatment after recovery from hepatic coma. On admission, he was fully conscious and had massive ascites. The serum bilirubin level was 22.1 mg/100 ml (direct, 12.0 mg/100 ml; the blood urea nitrogen level was 15 mg/100 ml. On the first and third days, he received furosemide, 80 mg by mouth; potassium chloride, 15 mEq three times a day, was started at the same time. After the second dose of furosemide, he became drowsy. The drug was stopped for that reason. Potassium chloride was continued in unaltered dosage. During the last three days of his life, oliguria developed. Quite suddenly, he became severely hypotensive and went into shock. An electrocardiogram showed first degree atrioventricular heart block and tall, symmetrical, peaker T waves. The serum potassium level was 7.7 mEq/liter. Potassium chloride was discontinued because of "hyperkalemia." In spite of treatment, the ECG evolved into a sine-wave pattern, and he died in cardiac arrest.

The patient was critically ill. However, it was believed that the proximate cause of death was hyperkalemia, induced by potassium chloride and potentiated by terminal oliguria.

CASE A.—A 78-year-old man with acute inferior myocardial infarction suddenly lost consciousness six days after onset of the heart attack. Immediately, epinephrine (1 part in 1,000 parts of water), 1 ml in 9 ml of normal saline solution, was injected into the heart, and the cardiac resuscitation team arrived as the injection was completed. Treatment included intravenously administered sodium bicarbonate, ventilation, and external cardiac massage. External direct-current shock resulted in conversion of ventricular fibrillation to ventricular tachycardia, which promptly reverted back to ventricular fibrillation. Two further attempts resulted in the same succession of events, and direct-current shock was thereafter ineffective.

It was claimed by the resuscitation team that epinephrine prevented successful conversion. However, the clinical pharmacologist felt that there was reasonable doubt. The case was *not* accepted as a drug-related death.

Results

This series included 6,199 patients representing six hospitals: one hospital for the treatment of chronic diseases, and five general medical hospitals.[1-4] The mean age of the patients was 58 years, and 61% were men. There were 744 patients who died in hospital (12%), and of these, 27 were judged to have died as a result of drug treatment. Thus, drug deaths accounted for 0.44% of the total series, or 3.6% of the hospital deaths.

The drug deaths are detailed in the Table. The major mechanisms were hyperkalemia (five cases), fluid overload (four cases), depression of the central nervous system (three cases), superinfection (three cases), hemorrhage (two cases), cardiac or respiratory arrest or both (two cases), dehydration and electrolyte depletion (two cases), hypoglycemia (two cases), anaphylaxis (one case), liver failure (one case), perforated duodenal ulcer (one case), and hypercalcemia (one case).

Drug categories implicated in the fatalities were as follows:

1. Potassium supplements (five deaths out of 1,949 patients exposed).
2. Intravenously administered fluids (four deaths out of 2,048 patients exposed).
3. Narcotic and sedative drugs (three deaths).
4. Antibiotics (three deaths out of 2,548 patients exposed).
5. Parenterally administered anticoagulants (two deaths out of 361 patients exposed).
6. Digitalis glycosides (one death out of 1,254 patients exposed).
7. Diuretics (two deaths out of 1,254 patients exposed).
8. Insulin preparations (two deaths out of 353 patients exposed).
9. Single deaths were attributed to neostigmine, asparaginase, cyproterone acetate, adrenal steroids, and diethylstilbestrol.

Five of the patients were considered terminally ill immediately prior to the onset of their reactions, and though drug therapy was judged responsible for their death, life was probably not appreciably shortened as a result. The majority, 15 patients, were severely ill, but it was believed that their lives, at the time of their reactions, were not otherwise in immediate danger. The remaining seven patients were moderately ill.

Comment

Most of the information on fatal drug reactions has in the past been accumulated either as a result of sporadic reports of cases in the medical literature or through adverse-reaction reporting systems, such as that operated by the US Food and Drug Administration or that operated by the British Committee on Safety of Drugs. Such information is incomplete and provides no quantitation of fatal ef-

Deaths Due to Administration of Drugs*

Cause of Death	Case No.	Drug(s)	Comments
Hyperkalemia	1	Potassium chloride	Woman, age 52. Diagnosis: Cirrhosis. Developed cardiac arrest while receiving potassium chloride 90 mEq/day. Serum potassium prior to death was 8.8 mEq/liter.
	2	Potassium chloride	Woman, age 58. Diagnosis: Metastatic carcinoma of breast. Receiving diuretics and potassium chloride, 60 mEq/day. Developed BUN of 53 mg/100 ml and serum potassium of 7.2 mEq/liter. ECG "consistent with hyperkalemia." Died suddenly shortly thereafter.
	3	Potassium chloride, spironolactone	Woman, age 55. Diagnosis: Mitral stenosis. Receiving diuretics, including spironolactone, and potassium chloride, 45 mEq/day. Death followed multiple bouts of ventricular tachycardia. Serum potassium on day prior to death was 7.1 mEq/liter.
	4	Potassium chloride	Man, age 55. Diagnosis: Cirrhosis. Receiving diuretics and potassium chloride, 45 mEq/day. Developed sudden hypotension. ECG revealed grade 1 atrioventricular block followed by sine-wave pattern. Serum potassium, 7.7 mEq/liter. Died in cardiac arrest.
	5	Potassium chloride	Woman, age 84. Diagnosis: Congestive heart failure. Receiving diuretics and potassium chloride 90 mEq/day. Admission BUN, 110 mg/100 ml. Suddenly developed severe arrhythmia and cardiac arrest. Serum potassium on day of death, 8.0 mEq/liter.
Fluid overload	6	5% dextrose in water, normal saline	Woman, age 60. Diagnosis: Metastatic carcinoma of breast. While receiving 3 liters of intravenously administered fluids with phosphate buffer and hydrocortisone for hypercalcemia, developed irreversible pulmonary edema.
	7	5% dextrose in water	Man, age 73. Diagnosis: Congestive heart failure. While receiving 2 liters of fluid for intractable vomiting, developed irreversible pulmonary edema.
	8	5% dextrose in water, normal saline, lactated Ringer's solution, blood	Man, age 48. Diagnosis: Cirrhosis. Received 8 liters of fluid, including blood transfusion of 2 liters, over 12-hr period for bleeding varices. Developed irreversible pulmonary edema during 8th liter.
	9	5% dextrose in water	Man, age 72. Diagnosis: Congestive heart failure. Receiving 4 liters of fluid over 60-hr period for dehydration. Oliguria was present. During 4th liter, developed irreversible pulmonary edema.
Depression of central nervous system	10	Morphine sulfate	Man, age 63. Diagnosis: Bronchopneumonia, obstructive lung disease. Received morphine sulfate, 5 mg, by intramuscular injection. Found dead 1 hr later.
	11	Chlorpromazine	Woman, age 55. Diagnosis: Severe chronic obstructive lung disease. Received chlorpromazine, 50 mg by intramuscular injection, for agitation. Within 30 min, developed coma and cardiac arrest during attempted intubation.
	12	Chlordiazepoxide	Man, age 48. Diagnosis: Chronic alcoholism. Received chlordiazepoxide, 200 mg/day for 3 days, to prevent delirium tremens. On day 4, developed severe drowsiness, vomited, aspirated, and developed cardiac arrest. Despite resuscitation, died in coma with aspiration pneumonia 2 days later.
Superinfection	13	Cephalothin sodium, oxacillin, ampicillin, kanamycin, Coly-mycin, adrenal steroids	Man, age 48. Diagnosis: Fever of unknown origin. Treated with multiple antibiotics and steroids for 6 weeks. Developed fulminating pneumonia and died. Autopsy revealed necrotizing pneumonitis, due to *Candida albicans*, and Waldenström's macroglobulinemia.

Deaths Due to Administration of Drugs

Cause of Death	Case No.	Drug(s)	Comments
Superinfection	14	Chloramphenicol, penicillin	Man, age 50. Diagnosis: Chronic alcoholism. Admitted with fever of unknown origin. Received aqueous penicillin for 2 days and chloramphenicol 2 gm/day by intramuscular injection, for 5 days, with improvement. Suddenly developed high fever, with gram-negative septicemia, and irreversible shock, followed by death 3 days later.
	15	Chloramphenicol, methylprednisolone	Man, age 55. Metastatic bronchogenic carcinoma. Treated for bronchopneumonia with chloramphenicol, 2 gm/day by intramuscular injection, and methylprednisolone, 240 mg/day, with initial improvement. On day 4, developed high fever and irreversible shock. Blood culture prior to death revealed *Escherichia coli* resistant to chloramphenicol.
Hemorrhage	16	Heparin sodium	Woman, age 67. Diagnosis: Pulmonary embolism. After heparin sodium therapy, 5,000 units by intravenous injection every 6 hr for 1 day, developed massive hemoptysis, followed by asphyxia and death. Autopsy revealed massive intrapulmonary hemorrhage.
	17	Heparin sodium	Woman, age 47. Diagnosis: Thrombophlebitis. Received heparin sodum, 10,000 units by intravenous injection, every 6 hr. On day 2, suddenly developed irreversible shock and died. Autopsy revealed massive retroperitoneal hemorrhage.
Arrhythmia	18	Digoxin	Man, age 79. Diagnosis: Cor pulmonale. Given digoxin on admission for heart failure. On day 7, developed complete heart block with intermittent sinus tachycardia. Died in ventricular fibrillation 1 hr later.
	19	Neostigmine	Woman, age 54. Diagnosis: Diabetes mellitus and pneumonia. Developed tachycardia shortly after admission. Received neostigmine, 0.5 mg, by intravenous injection to slow heart rate in order to identify arrhythmia. Administration of drug was followed immediately by multiple arrhythmias and cardiorespiratory arrest. Resuscitation was successful in achieving sinus rhythm, but patient remained in coma and died hours later.
Dehydration and electrolyte depletion	20	Spironolactone, acetazolamide, chlorothiazide, furosemide	Man, age 71. Diagnosis: Malignant ascites. Received combinations of diuretics for 8 days. On day 9, became severely dehydrated and febrile; this was followed by irreversible shock and death. Prior to death, serum sodium, 111 mEq/liter; serum chloride, 80 mEq/liter.
	21	Hydrochlorothiazide, furosemide	Woman, age 86. Diagnosis: Congestive heart failure. Received diuretics for 10 days. On day 10, there was severe dehydration. Serum sodium, 115 mEq/liter, and serum chloride, 72 mEq/liter. Death following shock and coma occurred the same day.
Hypoglycemia	22	Protamine zinc insulin suspension, isophane insulin suspension chlordiazepoxide, diazepam, diphenhydramine hydrochloride, atropine sulfate, phenobarbital	Man, age 69. Diagnosis: Diabetes mellitus. Developed insulin coma corrected by intravenous administered glucose. Recurrent severe insulin coma 2 days later. No recovery following intravenously administered glucose, and death occurred shortly thereafter. Blood glucose prior to death and after administration of glucose was 60 mg/100 ml (Somogyi). In the hours prior to death, received chlordiazepoxide diazepam, atropine, diphenhydramine and phenobarbital for agitation and sweating.
	23	Soluble insulin	Man, age 64. Diagnosis: Diabetic ketoacidosis. Admitted in diabetic coma and received 680 units of soluble insulin. Rapid clinical improvement occurred. However, hypoglycemia developed (blood glucose, 27 mg/100 ml) followed, on recovery after intravenously administered glucose, by acute myocardial infarction, pulmonary edema, and death.

Deaths Due to Administration of Drugs

Cause of Death	Case No.	Drug(s)	Comments
Anaphylaxis	24	Asparaginase	Man, age 48. Diagnosis: Metastatic bronchogenic carcinoma. Received 80,000 units of asparaginase in 50 ml of saline without complication. One week later, this treatment was repeated. After 5 ml of infusion, patient developed acute severe anaphylactic reaction and died.
Liver failure	25	Cyproterone acetate	Man, age 62. Diagnosis: Carcinoma of prostate. Receiving long-term therapy with cyproterone acetate. Admitted, while still receiving therapy, with jaundice which progressed to death in liver failure. Autopsy revealed cholestatic jaundice with eosinophilic inclusion bodies in parenchymal cells. Histologic diagnosis was drug-induced liver disease.
Perforated duodenal ulcer	26	Prednisolone, hydrocortisone	Man, age 61. Diagnosis: Metastatic bronchogenic carcinoma. Treated with steroids for 12 days. Suddenly developed fever, coma, and death on day 13. Autopsy revealed multiple duodenal ulcers, one of which had perforated.
Hypercalcemia	27	Diethylstilbestrol	Woman, age 64. Diagnosis: Metastatic carcinoma of breast. Received diethylstilbestrol, 5 mg/day, for 8 days. Serum calcium rose from 11 mg/100 ml to more than 20 mg/100 ml. Coma followed by death on 8th day of treatment.

*Drug-prescribing information is obtained by nurse-monitors directly from order written by attending physician. It is often not customary for physician to prescribe the form (or brand name) of drug to be given. For example, if an order is written for ampicillin, 500 mg every six hours by mouth, the hospital pharmacy may provide either the anhydrous or trihydrate form of drug. Furthermore, since stocks change and sometimes more than one form is in stock, there may be no consistency in the form provided from time to time, even for the same patient. Thus, in instances in which the precise form of drug was unknown, a drug such as ampicillin has been designated simply by that term without reference to specific form used.

fects, either for drugs in general or for specific drugs.[3]

Two limited series of consecutive patients monitored for drug-attributed deaths have been published. In 1966, Seidl et al[6] reported on a series of 714 consecutive medical inpatients. Seven were judged to have died of adverse drug reactions in the hospital (1.0%). Respiratory depression due to sedative and narcotic drugs accounted for two deaths, and other causes were superinfection (antibiotics), nodal tachycardia (digoxin), hypotension (methyldopa), and leukopenia (mercaptopurine). In another series involving 731 medical inpatients, Ogilvie and Ruedy reported 17 drug-attributed deaths (2.3%).[7] Twelve of these were ascribed to digitalis glycosides and four to superinfection, and one patient with obstructive airway disease died after receiving morphine.

The current communication concerns the first extensive series, to our knowledge, of consecutive medical inpatients carefully monitored for toxic effects of drugs, and it covers a broad range of hospitals, diseases, and drugs.

Drug-attributed deaths were relatively infrequent, occurring in 27 of 6,199 medical inpatients (0.44%), but they accounted for an appreciable proportion of hospital deaths (3.6%). In contrast to other reports, the present study points to fluid and electrolyte therapy as a major source of drug deaths. The data indicate that potassium chloride may be especially dangerous in the presence of complicating factors, such as renal failure, but that it can be lethal even when they are absent. Furthermore, this drug cannot be considered wholly safe when given by mouth or when given together with diuretic drugs which tend to produce potassium loss. In the total monitored series, a surprisingly high proportion of patients (31%) have received potassium chloride, but usage has varied widely among the monitored hospitals from 8% to 52%. This suggests that potassium chloride may be administered in some institutions more often than is necessary and that more stringent indications might result in a decreased mortality.

Much the same considerations apply to parenterally administered fluids. The administration of 5% dextrose in water, for example, has also varied between hospitals (range, 10% to 42%; mean, 27%). The rapid administration of large volumes of fluid in the presence of heart failure is particularly dangerous, but the risk of pulmonary edema is a real one, even in the absence of apparent predisposing factors.

Two of the three patients who died after sedative or narcotic treatment had obstructed airways. The hazards involved in giving such treatment to patients with respiratory tract disease, especially if they tend to retain carbon dioxide, are well known.

A relationship between antibiotics, adrenal steroids, and super-

infection was found in this study. The death rate due to this complication, while encouragingly low, nevertheless supports the principle that antimicrobial treatment should be reserved for specific indications and confined to necessary drugs. Concomitant adrenal steroid treatment should, if possible, be avoided.

Heparin sodium has been responsible for death in two out of the 361 patients exposed. Both were women, and one was 67 years old. In a previous study, it has been shown that bleeding due to heparin is most common among elderly women.[8]

Digoxin and neostigmine were each responsible for one death of a patient in cardiac arrest. It is reassuring to note the low recorded mortality with digoxin (one out of 1,254 patients). Elsewhere, it has been pointed out that the doses of digoxin used in the monitored hospitals appear to be appreciably lower than those given in studies in which a higher mortality has been reported.[9]

Diuretic drugs, especially the more potent ones such as furosemide, are not innocuous. The two deaths in this series emphasize the need for sound indications and for moderation and care in the use of these powerful agents.

Similar considerations apply to insulin. Hypoglycemia may prove fatal and is a medical emergency calling for prompt and vigorous treatment. Symptomatic treatment before the onset of coma with sedatives and other drugs is obviously contraindicated.

Anaphylaxis, liver failure, perforated ulcer, and hypercalcemia are all recognized as potentially fatal adverse drug effects, but in this series, they have been encountered infrequently. Drug deaths due to other mechanisms, such as renal damage or bone marrow depression, have not yet been observed.

It must be emphasized that all the drug deaths in this study concern cases in which cause and effect relationships were suspected by the attending physicians. There remains the problem of deaths in which drugs may have been responsible, but were not suspected. Techniques of event analysis[10] can provide for a preliminary approach to such problems and are feasible within a comprehensive monitoring system.[11,12]

Finally, the deaths reported here concern drugs administered in hospitals to medical patients. Consequently, this series does not reflect other drug deaths, such as those related to long-term drug usage (eg, analgesic nephropathy) or to delayed adverse reactions (eg, bone marrow aplasia due to chloramphenicol), which may be more common among outpatients.

Participating hospitals include Lemuel Shattuck Hospital, Peter Bent Brigham Hospital, Boston City Hospital (Tufts Service), and Boston Veterans Administration Hospital, Boston; Roger Williams General Hospital, Providence, RI; St. Joseph's and Westminister Hospitals, London, Ontario; and Hadassah Hebrew University Hospital, Jerusalem, Israel.

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Nonproprietary and Trade Names of Drugs

Cephalothin sodium—*Keflin*.
Furosemide—*Lasix*.
Methyldopa—*Aldomet*.

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HUMAN CHROMOSOME DAMAGE BY CHEMICAL AGENTS

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Two news items concerning the nation's health appeared last autumn. On October 18, 1969, Health, Education and Welfare Secretary Robert H. Finch announced a ban on cyclamate-containing foods because this artificial sweetening agent causes bladder cancer in rats. One week later, the presidents of three baby food companies announced they would voluntarily discontinue the use of the flavor-enhancer monosodium glutamate (MSG) because it produced brain damage in newborn mice. The removal of these two food additives will change the dietary habits of millions of people.

Agents which cause cancer or birth defects are easily recognized, even by the layman, as dangerous. But what about agents which cause more subtle damage such as genetic mutation or chromosome breakage. Because changes in the genes and chromosomes do not usually produce an immediate health hazard, they may go undetected for a lifetime or even for several generations. Yet, the human gene pool can become insidiously polluted.

This review concerns chemical agents which break chromosomes. Early in the writing of this article, it became apparent that a term was needed for the cumbersome phrase "chromosome-breaking agent." Already introduced into medical literature were such descriptive terms as carcinogen, leukemogen, mutagen, and teratogen. The word "chromosomoclastogen" is suggested; the Greek word root "-clast" means to break, fracture, or fragment. (Recall the terms osteoclast and iconoclast.) In this article, an abbreviated, more euphonious form, "clastogen" will be used.

A number of physical, chemical, and biological agents are known to break chromosomes. Physical clastogens include X rays, ultraviolet light, cold shock, magnetic fields, and sound waves. Certain genes, viruses, and protozoa are examples of biological clastogens. This paper will deal primarily with chemical clastogens. Although the effects on human chromosomes are emphasized, it will sometimes be necessary to refer to experiments on animal and plant chromosomes. These are not irrelevant. In fact, there are no known plant clastogens to which animal chromosomes have been found to be immune.

Many of the chemicals cited here have been tested on human cells only

under *in vitro* conditions, adding the agent to cultured lymphocytes or fibroblasts for varying times and concentrations. It is well recognized that these tests do not reflect the *in vivo* state. Auxiliary pharmacological tests with animals may suggest whether the agent is absorbed, concentrated in the serum, distributed widely in bodily tissues, or detoxified by the liver and excreted as a harmless substance. Nevertheless, *in vitro* tests are useful for screening many substances and determining levels of concentration which are toxic.

Some chemicals which are clastogenic may also be mutagenic. Others are teratogenic or carcinogenic as well as clastogenic. Thus, the agents overlap in their spectrum of effects. Their classification depends, at least in part, upon the choice of the test system and the vigor with which research efforts have been applied.

Most, if not all, of the chemical clastogens cause depression of mitotic rate. This is not surprising if one accepts the premise that broken chromosomes lead to loss of genetic material with accompanying lethality, and such cells would no longer be capable of dividing.

CLASSES OF CHEMICAL CLASTOGENS

Many different classes of compounds in man's modern environment have been shown to break chromosomes. Table 1 gives an alphabetical list of approximately 200 chemical and physical agents which are proven or suspected clastogens or mutagens. This list is subdivided in Tables 2 through 15 according to chemical relatedness or usage.

NUCLEIC ACID-RELATED COMPOUNDS

In Table 2, an alphabetical listing of the compounds in this group is given. The symbols used preceding the chemical name give an indication of the test system in which chromosome breaks have been reported. Commonly used abbreviations for drugs are in parentheses.

The literature on this subject is immense and cannot be reviewed in this article. Interested readers are referred to the excellent monograph by Kihlman listed in the bibliography.

As might be expected, the analogs of nucleic acid components are strong inhibitors of DNA and RNA synthesis. Thus, imperfect chromosome replication and chromosome breaks occur. Because of the strong inhibition of mitosis produced by many of these compounds, they have been used extensively in cancer chemotherapy and experimental biology studies. The beverage derivatives, caffeine, theophylline, and theobromine are discussed under the section on food ingredients.

ANTIBIOTICS

The streptomyces-derived antibiotics may be divided into three groups: inhibitors of DNA, RNA, and protein. Among the DNA inhibitors, azaserine, mitomycin C, phleomycin, and streptonigrin have been tested for

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TABLE 1. ALPHABETICAL LIST OF CHEMICAL AND PHYSICAL CLASTOGENS[a]

Table No.	Clastogen
14	A-8103 (see Piperazine)
12	Acetone
4	Acetophenon
4	Acetylsalicylic acid
10	Acridine
10	Acridine-half mustard (ICR 170)
10	Acridine orange
10	Acridine yellow
10	Acriflavin
9	Actidione
3	Actinomycin D
2	Adenine
2	Adenine deoxyriboside
14	Aflatoxin
11	Amethopterin (see Methotrexate)
10	5-Aminoacridine
11	Aminopterin
7	Apholate (see Aziridine)
13	Arsenates
4	Aspirin (see Acetylsalicylic acid)
10	Atabrine (see Quinacrine)
2	6-Azacytidine
3	Azaserine
14	Azathioprine
2	Azauridine
7	Aziridine
6	Benzo(a)pyrene
12	β-Mercaptoethanol
14	β-Mercaptopyruvate
8	β-Propiolactone
8	Bipyridine
4	Bonine (see Meclizine)
2	5-Bromodeoxyuridine
4	Bromolysergic acid
8	Busulfan (see Tetramethylenedimethanesulfonate)
2,5	Caffeine
13	Calcium deficiency
4	Cannabis
7	Captan
12	Carbon tetrachloride
12	Catechol
15	Centrifugal force
4	Chloral hydrate
6	Chloramine T
3	Chloramphenicol
4	Chlorpromazine
6	Chlorox (see Sodium hypochlorite)
9	Colcemid (see Demecolcine)
9	Colchicine
8	Cupferron (see Hydroxyphenylnitrosamine)
5	Cyclamate
12	Cyclohexane
5	Cyclohexylamine
8	Cyclophosphamide
2	Cytosine arabinoside
8	Cytoxan (see Cyclophosphamide)
3	Daunomycin
9	Demecolcine
7	Dichlorodiphenyltrichloroethane (DDT)
8	Dichloroethylmethylamine oxide
8	Diepoxybutane
8	Diepoxypropylether
8	Diethylsulfate
4	Dimenhydrinate
8	Dimethylsulfate
14	Dimethylsulfoxide (DMSO)
12	2,4-Dinitrophenol
4	Dramamine (see Dimenhydrinate)
15	Electromagnetic fields
8	Endoxan (see Cyclophosphamide)

[a] These are discussed in the text and listed in Tables 2 through 15 under various classifications.

TABLE 1—(*Continued*)

Table No.	Clastogen
4	Equanil (see Meprobamate)
12	Ethanol
2	8-Ethoxycaffeine
12	Ethyl acetate
8	Ethyl carbamide (see Urethane)
8	Ethylene oxide
8	Ethylenimine
8	Ethylmethanesulfonate
2	5-Fluorodeoxyuridine
15	Gamma rays
9	Gammexane
7	Hemel
7	Hempa
7	Hexamethylmelamine
7	Hexamethylphosphoramide
3	Hexamethylene tetramine
12	Hexane
8	Hydroxycarbamate (see Hydroxyurea)
14	Hydroxylamine
8	Hydroxyphenylnitrosamine
8	Hydroxyurea
12	Hyponitrites
10	ICR-170 (see Acridine half-mustard)
2	5-Iododeoxyuridine
14	Imuran (see Azathioprine)
15	Irradiated culture media
15	Irradiated foods
15	Irradiated plasma
4	Isocarboxazid
8	Isopropyl carbamate
8	Isopropylmethyl sulfonate
15	Isotopes: ^{131}I, ^{32}P, ^{3}H, ^{14}C
13	Lead excess
4	Lysergic acid diethylamide
13	Magnesium deficiency
7	Maleic hydrazide
3	Mandelamine (see Hexamethylene tetramine)
4	Marplan (see Isocarboxazid)
4	Meclizine
4	Meprobamate
2	6-Mercaptopurine
4	Mescaline
12	Metacresol
12	Metacresol purple
12	Metanitrophenol
12	Methanol
3	Methenamine mandelate (see Hexamethylene tetramine)
11	Methotrexate
8	Methoxychloroacridine
12	Methyl acetate
14	6-Methylcoumarin
10	Methylene blue
8	Methyl methane sulfonate
8	Methylnitronitrosoguanidine
8	Methylnitrosoguanidine
8	N-Methylphenylnitrosamine
8	Methylurethane
3	Mitomycin C
5	Monosodium glutamate
8	Mustard oxide (see Dichloroethylmethylamine oxide)
3	Nalidixic acid
12	Naphthalene
4	Nardil (see Phenylzine)
3	Negram (see Nalidixic acid)
10	Neutral red
4	Nialamide
4	Niamid (see Nialamide)
6	Nitric oxide
8	Nitrogen mustards
12	4-Nitrometacresol
12	6-Nitrometacresol
8	Nitrosomethylurethane
5	Nitrous acid

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TABLE 1—(*Continued*)

Table No.	Clastogen	Table No.	Clastogen
12	Orthocresol	3	Tetracycline
12	Orthocresophthalein	4	Tetrahydrocannabinol
12	Orthonitrophenol	8	Tetramethylenedimethane sulfonate
6	Ozone	2	Tetramethyl uric acid
4	*Para*-aminosalicylate	4	Thalidomide
12	Paracresol	2,5	Theobromine
9	Parafluorophenylalanine	2,5	Theophylline
12	Paranitrophenol	2	Thioguanine
3	Penicillin	4	Thorazine (see Chlorpromazine)
12	Perchloroethylene	15	Thorium dioxide
12	Phenol	15	Thorotrast (see Thorium dioxide)
8	Phenylurethane	2	Thymidine
4	Phenylzine	2	Thymine deoxyriboside
3	Phleomycin	4	Tigan (see Trimethobenzamide)
14	Piperazine	12	Toluene
9	Podophyllin	10	Toluidine blue
9	Podophyllotoxin	8	Trenimon
13	Potassium cyanide	7	Tretamine
13	Potassium excess	12	Trichloroethane
10	Proflavin	7	Triethylenemelamine
12	Propanol	7	Triethylenephosphoramide
3	Puromycin	7	Triethylenethiophosphoramide
10	Pyronine	8	Triethyleniminotriazine
13	Pyrophosphates	2	Trifluorothymidine
10	Quinacrine	4	Trimethobenzamide
10	Rivanol	7	Trimethylenephosphoramide
3	Rubidomycin (see Daunomycin)	10	Trypan blue
4	Scopolamine	15	Ultraviolet light
6	Sodium hypochlorite	8	Urethane
5	Sodium nitrite	15	Weightlessness
15	Sonic vibrations	15	X ray
3	Streptomycin		
3	Streptonigrin		
15	Temperature shock		

clastogenicity and all are extremely active at very low concentrations. For example, streptonigrin at a concentration of 0.01 μg/ml for 6 hours prior to harvest will produce marked damage to human chromosomes. These drugs also produce many exchange figures, suggestive of reciprocal translocations.

TABLE 2. NUCLEIC ACID-RELATED COMPOUNDS

+++	Adenine	+	5-Iododeoxyuridine (IUdR)
+++	Adenine deoxyriboside (AdR)	+++	6-Mercaptopurine (6MP)
+	6-Azacytidine	+	Tetramethyl uric acid (TMU)
+	Azauridine	+	Theobromine
+++	5-Bromodeoxyuridine (BUdR)	+	Theophylline
+++	Caffeine	+++	Thioguanine
+++	Cytosine arabinoside (CA)	++	Thymidine
+++	8-Ethoxycaffeine (EOC)	++	Thymine deoxyriboside (TdR)
+++	5-Fluorodeoxyuridine (FUdR)	?	Trifluorothymidine

+ = Clastogenic or mutagenic in microorganisms, fungi, higher plants, invertebrates, or vertebrates except mammals. Not tested in mammals.
++ = Clastogenic in nonhuman mammals. Not tested in humans.
+++ = Clastogenic in human cells, either *in vitro* or *in vivo*.
? = Not tested but suspected to be mutagenic or clastogenic.
– = Tested with negative results.

Mitomycin C is a bifunctional alkylating agent and acts to cross-link the two backbones of the Watson-Crick double helix of DNA. Streptonigrin has a monofunctional group but is still a very effective clastogen. Mitomycin C, phleomycin, and streptonigrin have been shown to induce a nonrandom distribution of breaks among the chromosomes, having an affinity for the centromere and heterochromatic regions. Somatic crossing-over has occurred in fungi and fruit flies treated with mitomycin C and phleomycin. Azaserine is clastogenic in plant root tips.

The RNA inhibitors actinomycin D and daunomycin (Rubidomycin) are also effective clastogens to human chromosomes. The mechanism of DNA and RNA inhibition is thought to be due to covalent bond formation with the bases, thus causing a permanent insertion of the compound into the nucleic acid molecules.

The protein inhibitors chloramphenicol, puromycin, streptomycin, and the tetracyclines have so far proved to be ineffective in breaking chromosomes. Parenthetically, chloramphenicol, puromycin, and streptomycin appear to offer some protection to *Drosophila* males from the mutagenic effects of X rays. The tetracyclines are strongly teratogenic in mice.

Two bacteriostatic agents used in urinary tract infections, methenamine mandelate (Mandelamine) and nalidixic acid (Negram), are clastogenic. Mandelamine has been tested on mouse fibroblasts *in vitro* while Negram is effective on the chromosomes of human lymphocytes *in vivo*. The antibiotics which have been tested are given in Table 3.

CENTRAL NERVOUS SYSTEM DRUGS

For convenience, these drugs will be classified according to their primary usage although there are overlapping effects. The analgesics, acetylsalicylic acid, acetophenon, and *para*-aminosalicylates are clastogenic in plants and

TABLE 3. ANTIBIOTICS

+++	Actinomycin D	+++	Nalidixic acid
+	Azaserine	+++	Negram (see Nalidixic acid)
–	Chloramphenicol	–	Penicillin
+++	Daunomycin (DM)	+++	Phleomycin
++	Hexamethylene tetramine	+++	Puromycin (PM)
++	Mandelamine (see Hexamethylene tetramine)	+++	Rubidomycin (see Daunomycin) (RM)
++	Methenamine mandelate (see Hexamethylene tetramine)	–	Streptomycin (SM)
+++	Mitomycin C (MC)	+++	Streptonigrin (SN)
		–	Tetracycline

dividing amphibian eggs. The sedatives, scopolamine and chloral hydrate, are plant clastogens, and scopolamine has also been demonstrated to break human chromosomes in HeLa cultures. The tranquilizers, chlorpromazine (Thorazine) and meprobamate (Equanil) have been effective clastogens on human lymphocytes *in vitro* but patients taking high doses of these drugs over long periods of time have not evidenced increased chromosome breakage. A number of CNS depressants produce a colchicine-like antimitotic effect on cells in culture but clastogenicity has not been investigated.

The antinauseants, meclizine (Bonine), dimenhydrinate (Dramamine), thalidomide, and trimethobenzamide (Tigan) are clastogenic in the onion root tip. Thalidomide is unquestionably able to break chromosomes in human lymphocytes *in vitro* but repeated tests in mouse and primate embryos and human babies deformed by thalidomide have shown no increased chromosome breaks *in vivo*.

The antidepressant drugs, or psychic energizers, have recently come under suspicion. Isocarboxazid (Marplan), nialamide (Niamid) and phenylzine (Nardil) have been shown to destroy the activity of transformant DNA in bacteria. Since a close correlation exixts between DNA inactivation and ability to break chromosomes, these compounds should be tested.

Finally, the hallucinogens require comment. Although there has been a great furor over clastogenicity of D-lysergic acid diethylamide (LSD), the number of reports of negative results from tests on patients' bloods is steadily increasing. These studies have been conducted in well-controlled clinical testing situations in which the time and concentration of the dose can be monitored. However, the *in vitro* effect of LSD remains, for the most part, unquestioned. In spite of the conflicting chromosome results, there is general agreement that LSD, bromolysergic acid, and mescaline are teratogenic to mice, rats, and hamsters at a very early period of pregnancy corresponding to about the 16th to 22nd day of human gestation. Cannabis is also questionably teratogenic and two substituted derivatives of tetrahydrocannibinol were recently reported to break human chromosomes *in vitro*.

Table 4 lists the drugs affecting the central nervous system which have been found to be, or are suspected of being, clastogenic.

TABLE 4. CENTRAL NERVOUS SYSTEM DRUGS

+	Acetophenon	?	Marplan (see Isocarboxazid)
+	Acetylsalicylic acid	+	Meclizine
+	Aspirin (see Acetylsalicylic acid)	+++	Meprobamate
+	Bonine (see Meclizine)	+++	Mescaline
+++	Bromolysergic acid	?	Nardil (see Phenylzine)
?	Cannabis	?	Nialamide
+	Chloral hydrate	?	Niamid (see Nialamide)
+++	Chlorpromazine	+	*p*-Aminosalicylate
+	Dimenhydrinate	?	Phenylzine
+	Dramamine (see Dimenhydrinate)	+++	Scopolamine
		+++	Tetrahydrocannibinol
+++	Equanil (see Meprobamate)	+	Thalidomide
?	Isocarboxazid	+++	Thorazine (see Chlorpromazine)
+++	Lysergic acid diethylamide (LSD)	+	Tigan (see Trimethobenzamide)
		+	Trimethobenzamide

FOOD INGREDIENTS, ADDITIVES, AND PRESERVATIVES

As listed under nucleic acid-related compounds (Table 2), the purine derivatives caffeine, theophylline, and theobromine are proven clastogens. These substances are naturally occurring in coffee, tea, cocoa, and cola beverages. It will be mentioned below, under physical clastogens, that foods preserved by irradiation are also suspected of being mutagenic.

Before the artificial sweetener, cyclamate, was discovered to be carcinogenic in rats, several reports had appeared in the literature describing clastogenic properties of this food additive. Additionally, the metabolic breakdown product of cyclamate, cyclohexylamine, is a potent clastogen. It has been suggested that cyclamates are metabolized *in vivo* by only about 20 per cent of the human population—those individuals who excrete cyclohexylamine in the urine. If this is the case, then there should be individual differences in chromosome break rates when cyclamate is ingested, but cyclohexylamine should be capable of producing chromosome breaks without regard to the genotype of the individual. It is little comfort to know that we may have spared lives from bladder cancer by banning cyclamate if we have destined many unborn children to suffer genetic disease from mutations and chromosomal breaks already stored in our germ plasm.

Sodium nitrate is used as a preservative and color enhancer in smoked meats such as frankfurters, sausage, ham and salmon. This compound is converted to nitrous acid in the stomach. Nitrous acid is mutagenic in lower organisms.

Although the flavor enhancer, monosodium glutamate (MSG) has not yet been tested for clastogenicity, it has recently been placed on the suspect list because it causes brain damage in newborn mice. Further tests on rhesus

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monkeys are under way. In the meantime, it has been suggested that pregnant women avoid MSG to prevent possible brain damage in their unborn babies. Excess MSG can cause symptoms of headache, dizziness, and chest pains ("Chinese restaurant syndrome") in adults, and injections of MSG into brains of adult mice cause irreversible damage. Table 5 summarizes the compounds discussed in this section.

TABLE 5. Food Derivatives and Additives

Beverage derivatives		*Food additives*	
+++	Caffeine	+++	Cyclamate
+++	Theobromine	+++	Cyclohexylamine
+++	Theophylline	?	Monosodium glutamate (MSG)
		+	Nitrous acid
		?	Sodium nitrite

Air and Water Pollutants

Smog is a killer. Cigarettes are killers. But very little is known of the mutagenic and clastogenic activity of the chemicals produced by industrial aerosols, internal combustion engines, and cigarette smoke. Nitric oxide increases the radiosensitivity of bacteria and it decomposes in the human stomach to nitrous acid, a proven mutagen. Ozone decomposes in aqueous solutions to produce active radicals which are considered to be biologically active products of irradiation of protoplasm. Ozone will induce chromosome breaks in human cell cultures. Benzo(a)pyrene, a combustion product of tobacco, is a proven carcinogen in mice but has not yet been tested for its effect on chromosomes.

Sodium hypochlorite (Clorox) and Chloramine T ($1\text{-}CH_3C_6H_4\text{-}4\text{-}SO_2NClNa\cdot 3H_2O$) have been tested in our laboratory for clastogenicity of human lymphocyte chromosomes *in vitro*. Although the bleaching agent, Clorox, is highly toxic to the cells in small amounts, increased chromosome breakage has not been demonstrated with the concentrations and exposure times tested thus far. Chloramine T, on the other hand, produces a small but significant increase in chromosome breaks when added in concentrations of 100 to 400 parts per million for 24 hours. Above 400 ppm it is lethal. It is pertinent to ask, then, whether the highly unstable chlorinated amines in chlorinated drinking water are increasing our load of mutations. Excess hypochlorites dumped into swimming pools and gulped by children raises the question of hereditary damage to the most precious segment of our present population. They will be the parents of the next generation.

Although little is known about these classes of compounds, air and water pollution should be feared for their genetic effects on future generations as well as for their contribution to a current increase in mortality rates. Table 6 summarizes the relevant compounds in this category.

TABLE 6. Air and Water Pollutants

?	Benzo(a)pyrene
+++	Chloramine T
?	Clorox (see Sodium hypochlorite)
+	Nitric oxide
+++	Ozone
?	Sodium hypochlorite (Clorox)

Insecticides and Plant Growth Inhibitors

A number of insect chemosterilants have been tested for mutagenicity. These alkylating agents are substituted derivatives of melamine or phosphoramide. They are highly mutagenic in viruses, bacteria, fungi, *Drosophila*, and wheat. They are also clastogenic in human lymphocyte cultures. They are extensively used as antileukemic drugs and as carcinostatic agents in chemotherapy.

The compound, captan, is widely used as a fungicide in agriculture. It has recently been shown to break chromosomes of human embryonic lung cultures. Although it is a known mutagen in lower organisms, it did not cause chromosome loss by the dominant lethal test in mice. This latter screening study, however, would not have detected effects unless the agent increased lethality in the offspring of treated male mice by fivefold. DDT, the common pesticide, has not yet been proven to be clastogenic in the concentrations tested.

Maleic hydrazide is a plant growth inhibitor which prevents sprouting of potatoes, onions, and carrots during storage. It is used by gardeners to retard growth of grass and ivy. It causes mutations in corn and pea plants and breaks chromosomes in *Vicia faba* root tips and cultured mouse fibroblasts. These compounds are summarized in Table 7.

Alkylating Agents

Nitrogen mustard was the first chemical mutagen to be discovered. In 1941 Auerbach and Robson found that mustard gas caused a variety of chromosome aberrations and point mutations in *Drosophila* but did not report their findings until 1946 because their work was classified during World War II. Meanwhile, a German scientist discovered that urethane (ethyl carbamate) produced chromosome breaks and rearrangements in *Oenothera*, the evening primrose. This was the beginning of the explosive era of testing highly activated chemical compounds which we are still witnessing today.

There are a number of compounds mentioned elsewhere in this review which act as alkylating compounds but they fit conveniently under other headings. Examples include triethylene melamine, thio-triethylenephosphoramide, and cyclamate. In this section are included strong alkylating agents

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TABLE 7. PESTICIDES, FUNGICIDES, PLANT GROWTH INHIBITORS

+++	Apholate (see Aziridine)
+++	Aziridine
+++	Captan
+	Dichlorodiphenyltrichloroethane (DDT)
+++	Hemel (HML)
+++	Hempa (HMP)
+++	Hexamethylmelamine (HML)
+++	Hexamethylphosphoramide (HMP)
++	Maleic hydrazide
+++	Tretamine (TEM)
+++	Triethylenemelamine (TEM)
+++	Triethylenephosphoramide (TEPA)
+++	Triethylenethiophosphoramide (Thio-TEPA)
+++	Trimethylenephosphoramide (TMPA)

which have a clastogen effect. These chemicals have been shown to be potent mutagens in microorganisms, higher plants, *Drosophila*, and house mouse.

Ethylmethanesulfonate (EMS) and methylmethanesulfonate (MMS) act to alkylate guanine in nucleic acids. Thus, methylated guanine splits off from the molecule, leaving a "gap" which can then be replaced by another nucleotide base. If, by chance, it is not guanine, then a point mutation occurs.

Ethylmethanesulfonate and MMS are used extensively as commercial reagents, adding alkyl groups to phenols, amines, esters, ethers, and other compounds. Likewise, methylnitronitrosoguanidine (MNNG), another widely used industrial alkylating agent, is one of the most powerful mutagens known to bacteria. It appears to be able to change every purine and pyrimidine in the DNA code. Although it has not caused chromosome breaks and rearrangements in the mouse, it is clastogenic in the onion root tip at very low concentrations.

The ethylenimine compounds (EI) are used in a wide variety of industrial processes such as fireproofing, crease-resistant fabrics, laundry starches, and shampoos. Ethylenimine causes breaks in human chromosomes in culture and appears to act differentially, producing its greatest effects during the stage of DNA synthesis.

Other powerful mutagens in this group include ethylene oxide (EO), diepoxybutane (DEB), diepoxypropylether (DEPE), β-propiolactone (BPL), as well as others listed in Table 8.

C-Mitotic Agents and Prophase Poisons

The name of this general class of compounds is derived from the prototype chemical, colchicine, a plant extract of *Colchicum autumnale*, which has

TABLE 8. ALKYLATING AGENTS

+	β-Propiolactone (BPL)
+	Bipyridine
+	Busulfan (see Tetramethylenedimethane sulfonate)
+	Cupferron (see Hydroxyphenylnitrosamine)
+++	Cyclophosphamide
+++	Cytoxan (see Cyclophosphamide)
+	Dichloroethylmethylamine oxide
+	Diepoxybutane (DEB)
+	Diepoxypropylether (DEPE)
+	Diethylsulfate
+	Dimethylsulfate
+++	Endoxan
+	Ethyl carbamide (see Urethane)
+	Ethylene oxide (EO)
+++	Ethylenimine (EI)
++	Ethylmethanesulfonate (EMS)
+	Hydroxycarbamate (see Hydroxyurea)
+	Hydroxylphenylnitrosamine
+	Hydroxyurea
+	Isopropyl carbamate
+	Isopropylmethyl sulfonate
+	Methoxychloroacridine
++	Methyl methane sulfonate (MMS)
+	Methylnitronitrosoguanidine (MNNG)
+	Methylnitrosguanidine (MNG)
+	Methylphenylnitrosamine (MPN)
+	Methylurethane
+	Mustard oxide (see Dichloroethylmethylamine oxide)
+++	Nitrogen mustards (HN2)
+	Nitrosomethylurethane (NMU)
+	Phenylurethane
+	Tetramethylenedimethane sulfonate
+++	Trenimon
+	Triethyleniminotriazine
+	Urethane

been used as a therapeutic agent for gout since pre-Biblical days. Before any mutagenic chemical agents were discovered, it was known that polyploidy could be induced in plants treated with colchicine. A primary action of this class of drugs is to inhibit mitosis at metaphase through effects on the centriole and spindle protein. In addition to these cellular effects, colchicine and its synthetic derivative, demecolcine (Colcemid), produce abnormalities of the chromosome including despiralization, centromere division, and, secondarily, breaks and gaps. Podophyllin and podophyllotoxin are colchicine derivatives with similar effects. These agents act on both plant and animal cells *in vivo* and *in vitro*. Parafluorophenylalanine induces mitotic haploidization in diploid fungi cultures and we have recently observed dicentric chromosomes in human lymphocyte cultures treated with this agent.

Actidione and gammexane block mitosis at an earlier stage of cell division (prophase). They are also clastogenic in the plant chromosomes tested. Table 9 lists some of the cell cycle poisons which have been shown to break chromosomes.

Acridines and Other Photodynamic Dyes

Acridine and its related dyes, acridine-orange and acridine-yellow, are mutagenic compounds in microorganisms. They are also clastogenic in *Drosophila* and higher plants, but the treatment system requires both visible

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TABLE 9. MITOTIC POISONS

	C-Mitotic Agents	*Prophase Inhibitors*
++	Colcemid (see Demecolcine)	+ Actidione
+++	Colchicine	+ Gammexane
++	Demecolcine	
+++	Parafluorophenylalanine	
+	Podophyllin	
+	Podophyllotoxin	

light and an oxygen substitute in order for the breakage reaction to occur. Quinacrine (Atabrine) is an antimalarial drug introduced during World War II. It is also an acridine derivative which is highly mutagenic to bacteriophage, bacteria, and fruit flies. The acridine half-mustard, ICR-170, is an anticancer agent with mutagenic properties. Three antiseptics which are acridine-related compounds are acriflavin, proflavin, and 5-aminoacridine. Proflavin breaks human chromosomes in HeLa cultures. Both acridine and acridine orange break chromosomes in human diploid fibroblasts *in vitro*.

Other photodynamic dyes which have been shown to be mutagenic in bacteria and/or clastogenic in plants are rivanol, methylene blue, toluidine blue, pyronine, and thiopyronine. Trypan blue is teratogenic in mice and a single dose injected into pregnant mice produces chromosomal abnormalities in the embryonic cells. The dyes mentioned in this section are listed in Table 10.

TABLE 10. PHOTODYNAMIC DYES AND RELATED COMPOUNDS

	Acridines		*Other Dyes*
+++	Acridine	+	Methylene blue
+++	Acridine orange	+++	Neutral red
+	Acridine yellow	+	Pyronine
+	Acridine half-mustard	+	Rivanol
+	Acriflavin	+	Toluidine blue
+	5-Aminoacridine	++	Trypan blue
+	Atabrine (see Quinacrine)		
+	ICR-170 (see Acridine half-mustard)		
+++	Proflavin		
+	Quinacrine		

ANTIFOLIC COMPOUNDS

The first antimetabolites to be discovered were folic acid antagonists. Aminopterin-treated cancer patients have increased chromosome breaks in cultured lymphocytes. Also, direct bone marrow examination of patients with folate deficiency and vitamin B_{12} deficiency have shown striking cytogenetic abnormalities. These changes can be reversed by treatment with B_{12}.

Methotrexate (amethopterin) is an antifolic compound which has long been an effective therapeutic agent for the treatment of leukemia and choriocarcinoma but recently it has had widespread acceptance among patients for its miraculous results in the long-standing, incapacitating, but nonlethal disease, psoriasis. It causes chromosome breaks in cultured human lymphocytes. Its chemical action is to compete with dihydrofolic acid for the enzyme dihydrofolate reductase, thus acting directly on thymidine biosynthesis.

Many antifolic compounds are teratogenic in birds and mammals and no doubt cause congenital defects in man. Table 11 gives the two compounds mentioned here.

TABLE 11. ANTIFOLIC CLASTOGENS

+++	Amethopterin (see Methotrexate)
+++	Aminopterin
+++	Methotrexate

ALCOHOLS AND OTHER ORGANIC SOLVENTS

Perhaps ethanol should be discussed under the section on foods and beverages but it is the only organic solvent listed in Table 12 that could be so classified. Its mutagenic effects in microorganisms are established but there are conflicting reports on its clastogenicity. For example, it has been shown to break chromosomes in the root tip test using the broad bean *Vicia faba*, but results in the onion root tip test under similar conditions were negative. Furthermore, ethanol causes specific breakage of the X chromosome in one

TABLE 12. ALCOHOLS AND OTHER ORGANIC SOLVENTS

Aliphatic Alcohols		+	Paranitrophenol
+++	β-Mercaptoethanol	+	Phenol
+	Ethanol	*Lacquer Thinners*	
?	Methanol	?	Ethyl acetate
?	Propanol	?	Methyl acetate
Benzyl Alcohols		*Cleaning Fluids*	
+	Cathechol	?	Carbon tetrachloride
+	2,4-Dinitrophenol	?	Naphthalene
+	Metacresol	?	Perchloroethylene
+	Metacresol purple	?	Trichloroethane
+	Metanitrophenol		
+	4-Nitrometacresol	*Airplane Glue Solvents*	
+	6-Nitrometacresol	?	Acetone
+	Orthocresol	+	Cyclohexane
+	Orthocresophthalein	?	Hexane
+	Orthonitrophenol	?	Toluene
+	Paracresol		

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species of grasshopper but no breaks could be demonstrated in a closely related species. Why have no studies been done on the clastogenic effects of ethanol *in vivo* in *Homo sapiens*?

The benzene derivatives, cresols, nitrophenols, and catechols are potent clastogens of onion chromosomes and phenol is mutagenic in microorganisms, higher plants, and *Drosophila*. Under suspicion are other organic solvents such as lacquer thinners, cleaning fluids, and airplane glue ingredients.

Inorganic Chemicals

The divalent cations, Ca^{++} and Mg^{++}, are important to the integrity of the chromosome. If cells are grown in calcium- and magnesium-deficient media the chromosomes have a tendency to uncoil and stretch and they become more fragile and are easily broken. If the monovalent cation, K^{+}, is added in excess, the heterochromatic regions of the chromosomes are attenuated and exaggerated.

Acute lead poisoning in children leads to irritability, vomiting, convulsions, coma, and death. Chronic lead poisoning produces microcytic anemia, the "lead line" of the gums, and encephalopathy. In these children, excess chromosome breaks have been demonstrated.

Certain inorganic anions are also capable of breaking chromosomes. Sodium diarsenate causes pulverization of human chromosomes *in vitro*, while hyponitrites and pyrophosphates act as clastogens on *Vicia faba* chromosomes.

Potassium cyanide reacts with heavy metals. Since aerobic respiratory enzymes contain heavy metals such as iron, copper, and zinc, KCN acts as an effective respiratory inhibitor. The inorganic elements, radicals, and compounds are given in Table 13.

TABLE 13. Inorganic Chemicals

Cations	
++	Calcium deficiency
+++	Lead excess
++	Magnesium deficiency
+	Potassium excess
Anions	
+++	Arsenates
+	Hyponitrites
+	Pyrophosphates
Salts	
+	Potassium cyanide

Miscellaneous Compounds

Several chemical compounds deserve mention even though they have not been included in the classifications listed previously. They are given in Table 14.

The anti-leukemic agents, piperazine (A-8103) and azathioprine (Imuran) produce severe damage to the chromosomes of bone marrow cells and circulating leukocytes in patients who have been treated with these drugs. Beta-mercaptopyruvate and 6-methylcoumarin reportedly break human chromosomes and *Vicia* root tip chromosomes, respectively.

Hydroxylamine produces severe shattering of Chinese hamster chromosomes in culture. This agent acts preferentially on certain regions of specific chromosomes. It is also a potent mutagen in bacteriophage, and several derivatives of hydroxylamine have strong carcinogenic properties.

Aflatoxin is produced by a mold which is found on peanuts and other agricultural products. It is a potent carcinogen and was recently reported

TABLE 14. Miscellaneous Compounds

+++	A-8103 (see Piperazine)
+++	Aflatoxin
+++	Azathioprine
+++	β-Mercaptopyruvate
?	Dimethylsulfoxide (DMSO)
++	Hydroxylamine (HA)
+++	Imuran (see Azathioprine)
+	6-Methylcoumarin
+++	Piperazine

to be highly mutagenic in the mouse dominant lethal test, presumably through chromosome damage to male germ cells.

Dimethylsulfoxide (DMSO) is a commercial solvent which has an unusual capacity to penetrate cells very rapidly, carrying other molecules with it. It is miscible with water and organic solvents. Although it is not known to be a clastogen it is of concern because it may serve as a vehicle for clastogens (which would not otherwise enter various bodily tissues) to penetrate the cell and exert damaging effects.

Physical Agents

Although this review is not concerned with physical clastogens, several remarks are appropriate. X ray was the first proven mutagen, causing point mutations in *Drosophila melanogaster*. All forms of radiant energy, including gamma rays from atomic bomb explosions and ultraviolet light, are potent

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mutagens and clastogens in all organisms tested, including man. Sonic vibrations and sudden temperature changes, especially cold shock, have been shown to break human chromosomes *in vitro*. With the recent biosatellite experiments in connection with space exploration, translocations (resulting from chromosome breakage and rearrangement) have been recovered from *Drosophila* exposed to added *G* forces, weightlessness, and electromagnetic fields.

With the increased use of isotopes in diagnostic medicine, it is pertinent to comment that radiation emitted from ^{131}I-, ^{32}P-, ^{3}H-, and ^{14}C-containing compounds will produce chromosome breaks in all of the tissues of the body where the isotope is distributed, including the gonads. Tritium-labeled nucleosides are clastogenic to human lymphocytes *in vitro* and ^{32}P-labeled compounds produce nonrandom breaks in heterochromatin in the root tips of *Vicia faba*.

Thorotrast deserves special comment. It was introduced in 1931 as a radiopaque contrast medium for arteriography and retrograde pyelography. When its toxic effects caused by release of isotopes in the ^{232}Th decay series were brought to attention, its use was immediately discontinued. However, approximately 50,000 patients received the drug. They are receiving up to 500 rads over a 25-year period since Thorotrast is incorporated into body tissues and eliminated at a negligible rate. It is stored chiefly in the reticuloendothelial system of the liver, spleen, bone marrow, and lymph nodes. Thus, it is not surprising that chromosome aberrations have been repeatedly demonstrated in lymphocyte cultures from these individuals.

Physical clastogens are thought to mediate their effects through chemical changes in the cell, producing unstable radicals, such as peroxides, which react with the DNA, RNA, or protein of the chromosome, causing disruption. Secondary clastogenic effects of radiant energy have been demonstrated by the production of chromosome breaks if the culture media or some of its components are heavily irradiated with gamma rays from a cobalt source before the cells are introduced into the broth. This, of course, would have significance in the preservation of foods by irradiation. Both irradiated amino acids and carbohydrates have been implicated as clastogens.

If plasma from an individual who has received total body irradiation is used in the culture medium of normal human lymphocytes, increased chromosome breakage occurs. Table 15 gives a listing of the agents mentioned in this section.

Genetic and Clinical Significance of Chromosome Breakage

The production of chromosome breaks is only the first step of a two-step process resulting in structural rearrangement of the genetic material. Breaks must be followed by healing of the broken ends in order for the chromosomes to survive and be transmitted to daughter cells. It is believed that most breaks heal by rejoining, resulting in restitution of the original configura-

TABLE 15. Physical Clastogens

+	Centrifugal force	+++	Temperature shock
+	Electromagnetic fields	+++	Thorium dioxide
+++	Gamma rays	+++	Thorotrast (see Thorium dioxide)
+++	Irradiated culture media		
+++	Irradiated foods	+++	Ultraviolet light
+++	Irradiated plasma	+	Weightlessness
+++	Isotopes: ^{131}I, ^{32}P, ^{3}H, ^{14}C	+++	X rays
+	Sonic vibrations		

tion. If, however, breakage at two sites is followed by the union of different chromosomal segments, then new chromosomes are formed. If these new structures are stable they can be perpetuated, but if unstable, they are ultimately lost in subsequent cell generations. Newly derived stable chromosomes are sometimes called "chromosomal mutations." In some instances, genetic information is gained or lost, while in other cases there is merely novel packaging of the old material with maintenance of a balanced genetic complement. In this latter case, the raw material for chromosomal evolution is provided by new linkage groups and position effects on genes which acquire new neighbors.

Figures 1 and 2 illustrate some of the common chromosomal rearrangements and how they are produced by breakage and rejoining. They are classified into two groups: stable and unstable. Only the unstable rearrangements (acentrics, dicentrics, and rings) are easily identified cytologically. They are usually scored when cells are examined for chromosome damage because they present strikingly abnormal configurations (Fig. 3). The stable rearrangements usually go unrecognized unless the time-consuming process of extensive karyotyping is carried out. Even then, only major shifts in chromosome length or centromere position will be perceived by our present cytologic methods. This difficulty in detecting stable abnormalities is not a serious drawback to assessment of damage because breakage without rehealing and healed unstable aberrations are an accurate index of the disruption of the genetic material.

It should be emphasized that the stable rearrangements (e.g., translocation mongolism) are those which are of concern to the physician and his patient because they can result in unbalanced genetic constitutions and they may be passed on to future generations if the changes occur in the germinal cells. This is one important reason why practitioners should be knowledgeable about possible clastogenic effects of the drugs they administer and insist on careful testing of both old and new pharmacologic agents. Thus, drugs should be considered potentially hazardous, just as radiation is so respected, lest today's clinicians unwittingly produce iatrogenic disease in tomorrow's children.

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DIAGRAMMATIC REPRESENTATION OF STABLE CHROMOSOMAL REARRANGEMENTS

Old Chromosome	New Chromosome	No. of Breaks	Cytologic Effect	Genetic Effect	Rearrangement
		2	Chromosome shortened	"E" segment lost	Deletion (Deficiency)
		2	Chromosome lengthened	"E" segment added	Duplication
		2	None	Change in gene order	Paracentric inversion
		2	Centromere position shifted	Change in gene order	Pericentric inversion
		2	Two morphologically altered chromosomes	Genes shuffled into new linkage groups	Reciprocal translocation
		3	Chromosome lengthened	"L" segment added	Insertion
		1*	Chromosomes are exactly metacentric	Entire chromosome arm is duplicated and other arm lost	Isochromosome

*Misdivision of centromere after chromosome replication.

FIG. 1

DIAGRAMMATIC REPRESENTATION OF UNSTABLE CHROMOSOMAL CONFIGURATIONS

Old Chromosome	New Chromosome	No. of Breaks	Cytologic Effect	Rearrangement
		2	Chromosome with two centromeres	Dicentric chromosome
		1	Chromosome segment lacking a centromere	Acentric fragment
		2	Two proximal ends of chromosome are joined	Ring chromosome

FIG. 2

MONITORING THE HUMAN POPULATION FOR INCREASED CHROMOSOME BREAKAGE

With the introduction of hundreds of new synthetic chemicals into man's environment each year there is a real danger that a "genetic catastrophe" may occur. At the present time, scientists have no reliable method for detecting such an event. But several possible avenues of approach are under consideration.

One method involves the detection of a sudden increase in dominant mutations which are visible at birth (e.g., achondroplastic dwarfism). Other methods are concerned with the systematic testing of chemicals which are suspected of being mutagenic or clastogenic. These are done by the onion or broad bean root-tip test, the mouse dominant lethal test, the host-mediated bacterial test, or the *in vitro* human lymphocyte clastogen test. These are only indirect experimental tests and would not detect a rapid increase in damage to the human gene pool which was unanticipated.

It was pointed out in the introduction that there is a partial overlap of chemicals for their oncogenicity, teratogenicity, mutagenicity, and clastogenicity. There is also incomplete species specificity, and *in vivo-in vitro* differences. Some of these differences are due to inadequate testing and our ignorance of mechanisms, but some are probably biological. Figure 4 diagrams some examples of interrelationships of bioassays which must be considered in evaluating genetic damage.

In order to detect an unsuspected rise in human chromosome breakage

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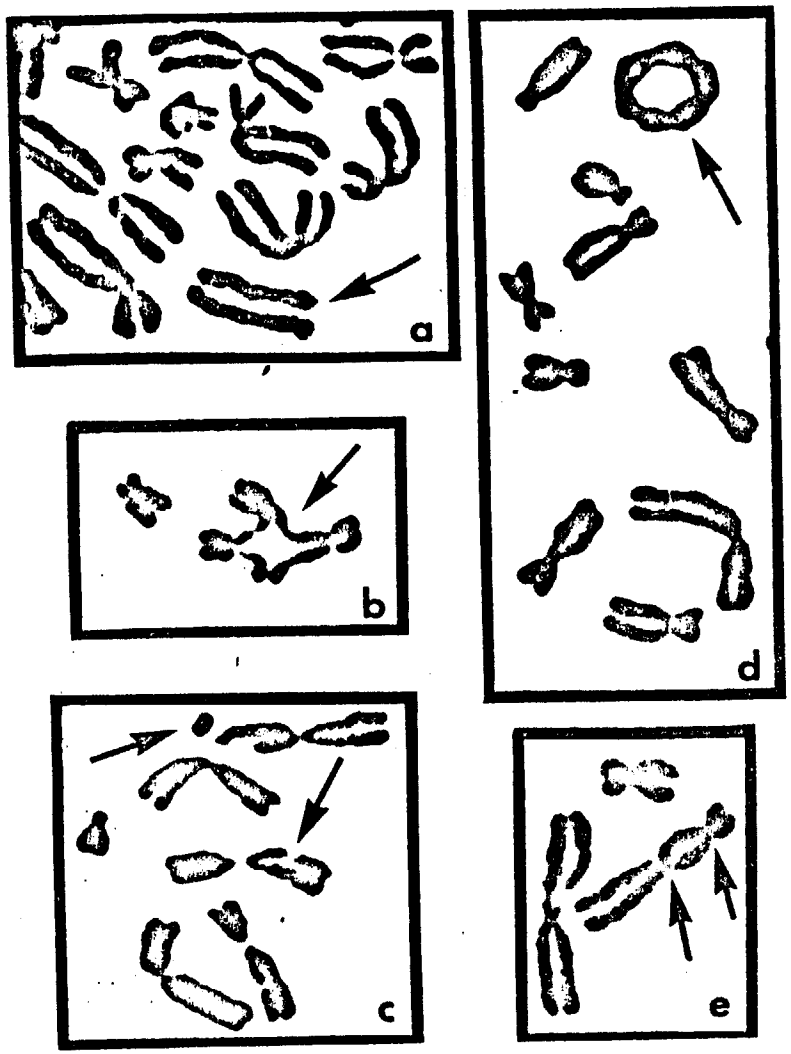

FIG. 3. Cytogenetic appearance of unstable chromosome aberrations which are easily scored in clastogen tests: a. long acentric fragment; b. chromatid exchange; c. displaced chromatid break (upper arrow) and aligned chromatid gap (lower arrow); d. ring chromosome; and e. dicentric chromosome.

INTERRELATIONSHIPS OF TEST SYSTEMS TO MONITOR BIOLOGICAL DAMAGE

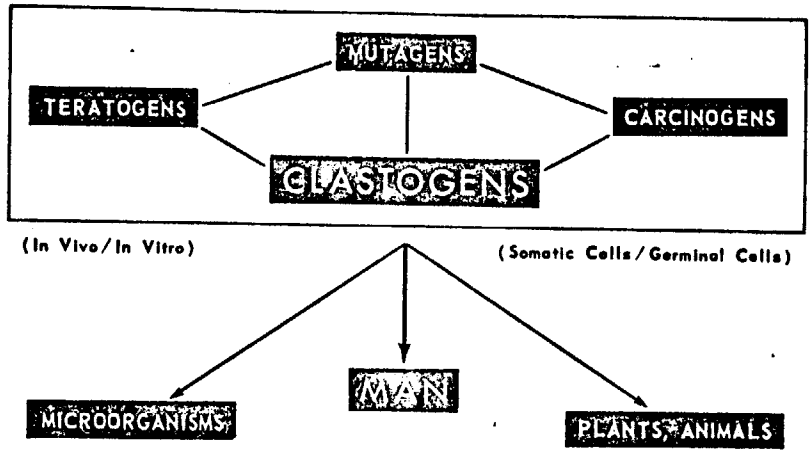

FIG. 4

(which is, theoretically, an accurate indicator of genetic change) we need first to know the incidence of "spontaneous" chromosome breakage in man. To date, there have been no large-scale surveys to obtain this information. An indirect estimate, however, derived from chromosomal abnormalities in the newborn population, is available (Table 16). Data combined from five laboratories give the incidence of numerical and structural aberrations in more than 13,000 newborns which have been karyotyped. These laboratories are located in Boston, Massachusetts; New Haven, Connecticut; Pittsburgh, Pennsylvania; London, Ontario; and Edinburgh, Scotland. The overall frequency of chromosome anomalies is approximately 1 in every 140 live births, or about half the frequency of human twinning. If the annual birth rate in the United States is 3.6 million, then 26,000 babies are born with chromosomal defects each year. A sudden doubling of this figure may go unnoticed unless large samples of neonates are monitored. Perhaps the simple expedient of requiring the registration of all mongol births in large hospitals where the diagnosis is not likely to be missed would be a cheap and effective approach which could sound a note of warning.

Approximately 33 percent of all detected spontaneous abortions in the first trimester of pregnancy are chromosomally abnormal. Since 15 percent of all detectable conceptuses are aborted, this means that at least 5 percent of all conceptions carry a chromosomal abnormality (approximately 200,000 per year in the United States). If a large sample of females of reproductive age could be monitored for an increased spontaneous abortion rate this might be a sensitive indicator of increased chromosomal abnormalities.

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TABLE 16. SUMMARY OF RESULTS OF FIVE NEWBORN SURVEYS FOR RECOGNIZABLE CHROMOSOMAL DEFECTS

Chromosomal Abnormality	No. Tested	No. Found	Abnormalities Per 1000 Live Births
A. Structural Changes	13,665	37	2.71
B. Numerical Changes in Autosomes			
Trisomy D	9,565	3	0.31
Trisomy E	9,565	2	0.21
Trisomy G	9,565	10	1.05
C. Numerical Changes in Sex Chromosomes			
XYY	13,665	11	0.80
XXY	13,665	14	1.02
XXYY	13,665	1	0.07
XXX	11,659	6	0.51
XO	7,559	2	0.26
XX male	13,665	1	0.07
Total abnormalities per 1000 live births			7.01

CONCLUSION

In terms of Darwinian fitness, the ability of the DNA to mutate must have a high selective value. Gene mutations and chromosomal changes are the raw materials of evolution and without them most species would stagnate, deteriorate, and eventually extinguish themselves. But the introduction of new mutations has been carefully controlled by Nature to occur at a rate which will allow the species to incorporate the few advantageous ones and weed out the deleterious mutants. An upset in this balance could spell disaster.

Man's most precious possession is his genetic heritage. If, in the short time we are allowed to store the genes for future generations, we carelessly squander our resources and poison our germ plasm with devastating mutations, then our heirs will be the losers.

ACKNOWLEDGMENTS

This review was written without specific reference citations which would have encompassed over 500 articles. To all of the authors of these articles I am greatly indebted. A short bibliography of major books and recent review articles is included here. A complete list of references may be obtained from the author upon request.

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Evaluation of the addition of potassium chloride to lidocaine as an anesthetic in dentistry

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Qualitative and quantitative differences were found in anesthesia produced by lidocaine, lidocaine with potassium chloride, and lidocaine with epinephrine in these tests conducted with two groups of dental patients and one group of volunteers. Of the three solutions, lidocaine with epinephrine seemed to have the most desirable qualities.

The incorporation of certain ions, such as potassium, may enhance the duration of action of local anesthetic drugs.[1, 2] One early report[3] of procaine and potassium chloride (KCl) used for dental anesthesia showed no significant difference in duration of the anesthesia as compared to that obtained when procaine was used alone. During investigation of the effects of the combination of various compounds with lidocaine, Bromage and others[4, 5] noted that potassium and CO_2 salts would enhance the duration, quality, and spread of extradural blockade. Hyaluronidase impaired these factors.

Animal investigations conducted in our laboratories[6] showed no difference in the onset and duration of systemic toxic manifestations in rabbits which were injected intravenously and guinea pigs which were injected intraperitoneally with solutions of 2% lidocaine and 2% lidocaine containing potassium chloride (180 mM). Microscopic examinations of skin, muscle, and nerve tissue sections of tissues that had been injected with these solutions revealed no histopathologic changes (Fig 1). Tissue specimens were obtained seven days from injection time, immediately after sacrifice. No obvious differences were noted in the degree of lymphocyte infiltration in the muscle fibers. Vacuolization of myelin was similar on both nerve fibers.

An evaluation of the qualities of anesthesia obtained in dental procedures by the addition of potassium chloride to lidocaine was undertaken.

Materials and methods

As in our previous studies, patients having dental surgical procedures under local anesthesia were compared with volunteers who were not having surgery. Comparison was made between 2% lidocaine, 2% lidocaine plus potassium chloride, and 2% lidocaine plus epinephrine. The study was conducted in three phases, all of which were tested in a double-blind fashion. The first two phases included a total of 123 patients undergoing various dental procedures. In the first phase, the effectiveness, onset, and duration of action of 2% lidocaine containing 1.5% KCl solution was compared to 2% lidocaine alone. In the

Fig 1 ■ Effects of intramuscular and intraneural injection of 2% lidocaine into sciatic nerve and surrounding muscle of rat (top left and right); effects of similar injection of 2% lidocaine with 1.5% KCl (bottom left and right).

Table 1 ■ Onset, depth, and duration of anesthesia from supraperiosteal injections on all tested teeth of volunteer group.

	2% Lidocaine		2% Lidocaine-1.5% KCl		2% Lidocaine-epinephrine 1:100,000	
	No.	%	No.	%	No.	%
Onset of anesthesia (min)						
0-5	13	39	36	52	21	58
6-12	1	3	10	15	7	19
13-18	0	...	6	9	6	17
19-30	0	...	0	...	0	...
>30	19	58	17	24	2	6
Total	33		69		36	
Depth of anesthesia (Vitalometer reading)						
0-4	1	3	3	4	0	...
5-9	19	58	27	40	1	3
10-13	2	6	9	13	3	8
14	11	33	30	43	32	89
Total	33		69		36	
Duration of anesthesia (min)						
0-10 (failure)	20	59	17	23	2	6
10-30	8	24	29	42	7	19
30-60	5	17	19	28	18	50
>60	0	...	4	7	9	25
Total	33		69		36	

Onset of anesthesia expresses time in minutes to reach maximum tolerated pain threshold. Depth of anesthesia is reported as measured with Vitalometer. Duration of anesthesia is time in minutes from onset until return of pain threshold to control level reading.

second phase, 2% lidocaine containing 1.5% KCl was compared to 2% lidocaine with epinephrine.

The patients were either outpatients having operative procedures or dental surgery at Denver General Hospital or inpatients at the dental clinic of National Jewish Hospital. These patients received either regional mandibular nerve blocks or supraperiosteal infiltration analgesia. After administering the prepared solution, the dentist performed the required procedure. The onset, duration, and depth of anesthesia were assessed subjectively according to when the patient felt numbness and objectively according to when the patient felt stimulation performed with a Burton Vitalometer.* The volunteer group and the outpatients at Denver General Hospital were asked to inform us when regular sensation returned, as determined by fingernail stimulation of the gingiva or disappearance of the numb feeling or both. They were also invited to comment on the injection and operative procedure. If they reported discomfort during the operative procedure, the anesthesia was considered a failure.

In order to rule out the variants involved in the first two phases (such as tooth pathology, tooth morphology, nerve vitality, and so forth), a third phase was added. In the third phase, the three solutions were again tested on a group of 33 volunteers. These volunteers were required to have vital maxillary anterior teeth which were free of dental pathology. After thorough drying of the teeth, a control pain threshold was obtained electrically for each tooth by means of the Burton Vitalometer with a dentifrice as the conductive medium. Then 2 ml of the prepared anesthetic solution was injected supraperiosteally over the apex of each lateral incisor. At five-minute intervals the pain threshold of the maxillary anterior teeth was tested with the Vitalometer until sensitivity reached a peak. Readings were then continued at 15-minute intervals until the control level was reattained.

Preparation of solutions

Twenty milliliter vials of 2% lidocaine were used. Lots of four vials were selected simultaneously; 2 ml was removed from two of them and the same volume of a 1.5% solution of KCl (2 mEq/ml) was added. After the manufacturer's labels were removed, the vials were numbered at random by a dental assistant unfamiliar with the study. Identification records were kept separated until the end of the study. A similar procedure was used

Table 2 ■ Results of superperiosteal injection of upper lateral incisors of volunteers.

	2% Lidocaine		2% Lidocaine-1.5% KCl		2% Lidocaine-epinephrine 1:100,000	
	No.	%	No.	%	No.	%
Onset of anesthesia (min)						
0-5	6	50	12	55	8	67
6-12	1	8	2	8	1	8
13-18	1	8	4	16	3	25
19-30	0	...	0	...	0	...
> 30	4	34	5	21	0	...
Total	12		24		12	
Depth of anesthesia (Vitalometer reading)						
0-4	1	8	0	...	0	...
5-9	5	42	8	13	0	...
10-13	0	...	3	12	1	8
14	6	50	13	55	11	92
Total	12		24		12	
Duration of anesthesia (min)						
0-10 (failure)	5	42	5	21	0	...
10-30	3	25	8	34	1	8
30-60	4	33	9	37	8	67
> 60	0	...	2	8	3	25
Total	12		24		12	

for the second phase in which ampules of 2% lidocaine with epinephrine 1:100,000 solution were used for comparison with vials containing lidocaine-KCl. For the third phase 36 vials of 20 ml were divided into three groups of 12, each containing 2% lidocaine, 2% lidocaine-KCl, or 2% lidocaine-epinephrine. After the manufacturer's labels were removed the vials were labeled with consecutive numbers. To maintain the blind character of the study, conventional cartridge syringes were not used.

Results

Onset, depth, and duration of anesthesia observed in the volunteer group are shown in Table 1. Failures in the group receiving lidocaine alone totaled 58%; in the group receiving lidocaine-KCl, 24%; in the group receiving lidocaine-epinephrine solution, 6%. Prompt anesthesia (onset in less than five minutes) occurred in more than 50% of all subjects receiving lidocaine-KCl or lidocaine-epinephrine, whereas only 39% of those receiving lidocaine alone had prompt anesthesia.

In the lidocaine-epinephrine group, less response to increasing electrical stimulus to teeth was noted in 89%. Although 33% of those receiving lidocaine alone and 43% of those receiving lidocaine-KCl attained a comparable depth of anesthesia, nevertheless 60% (20 teeth) of the group receiving lidocaine alone failed to have any anesthesia. Twenty-three percent (17 teeth) failed in the lidocaine-KCl group, but only 6% (2 teeth) in the lidocaine-epinephrine group. The longest duration of action occurred with lidocaine-epinephrine—25% of teeth tested remained anesthetized from 60 to 90 minutes. Because the injections of the three solutions were made over the lateral incisors, the onset, depth, and duration of anesthesia in only these teeth were compared. The results (Table 2) showed values somewhat similar to those obtained from evaluating the same parameters in the lateral incisors exclusively.

Failures and number of effective blocks, minimal, maximal, and mean duration of anesthesia for those patients receiving mandibular nerve blocks are listed in Table 3. Evaluations of the results from supraperiosteal injections are listed in Table 4. Complications and side effects encountered in both groups are also noted.

Discussion

From this study, it appears that the addition of KCl to lidocaine affords a longer duration of action than lidocaine alone, but not as long as lidocaine with epinephrine.

In the patients receiving supraperiosteal injections, the differences between the mean duration of anesthesia observed with the three solutions were not statistically significant. However, in comparing the mean duration in those who received mandibular nerve blocks, the difference was highly significant ($p<0.001$) when epinephrine was added to lidocaine alone, but only significant ($p<0.05$) when potassium was added. The difference noted between the mean duration of action for lidocaine plus KCl and lidocaine plus epinephrine was not significant.

A similar pattern was observed in the volunteer group. The difference in onset, depth, and duration of anesthesia obtained from the addition of potassium chloride to lidocaine was not as great as the difference obtained from the addition of epinephrine. The onset of anesthesia in the lateral teeth of the volunteer group and all upper anterior teeth was not significantly different.

The percent of failures noted in the groups of patients receiving lidocaine and lidocaine plus KCl was similar. However, when epinephrine was added, a lower incidence of failure was noted in both those receiving the mandibular blocks and those receiving supraperiosteal injections. In the lateral incisors of the volunteer group, the failures were also more frequent when lidocaine alone was used (42%) in contrast to lidocaine with KCl (22%) and lidocaine with epinephrine (0%).

The use of the Burton Vitalometer as described by Taylor[7] allowed us to make an objective evaluation of the depth of anesthesia in the volunteers who received supraperiosteal injections. Fifty percent of the individuals receiving lidocaine alone were without pain at the maximum readings of electrical stimulation obtainable. When KCl was added to lidocaine, 52% were without pain, and when epinephrine was added, 92%.

The clinical application of a solution such as the lidocaine-KCl mixture, although remote, may be valuable because of the possible contraindication to the use of vasoconstrictors in patients with cardiovascular disease.[8] The American Dental Association and the American Heart Association in a joint report[9] suggested that local anesthetics containing vasoconstrictors may be used in cardiac patients when administered carefully and with preliminary aspiration. However, in our view, the use of vasoconstrictors should be regarded as dangerous in patients who have severe advanced arterial hypertension or pheochromocytoma, or who are receiving mono-amino-oxidase inhibitor therapy.

Table 3 ■ Results of mandibular nerve blocks in patients.

	2% Lidocaine	2% Lidocaine 1.5% KCl	2% Lidocaine-epinephrine 1:100,000
Number of patients	12	27	20
Effective blocks	9 (75%)	21 (77%)	18 (90%)
Failures	3 (25%)	6 (23%)	2 (10%)
Minimal duration (min)	60	72	81
Maximal duration (min)	165	314	490
Mean duration (min)	106a	175b	198c
Complications	. . .	Burning sensation on lip (1)	Dizziness(2)

a-b = $p<0.05$
a-c = $p<0.001$
c-b = N.S.

Since Tainter and Throndson's study[3] on the use of procaine plus potassium, no other study has been conducted with the newer local anesthetics. Early work done by our group[6] revealed a longer duration of ulnar and digital blocks in volunteers who received lidocaine plus KCl than in those who received lidocaine alone. Also, bilateral superior laryngeal nerve blocks and transcricoid injection of 1% lidocaine-KCl produced adequate anesthesia for bronchoscopies in 40 patients without untoward effects.[10] It was felt that a convenient method of evaluation of this combination could be conducted in dentistry.

The results obtained from this study showed that statistically significant differences of duration of

Table 4 ■ Results of anesthesia indexes observed after supraperiosteal injections on patients with tooth pathology.

	2% Lidocaine	2% Lidocaine 1.5% KCl	2% Lidocaine-epinephrine 1:100,000
Total number of patients	14	34	16
Effective injections	8 (57%)	22 (65%)	15 (94%)
Failures	6 (43%)	12 (35%)	1 (6%)
Minimal duration (min)	60	80	95
Maximal duration (min)	160	184	282
Mean Duration (min)	115	126	147
Complications and side effects	. . .	Buccal hematoma(1)	Dizziness(1)

Differences between means of duration of action were not statistically significant.

Proposed mode of action

Movement of ions through the nerve membrane is considered to be one of the main steps in the process of excitation and the propagation of stimuli (Fig 2a, b). Impeding such movement will probably repress membrane depolarization and delay impulse transmission.

Local anesthetic drugs have the ability to block the propagation of nerve impulse distal to the portion of the nerve which has been in contact with a particular drug (Fig 2c, d). Changes of ion gradients (Na and K) are largely responsible for the transfer from resting to active membrane potential. Potassium is more concentrated inside; it tends to diffuse outward, making the outside more positive. By moving through the membrane, the negatively charged organic anions electrically attract potassium ions, counterbalancing their tendency to diffuse outward, and thus establish equilibrium.[11]

The concentration of sodium ions is greater outside the membrane. Sodium crosses slowly through the membrane, aided by the gradient and electrical forces, but it is again extruded by the cell to maintain equilibrium. In this way, sodium is continually circulated by the process called the "sodium pump." The delay in recovery of the resting potential can thus be explained in terms of the difference of K^+ concentrations. Blockage of a nerve impulse can be effectively produced by an accumulation of potassium ions on the outside of the neuron.[1] It is assumed that the blockade produced by lidocaine (Fig 2c, d) is reinforced and prolonged by the increase of K^+ concentration out of the nerve membrane as result of the administration of exogenous KCl (Fig 2e, f).

Previous trials incorporating potassium into local anesthetic solutions have not been completely successful.[2, 3, 12] Among the reasons may have been: difference in potassium concentration, the use of procaine (which is less diffusable than lidocaine), and acid solutions that would decrease the potency of the local anesthetic. A study of the combination of various concentrations of KCl with lidocaine and with other local anesthetics would be desirable.

Summary

Lidocaine, lidocaine with KCl, and lidocaine with epinephrine were compared as to duration, depth, and onset of anesthesia in dental patients and volunteers.

Qualitative and quantitative differences were noted between effects of the three solutions. The solution containing epinephrine seemed to have more desirable qualities. Lidocaine with KCl appears to produce anesthesia of longer duration than lidocaine alone, but its clinical usefulness is still not completely proved.

In terms of producing a local anesthetic which is physiologically compatible, contains no harmful additive, and achieves satisfactory duration, depth, and onset of anesthesia, the results of this study are encouraging.

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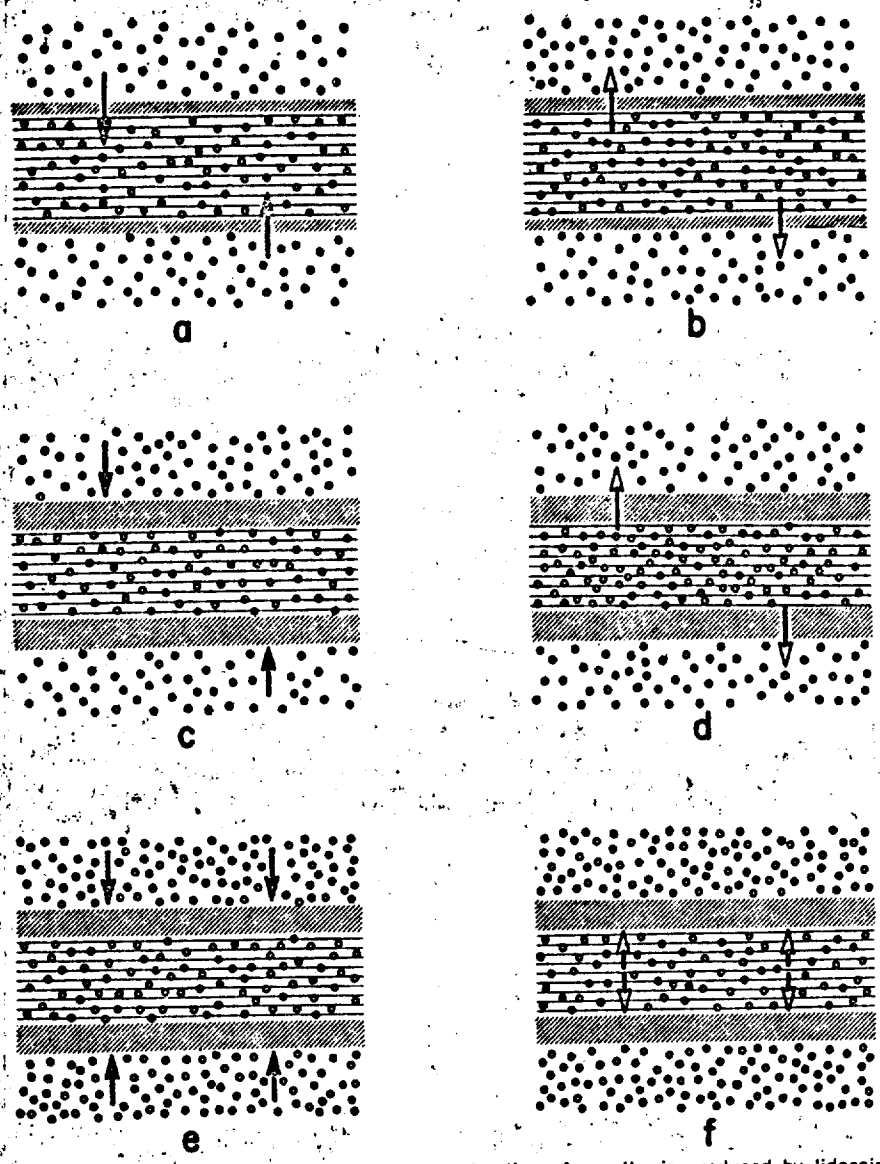

Fig 2 ■ Proposed mode of action in prolonging duration of anesthesia produced by lidocaine when exogenous potassium is added. During nerve stimulation, at depolarization, membrane becomes permeable; sodium ions (black dots) enter (a) while potassium ions (hollow dots) exit (b). Local anesthetics incorporate into lipoid phase of membrane, rendering it impermeable to sodium ions' passage (c) but potassium ions, because of difference in concentration on both sides of membrane, continue to cross from inside to outside of nerve (d). Addition of exogenous potassium "reinforces" block; Na^+ cannot go in (e); K^+ cannot go out (f).

action of the solutions were found only in connection with mandibular nerve blocks. One can theorize that the lack of consistency noticed for supraperiosteal injections in volunteers and patients was due to the slower absorption and area pooling effect of the anesthetic when epinephrine was used. This would decrease the amount of anesthetic rapidly absorbed through the maxillary areolar bone, thus allowing it to concentrate to achieve nerve contact and produce analgesic results.

The preservation of epinephrine-containing solutions requires a lower pH; this in time, makes a more concentrated solution of the local anesthetic necessary. When potassium chloride is added, the pH of the lidocaine solution is not changed and would seemingly be more readily accepted by the areolar tissue because of this nearly neutral pH.

Letters to the Journal

Salt Excretion in Sweat in Cystic Fibrosis

To the Editor:—Since the discovery of abnormally high levels of sodium and chloride in sweat in children with cystic fibrosis of the pancreas (CFP) by di Sant'Agnese et al in 1953, the sweat test has proved to be a valuable help in pediatrics. The finding of a high salt excretion by eccrine sweat glands in a child with steatorrhea associated with pulmonary disease makes the diagnosis of CFP certain. Most investigators give the upper normal limit of sodium at 70-80 mEq/liter and that of chloride at 50-60 mEq/liter. In CFP very high sodium values up to 120 mEq/liter and more are encountered, and chloride levels may be even higher. Salt loss by sweating is also important in adrenal cortical hypofunction (Addison's disease), but, in general, sodium and chloride concentrations are not as high as in CFP. On the other hand, pituitary hypofunction is not followed by abnormal salt excretion in sweat because adrenal aldosterone secretion is not disturbed.

Sweating can be induced by heat or by means of a pharmacological substance, ie, pilocarpine nitrate 0.2%. For practical reasons and for technical convenience, pharmacological methods are preferred. Our own results indicate that this method gives results comparable to thermal sweating. Since electrolyte composition of sweat varies greatly on different skin areas, sweat tests should always be performed on the same skin area, ie, palmar side of the forearm in older children and adults.

In adults the sweat electrolyte excretion is variable. Sodium and chloride concentrations have a wide range even in normal persons. High sodium levels up to 140 mEq/liter can be found. Screening tests in a random population have shown in 10% of cases sodium levels above 80 mEq/liter.[1] Several factors are responsible for these variations in adults. Among these, seasonal fluctuations are most important: in the northern hemisphere sodium and chloride levels are higher during the winter time. Electrolyte concentrations also increase with age and with high intake of salt. Experimental salt depletion, on the other hand, is followed by an important decrease of sweat sodium chloride due to the stimulation of aldosterone secretion. Such physiological variations of sweat electrolytes in adults indicate that a high sodium or chloride level found occasionally by means of a single sweat test cannot be considered a criterion for CFP, a fact which has not been sufficiently recognized in numerous publications on the problem of CFP in adults.[2]

There is good evidence that aldosterone can act directly on the sweat glands by lowering sodium excretion. This extrarenal action of aldosterone has recently been used in the diagnosis of CFP. It has been shown that abnormally high levels of sodium and chloride in sweat in children with CFP are only little influenced by aldosterone. Thus the excretion of these electrolytes remains pathological in experimental hyperaldosteronism during salt depletion or parenteral administration of a high dose (0.5-1.0 mg/10 kg of body weight) of aldosterone for four to five days. This particular behavior of sweat electrolytes is typical in CFP and may prove to be helpful for the diagnosis in adolescents and adults. Conversely, in normal persons, abnormally high values of sodium and chloride in sweat are lowered below the critical limit of 80 mEq/liter for sodium and 60 mEq/liter for chloride in these circumstances.

In *conclusion* it is doubtful whether cystic fibrosis of the pancreas is frequent in adults. Single sweat tests are not reliable for diagnosis except in children. Dynamic studies of sweat sodium and chloride excretion during salt depletion or aldosterone load during four to five days show characteristic differences between normal persons even with initially high sweat electrolyte levels and patients with cystic fibrosis of the pancreas.

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"Acute Effects of Excessive Consumption of Salt. Excess of the Na or Cl ions is ordinarily eliminated promptly; but even when they are retained, these ions produce only slight effects, for the amounts normally present are so large that the artificial introduction of ordinary amounts will not increase their ratio to a considerable extent. In this sense Na and Cl are the most indifferent of the ions, and their salts are used when the effects of other ions are to be tested. However, they are essential to life, and the absence of effects is due only to the fact that the variations which are introduced are relatively small...

"Prolonged ingestion of excessive salt tends to produce edemas, degenerative changes in the kidney and to less degree in other organs, and chronic arterial hypertension...

"Therapeutic restriction of salt income is used to suppress the formation of effusions in chronic congestive heart failure and in hepatic cirrhosis. It also greatly diminishes the polyuria of diabetes insipidus (Swann, 1939). In many patients with chronic essential hypertension, it lowers the excessive systolic and diastolic blood pressure and the symptoms improve; it is also beneficial in eclampsia...

"Effects of Limited Salt Income on Normal Individuals. The maintenance of a certain sodium and chloride content is indispensable to the body. Animal food contains a sufficiency of this salt; herbivorous and omnivorous diets are insufficient in this respect; but a greatly restricted salt income (as by an excessive milk diet) can be borne for a considerable time, being met by a corresponding decrease of excretion, so that the percentage of the salt in the blood is scarcely altered, and no general symptoms result. Dogs on a Cl-poor diet retain Cl or I more efficiently than on ordinary food (Sarvonat and Coemier, 1911). The diminished income of salt in fever seems to have no deleterious effect. Even when the excretion is further increased by liberal water diuresis, the patients show no particular craving for salt...

"Excessive salt depletion produces a syndrome of weakness, nausea, muscle cramps, dehydration, peripheral vascular collapse similar to that of traumatic shock, which may be fatal (Elkenton et at, 1946). This may be induced by excessive sweating in "heat cramps", by the loss of gastric juice in the persistent vomiting of pyloric and high intestinal obstruction; by the profuse diarrhea of cholera; and by deficiency of the suprarenal cortical hormone, and exceptionally by mere restriction of salt income. In hot climates, the capacity for work is impaired if the daily salt income is less than 15 Gm.; higher income has no advantage (H.L. Taylor et al., 1943)..."

Vascular Smooth Muscle Reactivity in Normotensive and Hypertensive Rats

Abstract. *Aortic strips from spontaneously hypertensive rats were less responsive than normal animals to the contractile effects of norepinephrine, serotonin, and potassium chloride but more reactive to the relaxant effects of the stimulant of beta receptors, isoproterenol. Thus, hypertension is not the result of an absence of beta receptor or a hypersensitivity of the vascular smooth muscle.*

Increased peripheral resistance of the vascular system is characteristic of hypertension in humans (*1*). We have compared the reactivity in vitro of thoracic aortas from normotensive rats with those from a strain of spontaneously hypertensive rats (SHR) (*2*). The thoracic aorta was used as an indicator of vascular reactivity because it can be readily prepared for the recording of pharmacologic responses, although the aorta exerts little, if any, effect on total peripheral response. The hypertension exhibited by SHR may be analogous to essential hypertension in humans because histopathological changes are absent, especially in the cardiovascular-renal system before the development of hypertension (*3*). Aortas from hypertensive rats are less reactive to norepinephrine, serotonin, and KCl than those from normotensive animals. In addition, thoracic aortas from both the hypertensive and normotensive rats have beta adrenergic as well as alpha adrenergic receptor sites.

The blood pressures of unanesthetized male Wistar rats of the spontaneously hypertensive strain and normotensive Wistar rats (Carworth Farms), 7 to 10 weeks old, were measured by the tail-plethysmographic method (*4*). The animals were killed by a blow on the head, and spirally cut thoracic aortic strips were prepared by the method of Furchgott and Bhadrakom (*5*). Each strip was suspended in an isolated-organ bath (10 ml) containing a modified Krebs bicarbonate solu-

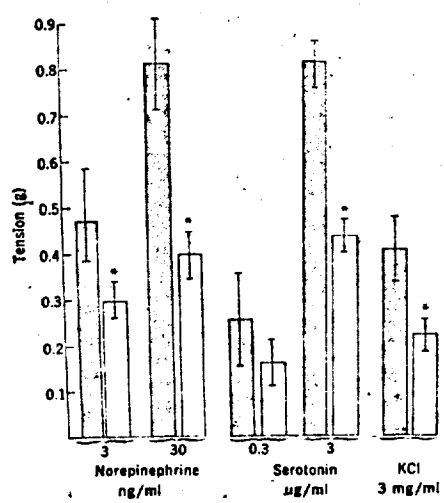

Fig. 1. A comparison of the contractile responses of thoracic aortic strips from five normotensive and nine spontaneously hypertensive rats to norepinephrine, serotonin, and KCl. Data given as means ± standard errors. The asterisks indicate statistically significant differences, $P < .05$. Shaded bars, normotensive animals; open bars, SHR.

Vascular smooth muscle from a strain of genetically hypertensive rats is less ive to specific (norepinephrine and in) and nonspecific (KCl) h muscle contractile substances than is comparable tissue from normotensive animals. The mechanism responsible for this abnormality may be related to the intrinsic contractility of the muscle, since all of the agonists were affected to about the same degree. On the other hand, stimulation of beta adrenergic sites in aortas from both hypertensive and normotensive rats with isoproterenol produced the classical relaxation. Thus, hypertension in the SHR is not due to the absence of beta receptors in the vascular tree. Reduction of alpha receptor activity plus slight enhancement of beta activity may be a compensatory mechanism to reduce the elevated blood pressure. Other compensatory mechanisms may be operative in the SHR; for example, the biosynthesis and release of catecholamines are reduced (*7*). However, since the tissue response to a nonspecific stimulant, such as KCl, is depressed, it would seem that in the SHR the responsiveness of the muscle itself to contract is reduced rather than there being a specific modi- n of the alpha and beta receptors.

d *et al.* (*8*) have shown an interaction between calcium and stimulating agents, such as epinephrine, and relaxing drugs, such as isoproterenol. The calcium content of vascular smooth muscle from SHR may perhaps be altered so that the effect of a stimulating drug is reduced while that of the relaxing agent is enhanced.

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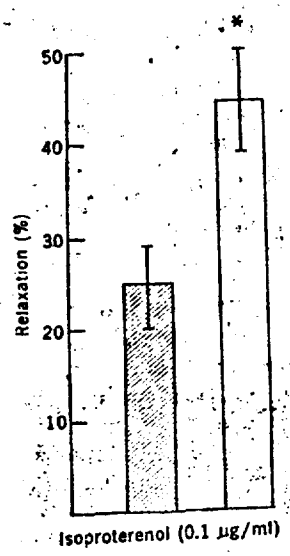

Fig. 2. A comparison of the relaxant beta responses of thoracic aortic strips from four normotensive and seven spontaneously rtensive rats to isoproterenol. The c tissue was made to contract with g of serotonin per milliliter. Data given s means ± S.E. The asterisk indicates a statistically significant difference, $P < .05$. Shaded bar, normotensive animals; open ar, SHR.

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Sodium uptake in various diets and its relation to salt fever in infants

by

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The specific function of sodium has not so far been precisely defined, either in biochemistry or in physiology. It is, however, a well known fact and one which is of great practical importance, that sodium is the leading cation of the extracellular fluid and the regulation of its concentration in this fluid in adults is among the most precise of homeostatic mechanisms. With regard to the regulation of the uptake of electrolytes, sodium occupies a special position in that, as has been stressed by Bunge, it is added to the human diet as the only inorganic salt in the form of sodium chloride. The total quantity of sodium ingested by man in one day varies considerably. It depends largely upon individual and geographic dietary peculiarities (Abbot; Ashe and Mosenthal; Dahl; Hawks, Bray et al; Randall; Talbot, Crawford et al.).

In view of the fact that the clinical picture of sodium deficiency in the diet has not so far been described, we do not have an exact picture of the optimum or the minimum sodium requirement of the human organism in different age segments. However, there are an increasing number of data on the possible connection between prolonged ingestion of large quantities of NaCl and pathologic alterations in the cardiovascular system and hypertension (Dahl, Heine et al.; Kerpel-Fronius; Smirk). In infants, the osmoregulating function of whose kidneys is not yet fully developed and who are thus not capable of maintaining water and salt homeostasis adequately, even brief excess ingestion of salt leads to salt fever, as well as to other effects (Martinek, Stanincova et al., Rietschel). It is known that all artificial baby food preparations, including various types of humanized milk, contain more sodium than mother's milk (Berger; Macy, Kelly et al.). The need to establish the optimal sodium content in food is thus obvious in the case of infant foods.

In our studies we have attempted 1. to establish differences in the sodium intake of breast-fed and artifically fed infants according to normal Czechoslovakian standards, 2. to determine the minimum sodium requirement and 3. to establish the relationship between the various forms of Na intake and the development of salt fever. Some of the results have already been published elsewhere as a preliminary report (Janovsky, Martinek et al.).

Methods

1. Determination of Na intake with various infant diets

The concentration of Na was determined in mature mother's milk, butter milk (Lakton), dried cow's milk (Sunar), as well as in a Czechoslovakian preparation recently developed and in a vegetable soup. Ten samples were used for each determination. Next the Na intake per 1 kg body weight per 24 hours was calculated in infants from the 15th day of life (after compensation of loss of birth weight) until the end of the 5th month after birth, with an average milk intake of 150 ml/kg body weight/24 hours. The replacement of a portion of dry cow's milk by vegetable soup was taken as being equivalent to the ingestion of 150 ml/kg/day of the mixture in question.

2. Calculation of the necessary Na retention in infants

The Na content was estimated in 15 day-old to 5 months old infants from the Na value for an average body weight of 1 kg and the required Na retention was calculated on the basis of the average weight increases per day. The absolute weight increases were calculated on the basis of the body weight of Prague boys and girls given in the anthropometric study of Syrovatka and Sobova. The average daily weight increases in the group studied was 38.5 g from the time of compensation of the birth weight loss until the end of the first month, 36.5 g during the 2nd month, 29.4 g in the 3rd month, 27.5 g in the 4th month and 22.6 g in the 5th month. One kg body

mass of an infant contains 70-80 mEq Na (Forbes). The calculation for all age groups studied was based on a mean value of 75 mEq; for every weight increase, depending on its size, the corresponding proportion of this value was postulated.

3. Chemical determination of the Na concentration

On the one hand we used milk ash (dissolved in 10% HNO_3), and on the other urine (diluted 1:10-1:50) and worked with a Zeiss III flame photometer, with mixed standards, containing K as well as Na. For 24 hour urines we used only full-term boys and we determined the urine concentration of Na in 49 breast-fed infants aged 15 days to 3 months, in 49 artificially fed infants aged 15 days to 4 1/2 months, and in 15 infants fed with humainzed milk aged 15 days to 3 months.

4. Control of salt fever

The onset of the fever was followed in two groups of full-term, healthy infants, 20 of them breast-fed, weighing 3748±690 g and 15 fed with dried butter milk weighing 4004± 557 g. The infants in both groups were 15-42 days old. The quantity of....

pp. 58 & 59 of text missing.

[The quantity of] milk ingested was determined on 3 control days: in breast-fed infants by weighing weight differences, and in artificially fed infants by making readings of the bottle contents with an accuracy of ±5 ml. Based on the average quantity of milk consumed on the 3 control days during single feedings (both breast-fed and artificially fed infants were fed six times per day) we added 80 mg NaCl per 100 ml milk on theday of experimentation in the following manner: shortly before nursing, the corresponding quantity of NaCl dissolved in a few ml breast milk was administered into the stomach by means of a stomach tube. In the case of the artificially fed infants the entire quantity of salt was introduced in this manner in butter milk. The rectal temperature was measured before each feeding and at 9 a.m. and 9 p.m. the infants were weighed with an accuracy of ±10 g. With rectal temperatures of 38.5°C and more we immediately stopped the administration of NaCl and gave the infants suitable quantities of tea. Subsequently the temperature invariably dropped again spontaneously within a few hours.

The statistical evaluation took place with the Student Test. The values given after the ±sign show the standard deviation.

Results

Breast milk contains 7.0±1.9 mEq Na/l, dried butter milk and dried cow's milk 22.0±40 and humanized milk 11.0±1.2 mEq. In the vegetable soup the concentration is 80.0±25.0 mEq Na/l. If from the second half of the first month of its life to the end of the 5th month the infant consumes an average of 150 ml/kg

body weight/24 hours, it ingests 1.1 mEq Na/kg/24 hours with breast milk. According to Czechoslovakian standards for artificial feeding with dried butter milk, the artificially fed infant consumes 2.9 mEq Na, with 2/3 cow's milk 1.92 mEq Na, with 3.4 cow's milk 2.22 mEq and with whole cow's milk 2.9 mEq Na. With vegetable soup (instead of a portion of whole milk) the infant ingests 4.7 mEq Na/kg body weight/24 hours. With humanized milk the infants ingest 1.6 mEq Na/kg body weight/24 hours (Fig. 1).

Sodium retention

In infants aged 15 days to the end of the 5th month the sodium retention required to cover their needs for growth increase gradually drops from 0.68 mEq Na to 0.22 mEq Na/kg/24 hours (Fig. 1). For this reason the percentage of retained sodium is also reduced. Its level is determined by the Na concentration of the different types of food (Fig. 1).

The Na concentration in the urine and the sodium clearance are shown in the table for breast-fed infants and those fed artificially with dried buttermilk, whole cow's milk or humanized milk. The values of the urinary concentration established for breast-fed infants and those fed with non-adapted cow's milk preparations are in accord with the observations described in so far that the artificially fed infants excrete more electrolytes through the kidneys than the breast-fed infants (Brock). Our observation that the Na clearance is low in infants is basically in agreement with those of McCance and Young. From the standpoint

of the involvement of the kidneys in the maintenance of homeostasis with different types of nutrition, humanized milk is closest to breast milk. This is indicated by the Na concentration in the urine as well as by the Na clearance value (Table).

Salt fever

By addition of NaCl the ingestion of Na in breast-fed infants was increased to 20.2 mEq/kg body weight/24 hours (Fig.1). Recalculated, this amounts to 464.4 mg Na [contained in 1,108 mg NaCl].

Fig. 1. Average Na ingestion in mEq/kg body weight/24 hours in infants fed with various types of food from the 15th day of life until the 5th month and in adults. Striated area shows the retained sodium in mEq/kg body weight/24 hours. Key: 1. Na ingestion in mEq/kg body weight/24 hours. 2. Month. 3. Whole cow's milk. 4. Vegetable soup. 5. Buttermilk. 6. Humanized milk. 7. Breast-fed infants. 8. Average Na ingestion. 9. Minimum Na ingestion. 10 Adults.

Table

Na concentration in the urine (U_{Na}) and Na clearance (C_{Na}) in infants of different ages and on different diets. Key: 1. Age. 2. Number. 3. Type of food. 4. Days. 5. Months. 6. Breast-fed. 7. Fat-free buttermilk. 8. Dried cow's milk. 9. 2/3 cow's milk. 10. Humanized milk. 11. Whole cow's milk.

... contain 1.108 mg NaCl. The average maximum body temperature reached was 37.5±0.4° in breast-fed infants (Fig. 2). The increase compared to the average maxima for the control days was statistically significant ($p < 0.01$). In the group of infants fed with dried butter milk the administration of NaCl had to be discontinued earlier than in the breast-fed infants due to the rise in temperature, as a result of which the average Na uptake reached was 10.0 mEq/body weight/24 hours, corresponding to 230 mg Na, contained in 584.2 mg NaCl. The difference in the Na uptake between the two groups is statistically significant ($p < 0.01$). The average maximum temperature in the artificially fed infants was 38.4±0.6°. This value is significantly higher ($p < 0.003$) than in the breast-fed group.

Discussion

The success of an experiment to determine the optimal sodium uptake values, and to assess the significance of any Na ingestion beyond that, lies in the accuracy of data relating to the requirement of the growing organism for Na retention. In the calculated retention value an error must be taken into account, since there are few basic systematic data on the Na content in the body mass in various age segments of infants. The retention calculated only for the weight increase probably represents the minimum value, since the Na incorporated into the morphologically maturing tissue is not included. For this

reason the data obtained by us must be regarded as approximate values. They are, nevertheless, of decisive importance. Their tendency to change during postnatal ontogenesis is in accord with the well-known phenomenon of the drop in Na content of the body during the first months after birth. This can be attributed to the reduction of the extracellular fluid space (Friis-Hansen) and the reduction of the sodium space (Fellers, Barnett et al.). A similar dependence between the Na content and age was found by Jelinek in the postnatal development of rats. The theoretical calculation of sodium retention needs should correspond to the analytical calculations. It appears, however, that even the most precise analytical studies, together with the determination of renal and extrarenal Na losses, particularly in artificially fed infants, yield very high values (Klinke; Swanson; Swanson and Iob; Hoffman, Parmelee et al.). This may be due to the fact that even a highly refined method of mineral analysis in an experiment to determine complete extrarenal losses is subject to limitations of a technical nature.

Still, even the limited accuracy of our observations of Na retention allows us to make use of the fact that the infantile period is the only stage in human life in which the organism regulates the ingestion of food, minerals and water without artificial intervention and individual habits, in such a way that the composition of mother's milk completely covers Na requirements. This conclusion is not only based on data regarding Na mineral requirements, but on the fact that no clinical manifestation of a pure nutritional Na deficiency is

known. Yet in all probability such manifestations should exist, since the reduced ingestion of Na, as has been demonstrated in animal experiments, gives rise to an inhibition of growth (Orent-Keiles, Robinson, et al.) and a sodium deficiency in infants represents the most common form of disturbance in the salt and water balance (Polacek; Kerpel-Fronius). The function of the nephron in the infantile kidney is apparently adjusted to the relatively low uptake of Na from mother's milk and the necessary retention is assured by the fact that the sodium retained from the ingested quantity maintains its high percentage. It is apparent from Fig. 1 that this percentage drops from 60 to 20% according to the age of the infant. This high proportion corresponds to the urinary excretion of a low Na concentration and the low Na clearance value (Table). Since mother's milk guarantees a sufficient Na supply for the infant, any higher ingestion can be regarded as superfluous. Infants fed artificially with dried butter milk or dried whole cow's milk ingested 2-6 times more Na than breast-fed infants; infants fed with humanized milk ingested only 1.4 times more than breast-fed infants (Fig. 1). Particular attention should be paid to the observation that when a portion of whole milk was replaced by vegetable soup, the ingestion of sodium was 4.3 times higher than in the breast-fed infants. No doubt this value is extremely variable, since there are no uniform determinations for the preparation of infant vegetable soups. Czechoslovakian specifications (Kolektiv Autoru) recommend not

adding salt to the vegetable soup, but in pediatric practice medical instructions on the subject are variable. Our investigations have revealed that in some institutions the soup was salted.

Comparison of the Na balance of infants and adults is also useful. It is apparent from many studies that the ingestion of sodium varies considerably in adults (Forbes). Based on the works cited above we have calculated a mean ingestion of 2.21 mEq Na/kg body weight/24 hours (Fig. 1). Assuming theoretically that the ingestion of Na were replaced by sodium chloride, its quantity in an adult weighing 70 kg would be 9.03 g NaCl. According to Abbot, the minimum ingestion of Na is 0.99 mEq Na/kg body weight/24 hours, or 4.03 g NaCl for an adult weighing 70 kg. Even if this is merely an orientating value, it is nevertheless striking that it is close to the average ingestion value of 1.1 mEq Na/kg body weight/24 hours observed in breast-fed infants (Fig. 1). In his monograph, Mach states that a daily ingestion of 3 g NaCl should be sufficient for an adult. Therefore we would seem to be justified in assuming that in adult humans (in certain geographical locations) the average ingestion of Na is excessive. Yet the infant fed with dried butter milk or dried whole cow's milk ingests relatively even more Na than the average consumption of the human adult. The salting of vegetable soup leads even to more than double the Na ingestion by infants compared to the average ingestion of the adult human, which means that

this represents the highest Na ingestion per kg body weight in the subject's entire life.

Compared to the kidneys of breast-fed infants, those of artificially fed infants are required to excrete urine of two to three times higher concentration. For this reason the portion of ingested Na which is retained is lower in infants on an artificial diet than in the breast-fed infants and the Na clearance is simultaneously higher (Table). Thus the nephron must balance the intrarenal Na on a different level in artificially fed infants than in breast-fed infants. The question arises whether the high uptake of Na which takes place for a few months after birth can influence renal function as the most important effector of osmoregulation, or may even be linked in some way to pathologic phenomena that might be manifested at a later stage of development. Clearcut replies to these questions have not yet been found, nor are they to be expected in the foreseeable future. There are, however, many experimental results, obtained mostly on rats, according to which early weaning has a marked influence on the further development of animals and furthermore increases their sensitivity to experimentally induced pathologic states (Krecek; Kraus, Andel et al.; Kraus and Popp; Kubat, Fabry et al., Novakova, Koldovsky et al., Novakova, Koldovsky et al.). These findings place particular emphasis on the question of to what extent artificial infant diets, like the early weaning of rats, could endanger the further development of the child.

To be sure, not only the sodium balance can be cited as criterion of the suitability of an artificial diet. It does, however, seem justified to assume that a high uptake of sodium together with a high protein consumption may be the reason for maintaining homeostasis of water and osmotically active substances by means of osmotic diuresis (Martinek, Janovsky et al.). The humanized milk preparation used by us is accompanied by a low Na and protein consumption.

The high Na uptake resulting from dry butter milk or non-adapted cow's milk is not only of theoretical importance, since in the artificially fed child salt fever can be elicited by lower NaCl quantities than in the breast-fed child. This type of fever is apparently a feature of thirst exsiccosis (Rietschel; Kerpel-Fronius) but its origin has not been clearly established. In considering the possible origin of the increased "sensitivity" of artificially fed infants to added NaCl the following factors should be considered above all: 1. The average concentrating ability of the kidneys of newborns and infants is lower compared to that of the adult organism (König and Lederer; Heller; Polacek, Vocel et al.). 2. The urinary osmolarity is higher in artificially fed infants than in breast-fed infants. 3. The capacity of a child's kidney suddenly to excrete excess quantities of ingested electrolytes is lower in artificially fed infants. This could be the reason for the more rapid onset of hyperelectrolytemia, which may be accompanied by salt fever. Also it is possible that prolonged uptake of osmotically

active substances and their sources with an artificial diet, leading to some degree of "supermineralization", could promote a freer development of thirst exsiccosis upon increased NaCl uptake. This is manifested by the variable nutrition offered by the ingestion of concentrated milk, with which dehydration is slowest among breast-fed children. If dehydration is produced by the administration of concentrated humanized milk, it is also slower than upon ingestion of concentrated butter milk or concentrated whole cow's milk (Janovsky, Martinek et al.).

The complex problems of an adequate artificial diet can only partly be solved by the study of the sodium balance. The present results nevertheless prove that in efforts to "humanize" milk the sodium content must be taken into account. This standpoint would seem to be a logical part of any effort aimed at an early prevention of possible later consequences of an unsuitable artificial diet.

Summary

The amount of Na necessary to cover the sodium supply upon increased body weight drops from 0.68 mEq/kg body weight / 24 hours in 15 day old infants to 0.22 mEq in 5 months old infants. This Na requirement is fully covered by the uptake of Na from breast milk owing to the high positive Na balance assured by the kidneys. In artificially fed infants the Na uptake per kg body weight is relatively higher than the average uptake

in adults. Upon salting vegetable soup the Na uptake probably reaches the highest level in the subject's life. Upon further increase of Na uptake, salt fever develops more rapdily in infants fed artificially than in breast-fed infants. Compared to non-humanized cow's milk preparations, the humanized milk preparation studied proved to be an adequate substitute for breast milk with regard to the Na balance. The possible consequences of an excess Na uptake by artificially fed infants are discussed.

Figures

1. Missing

2. Relationship between body temperature and Na ingestion in a group of 20 breast-fed and 15 artificially fed infants aged 15-42 days. Individual columns: Days of observation, white columns: normal NaCl uptake, striated columns: increased NaCl uptake. Key: 1. Body temperature. 2. Na uptake/kg body weight/24 hours. 3. breast-fed. 4. artificially fed.

Bibliography

See pp. 65-66.

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Die Natriumaufnahme bei verschiedener Ernährung und ihre Beziehung zum Salzfieber bei kleinen Säuglingen

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Die spezifische Funktion des Natriums ist bisher weder in der Biochemie noch in der Physiologie genau definiert worden. Es ist aber eine gut bekannte und in praktischer Hinsicht sehr bedeutsame Tatsache, daß das Natrium das führende Kation der extracellulären Flüssigkeit ist und die Regulierung seiner Konzentration in dieser Flüssigkeit bei Erwachsenen zu den genauesten homeostatischen Mechanismen gehört. In bezug auf die Regulierung der Elektrolyteneinnahme nimmt das Natrium insofern eine besondere Stellung ein, als es — wie schon BUNGE betonte — in Form von Kochsalz als einziges anorganisches Salz der menschlichen Nahrung zugesetzt wird. Die Gesamtmenge des in einem Tag eingenommenen Natriums ist beim Menschen sehr verschieden. Sie ist weitaus von individuellen und geographisch bedingten dietären Besonderheiten abhängig (ABBOT; ASHE u. MOSENTHAL; DAHL; HAWKS, BRAY u. Mitarb.; RANDALL; TALBOT, CRAWFORD u. Mitarb.). Angesichts der Tatsache, daß das klinische Bild der Nutritionskarenz des Natriums noch nicht beschrieben wurde, gibt es auch keine genauen Vorstellungen weder über den optimalen noch den minimalen Natriumbedarf des menschlichen Organismus in seinen einzelnen Altersstufen. Es mehren sich jedoch Angaben über mögliche Zusammenhänge einer langdauernden hohen Einnahme von NaCl mit den pathologischen Veränderungen des kardiovasculären Systems sowie mit der Hypertonie (DAHL, HEINE u. Mitarb.; KERPEL-FRONIUS; SMIRK). Beim kleinen Säugling, dessen Nieren ihre osmoregulatorische Funktion noch nicht vollkommen ausgebildet haben, und somit nicht in der Lage sind, die Wasser- und Salzhomeostase im erforderlichen Ausmaß aufrecht zu erhalten, führt schon eine kurzfristige, übermäßige Salzeinnahme außer anderen Auswirkungen zu Salzfieber (MARTÍNEK, STANINCOVÁ u. Mitarb.; RIETSCHEL). Es ist bekannt, daß alle Präparate der künstlichen Säuglingsernährung einschließlich verschiedener Sorten humanisierter Milch mehr Natrium enthalten als die Brustmilch (BERGER; MACY, KELLY u. Mitarb.). Die Notwendigkeit, sich

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über den optimalen Natri
wird somit schon bei der

In unserer Arbeit unt
der Na-Einnahme bei d
tschechoslowakischen No
2. den minimalen Bedarf
zwischen den verschiede
Entstehen des Salzfiebers
reits anderwärts als vorlä
TÍNEK u. Mitarb.).

1. Ermittlung der Na-Ein
die Konzentration von Natr
in Kuhtrockenmilch (Sunar
slowakischen Präparat und i
Untersuchungen wurden jew
lingen ab 15. Lebenstag (na
des 5. Monats nach Geburt.
durchschnittlicher Milchzuful
Der Ersatz von einer Kuh
Einnahme von 150 ml/kg ent

2. Die Berechnung der not
Na-Gehalt in 15tägigen bis
mittleren Körpergewichts ab
Grund durchschnittlichen Ge
Gewichtszunahmen wurden a
SYROVÁTKA u. ŠOBOVÁ angeg
chen berechnet. Die durchsch
der untersuchten Gruppe vo
verlustes bis Ende des 1. Leb
3. Monat 29,4 g, im 4. Monat
kleinen Säuglings enthält 70
allen untersuchten Altersgrup
jeweiligen Gewichtszuwachs w
dieses Wertes vorausgesetzt.

3. Die chemische Bestimm
Asche der Milch (aufgelöst in 1
und arbeiteten mit einem Zei
Standarten, die außer Na au
24stündigem Harn benutzten
konzentration von Na bei 49 g
naten, bei 49 künstlich ernährt
und bei 15 mit humanisierter
bis 3 Monaten.

4. Kontrolle des Salzfieb
Gruppen reifer, gesunder Säu
und bei 15 mit Trockenbutte
Die Säuglinge beider Gruppe

3152

über den optimalen Natriumgehalt der Nahrung Klarheit zu verschaffen, wird somit schon bei der Säuglingsernährung offenbar.

In unserer Arbeit unternahmen wir den Versuch, 1. Unterschiede in der Na-Einnahme bei der Ernährung von Brustkindern sowie gemäß tschechoslowakischen Normen künstlich ernährten Kindern festzustellen, 2. den minimalen Bedarf von Natrium zu ermitteln und 3. die Beziehung zwischen den verschiedenartigen Formen der Na-Einnahme und dem Entstehen des Salzfiebers aufzuklären. Ein Teil der Ergebnisse wurde bereits anderwärts als vorläufige Mitteilung veröffentlicht (JANOVSKÝ, MARTÍNEK u. Mitarb.).

Methoden

1. Ermittlung der Na-Einnahme bei verschiedener Säuglingsernährung. Es wurde die Konzentration von Natrium in reifer Muttermilch, in Buttermilch (Lakton), in Kuhtrockenmilch (Sunar), ferner in einem zur Zeit entwickelten tschechoslowakischen Präparat und in einer Gemüsesuppe festgestellt. Für die einzelnen Untersuchungen wurden jeweils zehn Proben benutzt. Daraufhin wurde bei Säuglingen ab 15. Lebenstag (nach Ausgleich des Geburtsgewichtsverlustes) bis Ende des 5. Monats nach Geburt, die Na-Einnahme/1 kg Körpergewicht/24 Std bei durchschnittlicher Milchzufuhr von 150 ml/1 kg Körpergewicht/24 Std berechnet. Der Ersatz von einer Kuhtrockenmilchportion durch Gemüsesuppe wurde als Einnahme von 150 ml/kg entsprechender Mischung pro Tag angerechnet.

2. Die Berechnung der notwendigen Na-Retention bei Säuglingen. Es wurden der Na-Gehalt in 15tägigen bis 5monatigen Säuglingen aus dem Na-Wert für 1 kg mittleren Körpergewichts abgeschätzt und der Bedarf an retiniertem Natrium auf Grund durchschnittlichen Gewichtszunahmen pro Tag berechnet. Die absoluten Gewichtszunahmen wurden auf Grund der in der anthropometrischen Studie von SYNOVÁTKA u. ŠOBOVÁ angegebenen Körpergewichte der Prager Jungen und Mädchen berechnet. Die durchschnittlichen täglichen Gewichtszunahmen betrugen bei der untersuchten Gruppe vom Zeitpunkt des Ausgleichs des Geburtsgewichtsverlustes bis Ende des 1. Lebensmonats 38,5 g, im Laufe des 2. Monats 36,5 g, im 3. Monat 29,4 g, im 4. Monat 27,5 g und im 5. Monat 22,6 g. 1 kg Körpermasse des kleinen Säuglings enthält 70—80 mEq Na (FORBES). Der Kalkulation wurde bei allen untersuchten Altersgruppen der Mittelwert von 75 mEq zugrundegelegt; im jeweiligen Gewichtszuwachs wurde gemäß seiner Größe der entsprechende Anteil dieses Wertes vorausgesetzt.

3. Die chemische Bestimmung der Na-Konzentration. Wir nahmen einerseits Asche der Milch (aufgelöst in 10% HNO_3), andererseits Harn (1:10—1:50 verdünnt) und arbeiteten mit einem Zeiß III-Flammenphotometer, und zwar mit gemischten Standarten, die außer Na auch K enthielten. Für die Entnahme von komplettem 24stündigem Harn benutzten wir nur reife Jungen und bestimmten die Harnkonzentration von Na bei 49 gestillten Säuglingen im Alter von 15 Tagen bis 3 Monaten, bei 49 künstlich ernährten Säuglingen im Alter von 15 Tagen bis $4^1/_2$ Monaten und bei 15 mit humanisierter Milch ernährten Säuglingen im Alter von 15 Tagen bis 3 Monaten.

4. Kontrolle des Salzfiebers. Wir verfolgten den Beginn des Fiebers bei zwei Gruppen reifer, gesunder Säuglinge, und zwar bei 20 gestillten von 3748 ± 690 g, und bei 15 mit Trockenbuttermilch ernährten von 4002 ± 557 g Körpergewicht. Die Säuglinge beider Gruppen waren 15—42 Tage alt. Die Menge der von ihnen

ausgetrunkenen Milch ermittelten wir an 3 Kontrolltagen: bei gestillten Säuglingen durch Abwägen der Gewichtsdifferenz, bei künstlich ernährten mittels ± 5 ml genauen Ablesen des Flascheninhaltes. Entsprechend der bei den einzelnen Fütterungen in 3 Kontrolltagen ausgetrunkenen mittleren Milchmenge (gestillte und künstlich ernährte tranken sechsmal täglich) setzten wir am Versuchstag auf jede 100 ml Milch 800 mg NaCl in folgender Weise zu: kurz vor dem Stillen reichten wir die entsprechende, in einigen Milliliter Muttermilch aufgelöste Menge NaCl mittels Sonde in den Magen. Bei den künstlich ernährten wurde auf diese Weise die ganze entsprechende Portion Buttermilch zugeführt. Vor jeder Fütterung wurden Rectaltemperaturen gemessen und um 9.00 und 21.00 Uhr wurden die Säuglinge mit einer Genauigkeit von ± 10 g gewogen. Bei Rectaltemperatur von 38,5°C und mehr stellten wir die Zufuhr von NaCl sofort ein und boten den Säuglingen ausreichende Mengen Tee an. Die Temperatur sank in der Folge immer spontan binnen einiger Stunden.

Die statistische Auswertung führten wir mit Students Test durch. Die hinter den Werten angeführten Zeichen ± zeigen Abweichungen vom Standard an.

Ergebnisse

Einnahme von Na bei verschiedener Ernährung. Die Brustmilch enthält 7,0 ± 1,9, die Trockenbuttermilch und Kuhtrockenmilch 22,0 ± 4,0 und humanisierte Milch 11,0 ± 1,2 mEq Na/l. In der Gemüsesuppe beträgt die Konzentration 80,0 ± 25,0 mEq Na/l. Trinkt der Säugling ab der zweiten Hälfte des 1. Monats bis Ende des 5. Monats durchschnittlich 150 ml/kg Körpergewicht/24 Std, so erhält er durch die Brustmilch 1,1 mEq Na/kg/24 Std. Der künstlich ernährte Säugling nimmt gemäß tschechoslowakischen Normen für künstliche Ernährung mit der Trokkenbuttermilch 2,9 mEq Na, mit der Zweidrittelkuhmilch 1,92 mEq Na, mit der Dreiviertelkuhmilch 2,22 mEq Na, mit der vollen Kuhmilch 2,9 mEq Na ein. Mit der Gemüsesuppe (anstatt einer Portion Vollmilch) erhält er 4,7 mEq Na/kg Körpergewicht/24 Std. Mit humanisierter Milch werden dem Säugling 1,6 mEq Na/kg Körpergewicht/24 Std zugeführt (Abb. 1).

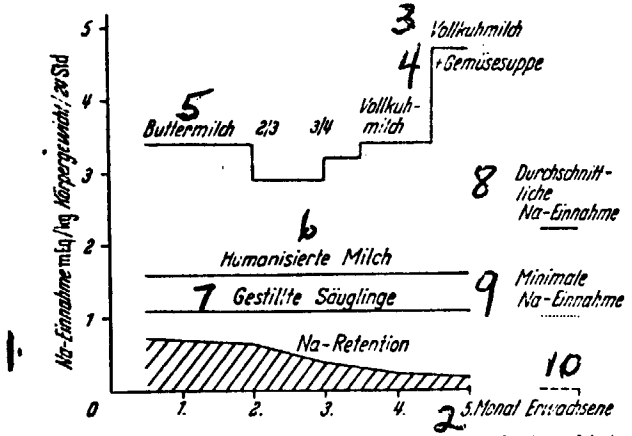

Abb. 1. Durchschnittliche Na-Einnahme in mEq/kg Körpergewicht/24 Std bei verschiedenartig ernährten Säuglingen vom 15. Tag bis zum 5. Lebensmonat und bei Erwachsenen. Gestrichelte Fläche bedeutet retiniertes Natrium in mEq/kg Körpergewicht/24 Std

Natriumretention. Bei Säuglingen im Alter von 15 Tagen bis Ende des 5. Monats sinkt der Bedarf an retiniertem Natrium, das für die Deckung seines Anteils im Gewichtszuwachs erforderlich ist, stufenweise von 0,68 mEq Na auf 0,22 mEq Na/kg Körpergewicht/24 Std (Abb. 1). Aus diesem Grunde geht auch der Prozentsatz von retiniertem Natrium zurück. Seine Höhe ist durch die Na-Konzentration der einzelnen Nahrungstypen bedingt (Abb. 1).

Die Konzentration von Na im Harn und die Natrium-Clearance ist bei gestillten, bei künstlich mit Trockenbuttermilch, Kuhvollmilch oder humanisierter Milch ernährten Säuglingen in der Tabelle angeführt. Die

Tabelle. *Na-Konzentration im Harn (U_{Na}) und Na-Clearance (C_{Na}) bei Säuglingen verschiedenen Alters und Ernährung*

Alter	Zahl	Ernährung	U_{Na}	SD	C_{Na}	SD
15—30 Tage	24	gestillt	10,2	5,7	0,113	0,0017
15—30 Tage	13	Buttermilch, entfettete Trockenkuhmilch	20,7	7,6	0,224	0,0017
1—3 Monate	25	gestillt	7,4	5,2	0,098	0,0019
1—3 Monate	20	Zweidrittel Kuhmilch	21,9	8,7	0,286	0,0026
1—3 Monate	15	humanisierte Milch	13,5	7,7	0,152	0,0042
3,5—5 Monate		Vollkuhmilch	27,1	11,6	0,305	0,0039

festgestellten Werte der Harnkonzentration bei gestillten sowie mit nichtadaptierten Kuhmilchpräparaten ernährten Säuglingen stimmen mit den schon beschriebenen Beobachtungen insofern überein, daß die künstlich ernährten Säuglinge mehr Elektrolyte durch die Nieren ausscheiden als die gestillten (Brock). Auch unsere Feststellung, daß die Na-Clearance bei den Säuglingen niedrig ist, stimmt im Grunde mit den Beobachtungen von McCance u. Young überein. Vom Standpunkt der Anteilnahme der Nieren an der Aufrechterhaltung der Homeostase bei verschiedenen Nahrungstypen steht das Präparat der humanisierten Milch der Brustmilch am nahesten. Darauf weisen die Na-Konzentration im Harn und auch der Clearancewert von Na hin (Tabelle).

Das Salzfieber. Durch Zugabe von NaCl wurde bei der Gruppe der gestillten Säuglinge die Einnahme von Na auf 20,2 mEq/kg Körpergewicht pro 24 Std erhöht (Abb. 2). Umgerechnet sind es 464,4 mg Na, die in

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1,108 mg NaCl enthalten sind. Das Mittel der erreichten Körpertemperaturmaxima betrug bei den gestillten Säuglingen 37,5 ± 0,4°C (Abb. 2). Die Erhöhung ist gegenüber dem Maximamittel der Kontrolltage statistisch signifikant ($p < 0{,}01$). Bei der Gruppe der mit Trockenbuttermilch ernährten Säuglinge mußten infolge des Körpertemperaturanstieges die NaCl-Zugaben früher unterbrochen werden als bei den gestillten Säuglingen, weshalb die erreichte mittlere Na-Einnahme 10,0 mEq/kg Körpergewicht/24 Std betrug; dies entspricht 230 mg Na, enthalten in 584,2 mg NaCl. Die Differenz in der Na-Einnahme zwischen beiden Gruppen ist

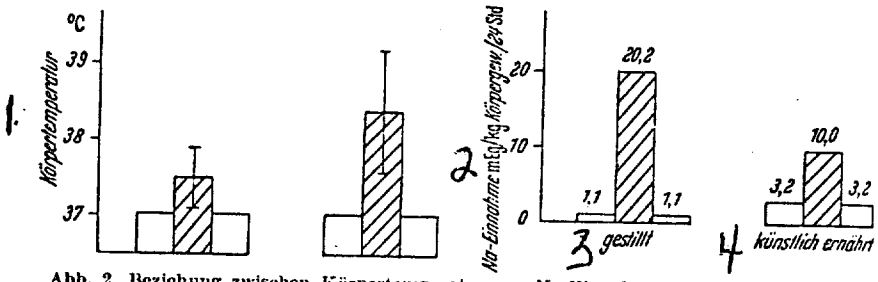

Abb. 2. Beziehung zwischen Körpertemperatur zur Na-Einnahme bei einer Gruppe von 20 gestillten und 15 künstlich ernährten Säuglingen, 15—42 Tage alt. Einzige Säulen: Beobachtungstage, weiße Säulen: normale NaCl-Einnahme, gestrichelte Säulen: erhöhte NaCl-Einnahme

statistisch signifikant ($p < 0{,}01$). Die mittlere maximale Temperatur betrug bei den künstlich ernährten Säuglingen 38,4 ± 0,6°C. Dieser Wert ist signifikant höher ($p < 0{,}003$) als bei der gestillten Gruppe.

Besprechung der Ergebnisse

Der Erfolg eines Versuches, den optimalen Wert der Natriumeinnahme zu bestimmen und die Bedeutung einer darüber hinausreichenden Na-Zufuhr zu bewerten, ist durch die Genauigkeit der Angaben über den Bedarf des wachsenden Organismus an retiniertem Na bedingt. Beim kalkulierten Retentionswert muß eine Fehlerbelastung in Kauf genommen werden, da nur wenig systematische Angaben über den Gehalt von Na in der Körpermasse bei verschiedenen Altersgruppen der Säuglinge zugrundeliegen. Die nur für die Gewichtszunahme berechnete Retention dürfte den minimalen Wert darstellen, da der Anteil des in das morphologisch ausreifende Gewebe inkorporierte Na nicht erfaßt wird. Aus diesem Grunde müssen die von uns gewonnenen Angaben als approximativ betrachtet werden. Nichtsdestoweniger sind sie von maßgebender Bedeutung. Die Tendenz ihrer Veränderungen innerhalb der postnatalen Ontogenese entspricht dem bekannten Phänomen der Erniedrigung des Körpergehalts an Na in den ersten Monaten nach der Geburt. Das ist auf die Verringerung des Raumes der extracellulären Flüssigkeit (FRIIS-HANSEN) und jene des Natriumraumes (FELLERS, BARNETT u. Mitarb.)

urückzuführen. Ähnliche
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GER u. Mitarb.; ROMINGER
MAN, PARMELEE u. Mitarb.
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zurückzuführen. Ähnliche Abhängigkeit von Na-Gehalt und Alter fand ZELINEK in der postnatalen Entwicklung bei Ratten. Die theoretischen Berechnungen des Retentionsanspruches für Na sollten mit den Bilanzkalkulationen übereinstimmen. Es scheint jedoch, daß auch genauest durchgeführte Bilanzuntersuchungen, verbunden mit Ermittlung der renalen sowie extrarenalen Na-Verlusten, insbesondere bei künstlich ernährten Säuglingen sehr hohe Werte liefern (KLINKE; ROMINGER, BERG u. Mitarb.; ROMINGER u. MEYER; SWANSON; SWANSON u. IOB; HOFFMAN, PARMELEE u. Mitarb.). Das könnte damit zusammenhängen, daß selbst eine höchst verfeinerte Methodik der Mineralbilanzierung bei einem Versuch komplette extrarenale Verluste zu bestimmen, Grenzen technischen Charakters ausgesetzt ist.

Doch auch die beschränkte Genauigkeit unserer Beobachtungen der Na-Retention läßt die Möglichkeit offen, aus der Tatsache Nutzen zu ziehen, daß die Säuglingsperiode die einzige Etappe im Leben des Menschen ist, in welcher der Organismus ohne artefizielle Eingriffe und individuelle Angewohnheiten die Zufuhr von Nährstoffen, Mineralien und Wasser derart reguliert, daß die Zusammensetzung der Muttermilch den Bedarf an Na vollkommen decken kann. Diese Folgerung stützt sich nicht nur auf die Angaben über den Mineralbedarf an Na, sondern auch auf die Tatsache, daß keine klinische Offenbarung einer reinen Nutritionsdefizienz von Na bekannt ist. Solche müßten aller Wahrscheinlichkeit nach erscheinen, da die erniedrigte Einnahme von Na, wie am Tierexperiment nachgewiesen, Wachstumsinhibition hervorruft (ORENT-KEILES, ROBINSON u. Mitarb.) und Na-Defizit bei Säuglingen den üblichsten Typus einer Wasser- und Salzhaushaltsstörung darstellt (POLÁČEK; KERPEL-FRONIUS). Die Funktion des Nephrons ist in der Säuglingsniere offensichtlich der relativ niedrigen Einnahme von Na in der Brustmilch angepaßt und die erforderliche Retention dadurch gesichert, daß das von der eingenommenen Menge retinierte Natrium seinen hohen Prozentanteil aufrecht erhält. Aus der Abb. 1 geht hervor, daß dieser Prozentanteil je nach Alter des Säuglings von 60 auf 20% herabsinkt. Diesem hohen Anteil entspricht die Harnausscheidung von niedriger Na-Konzentration und der niedrigere Wert der Na-Clearance (Tabelle). Da die Brustmilch den Säuglingen eine genügende Zufuhr von Na garantiert, kann jede höhere Einnahme als überflüssig erachtet werden. Künstlich mit Trockenbuttermilch oder getrockneter Kuhvollmilch ernährte Säuglinge empfangen 2,6mal mehr Na als gestillte Säuglinge; mit humanisierter Milch ernährte Säuglinge nur 1,4mal mehr Na als die gestillten (Abb. 1). Besondere Aufmerksamkeit gebührt der Feststellung, daß bei Ersatz einer Portion Vollmilch durch Gemüsesuppe die Natriumeinnahme 4,3mal höher ist als die bei gestillten Säuglingen. Dieser Wert dürfte zweifellos sehr variabel sein, da es keine einheitlichen Bestimmungen für

die Zubereitung der Säuglingsgemüsesuppe gibt. Die tschechoslowak
schen Vorschriften (Kolektiv Autoru) empfehlen zwar das Nachsalze
der Gemüsesuppe nicht, doch sind in der pädiatrischen Praxis die ärz
lichen Weisungen uneinheitlich. Unsere Ermittlungen ergaben, daß i
manchen Instituten die Suppe nachgesalzt wird.

Von Nutzen ist auch der Vergleich des Na-Haushalts bei Säuglingen mit jenem der Erwachsenen. Aus zahlreichen Studien geht hervor, da die Einnahme von Natrium bei Erwachsenen sehr verschieden ist (Fo bes). Auf Grund der bisher zitierten Arbeiten berechneten wir die mit lere Einnahme von 2,21 mEq Na/kg Körpergewicht/24 Std (Abb. 1). B theoretischer Annahme, daß die Na-Zufuhr durch Kochsalz ersetzt wär würde ihre Menge bei einem 70 kg schweren Erwachsenen 9,03 g NaC betragen. Das Minimum der Einnahme von Na beträgt laut Abbo 0,99 mEq Na/kg Körpergewicht/24 Std, d. h. 4,03 g NaCl bei einem 70 k schweren Menschen. Wenn dies auch nur einen orientierenden Wert dar stellt, muß es auffallen, daß dieser dem mittleren, bei gestilltem Säuglin festgestellten Einnahmewert von 1,1 mEq Na/kg Körpergewicht/24 St naheliegt (Abb. 1). Mach führt in seiner Monographie an, daß einem Er wachsenen die Tageseinnahme von sogar 3 g NaCl genügen müßte. E erscheint deshalb die Annahme begründet zu sein, daß auch beim erwach senen Menschen (in bestimmten geographischen Lagen) die mittlere Ein nahme von Na übermäßig hoch ist. Und dennoch nimmt der mit Trocken buttermilch oder trockener Kuhvollmilch ernährte Säugling relativ noch mehr Na ein als es dem mittleren Einnahmewert des erwachsenen Menschen entspricht. Das Nachsalzen der Gemüsesuppe führt bei Säuglingen zu einer gegenüber der mittleren Einnahme des erwachsenen Menschen sogar mehr als zweifachen Na-Einnahme, was bedeutet, daß dies die höchste Na-Zufuhr auf ein Kilogramm Körpergewicht im ganzen Leben darstellt.

Im Vergleich mit den Nieren der gestillten Säuglinge sind diejenigen der künstlich ernährten Säuglinge genötigt, Harn von zwei- und mehrfach höherer Konzentration auszuscheiden. Aus diesem Grunde ist der von der eingenommenen Na-Menge retinierte Anteil bei künstlich ernährten Säuglingen niedriger als bei gestillten und die Na-Clearance gleichzeitig höher (Tabelle). Der Nephron muß somit die intrarenale Bilanz von Na bei künstlich ernährten Säuglingen auf einem anderen Niveau durchführen als bei Brustkindern. Es stellt sich die Frage, ob die für einige Monate nach der Geburt andauernde hohe Einnahme von Na die Funktion der Niere als wichtigsten Effektors der Osmoregulation beeinflussen kann, oder sogar in einem gewissen Zusammenhang mit den sich eventuell auf einer späteren Entwicklungsstufe offenbarenden pathologischen Erscheinungen steht. Eindeutige Antworten auf diese Fragen können noch nicht gegeben werden, und sie sind in der nahen Zu-

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kunft kaum zu erwarten. Es gibt jedoch zahlreiche experimentelle, insbesondere an Ratten gewonnene Ergebnisse, laut denen das frühzeitige Abstillen die weitere Entwicklung der Tiere markant beeinflußt, und darüber hinaus ihre Empfindlichkeit gegenüber experimentell provozierten pathologischen Zuständen erhöht hat (KREČEK; KRAUS, ANDĚL u. Mitarb.; KRAUS u. POPP; KUBÁT, FÁBRY u. Mitarb.; NOVÁKOVÁ, KOLDOVSKÝ u. Mitarb.; NOVÁKOVÁ, KOLDOVSKÝ u. Mitarb.). Diese Funde stellen die Frage in den Vordergrund, inwiefern die künstliche Säuglingsernährung, ähnlich dem frühzeitigen Abstillen bei Ratten, die weitere Entwicklung des Kindes gefährden könnte.

Es ist gewiß nicht allein der Natriumhaushalt, der zum Kriterium der Angemessenheit der künstlichen Ernährung erhoben werden kann. Die Annahme erscheint jedoch als berechtigt, daß die hohe Zufuhr von Natrium zusammen mit der hohen Einnahme von Eiweißstoffen wahrscheinlich der Grund dafür sind, die Homeostase des Wassers und der osmotisch aktiven Substanzen mittels osmotischer Diurese aufrecht zu erhalten (MARTÍNEK, JANOVSKÝ u. Mitarb.). Das von uns benützte Präparat der humanisierten Milch führt eine niedrigere Na- und Eiweißstoffeinnahme mit sich.

Die durch Trockenbuttermilch oder nichtadaptierte Kuhmilch vermittelte hohe Na-Einnahme ist nicht nur von theoretischer Bedeutung, da beim künstlich ernährten Säugling Salzfieber durch geringere Zugaben von NaCl hervorgerufen werden kann als beim gestillten Säugling. Diese Art des Fiebers ist offensichtlich ein Attribut der Durstexsiccose (RIETSCHEL; KERPEL-FRONIUS) und sein Entstehen wurde noch nicht eindeutig aufgeklärt. Bei Erwägungen über den möglichen Ursprung der erhöhten „Empfindlichkeit" der künstlich ernährten Säuglinge gegenüber den NaCl-Zugaben müssen vor allem folgende Faktoren berücksichtigt werden: 1. Die mittlere Konzentrationsfähigkeit der Nieren des Neugeborenen und des kleinen Säuglings ist im Vergleich mit dem erwachsenen Organismus niedriger (KÖNIG u. LEDERER; HELLER; POLÁČEK, VOGEL u. Mitarb.). 2. Die Harnosmolarität ist bei künstlich ernährten Säuglingen höher als bei gestillten. 3. Die den Nieren des Kindes im Vorrat zur Verfügung stehende Kapazität, übermäßige Mengen plötzlich zugeführter Elektrolyten auszuscheiden, ist bei den künstlich ernährten Säuglingen kleiner. Dies könnte der Grund sein für ein rascheres Eintreten der Hyperelektrolytämie, deren Begleiterscheinung das Salzfieber sein kann. Außerdem kann nicht ausgeschlossen werden, daß eine langfristige, mit künstlicher Ernährung erfolgende Zufuhr von osmotisch aktiven Stoffen und ihren Quellen, die einen gewissen Grad von „Supermineralisation" herbeiführt, bei erhöhter Einnahme von NaCl einer freieren Entwicklung der Durstexsiccose Vorschub leisten kann. In diesem Sinne offenbart sich die verschiedenartige Ernährung auch bei der Ein-

nahme von konzentrierter Milch, mit welcher die Dehydrierung bei gestillten Säuglingen den langsamsten Verlauf hat. Wird Dehydrierung durch Zufuhr von konzentrierter humanisierter Milch hervorgerufen, verläuft sie ebenfalls langsamer als bei der Einnahme von konzentrierter Buttermilch oder konzentrierter Vollkuhmilch (Janovský, Martínek Mitarb.).

Die komplexe Problematik einer adäquaten künstlichen Ernährung kann durch eine Untersuchung des Natriumhaushalts nur teilweise erfaßt werden. Die vorliegenden Ergebnisse bezeugen nichtsdestoweniger, daß das Bestreben, Milch zu „humanisieren", folgerecht auch den Natriumgehalt im Auge behalten muß. Dieser Standpunkt erscheint auch als logischer Bestandteil aller Bestrebungen um frühzeitige Vorbeugung eventuell möglicher späterer Folgen einer nichtadäquaten künstlichen Ernährung.

Zusammenfassung

Der Bedarf an retinierter Na-Menge für die Deckung des Natriumanteils am Gewichtszuwachs sinkt von 0,68 mEq/kg Körpergewicht pro 24 Std bei 15tägigen auf 0,22 mEq bei fünfmonatigen Säuglingen. Dieser Bedarf wird durch die Na-Einnahme in der Frauenmilch dank der durch die Nieren gesicherten hohen positiven Na-Bilanz vollkommen gedeckt. Bei künstlich ernährten Säuglingen ist die Na-Einnahme pro Kilogramm Körpergewicht relativ höher als die durchschnittliche Einnahme bei Erwachsenen. Bei Nachsalzen der Gemüsesuppe nimmt die Na-Einnahme wahrscheinlich ihre höchsten Werte im ganzen Leben an. Bei weiterer Steigerung der Na-Zufuhr entsteht bei Säuglingen Salzfieber, und zwar rascher bei künstlich ernährten als bei den gestillten. Im Vergleich zu nichthumanisierten Kuhmilchpräparaten erwies sich das untersuchte Präparat humanisierter Milch in bezug auf den Na-Haushalt als adäquaterer Ersatz der Frauenmilch. Es werden Ergebnisse besprochen, die auf mögliche Folgen der erhöhten Na-Einnahme bei künstlicher Säuglingsernährung hindeuten.

Summary

The amount of Na necessary to cover the increase in body weight is 0.68 mEq/kg b. w./24 hrs in infants aged 15 days and 0.22 mEq in infants aged 5 months. This requirement for Na is supplied by breast milk since the kidney retain Na. Artificially fed infants consume more Na/kg b. w. more than adults even and if added vegetable soup is salted salt intake is the highest ever. If further NaCl is added to the diet salt fever appears sooner in artificially than in breast fed infants. The preparation of humanized milk used in this study is a more adequate substitute for breast milk than normal buttermilk or cow's milk. The possible significance of excess salt intake by artificially fed infants is discussed.

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Dietary Salt and Essential Hypertension

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Dietary intake of sodium chloride was studied in 717 patients with essential hypertension and 819 unequivocally normotensive patients. In the normotensive group 21.2 percent (174 of 819) stated that they added salt to food before tasting it, whereas 22.7 percent (163 of 717) of the hypertensive group had this habit. Of 43 hypertensive women who added salt to food before tasting it, 17 (39.5 percent) had diastolic blood pressure greater than 120 mm Hg, compared to 56 (17.2 percent) of 325 hypertensive women who did not add salt before tasting the food ($P < 0.0006$). Of 43 hypertensive women who added salt before tasting, 5 (11.6 percent) had either or both retinal hemorrhages and exudates with or without papilledema; of 314 women who did not add salt before tasting, only 8 (2.6 percent) had retinopathy of this severity ($P < 0.003$). Salt consumption did not influence the severity of hypertension in men as reflected by diastolic blood pressure or hypertensive retinopathy. However, hypertensive heart disease manifested by electrocardiographic evidence of left ventricular hypertrophy or roentgenographic evidence of cardiomegaly, or both, was encountered in 47 (39.2 percent) of 120 hypertensive men who salted their food before tasting it, compared to 51 (22.3 percent) of 229 hypertensive men who did not add salt before tasting ($P < 0.0008$). Salt consumption made no difference with regard to the prevalence of hypertensive heart disease among women.

Hypertension was more prevalent among patients with a family history of hypertension than among those without such a history. No significant differences were found between hypertensive and normotensive patients in regard to dietary history of salt consumption. However, dietary salt consumption may play a role in determining the severity of hypertension once it has developed.

The relation between the sodium ion and the development of hypertension remains obscure. An early scientific observation that dietary restriction of sodium chloride might ameliorate human hypertension was published by Ambard and Beaujard[1] in 1904. In 1940 Grollman et al.[2] demonstrated in animal experiments that adrenocortical steroids produced hypertension only when additional salt was provided. Kempner[3,4] favorably influenced hypertensive disease through the use of a rice and fruit diet; it was later shown[5] that the effect of the diet was due primarily to its low sodium content.

It is generally believed that the level of blood pressure is determined by many factors, both constitutional and environmental. Dahl and co-workers[6-11] have proposed and demonstrated by investigations in animals and man that excessive ingestion of salt may be a factor in the development of hypertension. From popula-

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TABLE I

Relation Between Subjective Estimate of Salt Consumption and Urinary Sodium Excretion in 47 Patients with Essential Hypertension

Patient's Description of Salt Consumption	no. of Patients	Average Urinary Sodium (mEq/24 hours)	Standard Deviation
Group A (salt not added before tasting)	31	111*	51
Salt never added	12	111	62
Salt added occasionally	8	93.5	47
Salt added to taste	11	124	40
Group B (salt added before tasting)	16	151*	58

* P = <0.05.

tion studies in various geographic areas Dahl[12] demonstrated a significantly lower prevalence of hypertension in societies and racial groups with a low content of sodium in their diets than in groups with a high dietary content of sodium.

The purpose of this study was to determine in a large group of patients the relation of dietary intake of sodium chloride to the prevalence and severity of hypertension, with consideration of the additional influence of genetic predisposition to hypertension (as demonstrated by a positive family history of hypertension).

Materials and Methods

Each of 1,536 out-patients who consulted one of us (R. W. G.) for complete diagnostic evaluation during a 4 year period from 1961 to 1965 was questioned by this investigator in regard to dietary sodium habits, and then was assigned to 1 of the following 4 categories: category 1, patients who never added salt to their food under any circumstances; category 2, patients who did not usually add salt to their food; category 3, patients who added salt to their food to suit their tastes; and category 4, patients who habitually added salt to their food before tasting it.

Dietary salt habits appeared to be life-long insofar as patients could remember. On the advice of their physicians, a few patients had been on low sodium diets for several weeks to several years at the time of initial interview. For purposes of classifying these patients, only the dietary salt habits before enforced limitation were considered.

The quantity of sodium in a 24 hour urine specimen was measured for each of 47 of the patients under conditions of their usual diets and at a time when they were taking no medications. Patients who were limiting the salt in their diet on advice of their physicians were not selected for this part of the study. Of these patients, 32 had essential hypertension. The purpose of this portion of the study was to determine whether the amount of sodium excreted correlated with the patient's estimate of the amount of sodium ingested.

The following information was also obtained from the patients' records: age, sex, initial blood pressure (supine, sitting and standing), height, weight, duration of hypertension when present, antipressor therapy, if any, family history of hypertension or vascular disease, and ophthalmoscopic grouping of hypertension according to the Keith-Wagener-Barker classification. Patients with secondary hypertension were excluded from the study. Hypertension was defined as initial systolic blood pressure greater than 140 mm Hg or initial diastolic pressure greater than 90 mm Hg, or both.

Correlative analyses were made of the severity of hypertension (as estimated from ophthalmoscopic changes, diastolic blood pressure, electrocardiographic changes, and cardiac size and configuration on roentgenogram) with dietary salt habits. The prevalence of a positive family history of hypertension was estimated for each dietary group.

Results

Of the 1,536 patients of this study, 717 had essential hypertension, and 819 were unequivocally normotensive.

Urinary sodium excretion: Of the 47 subjects whose urinary sodium excretion level was studied, 16 patients (group B) added salt to their food before tasting. Their average sodium excretion level was 151 mEq/24 hours (Table I). Of 31 subjects who did not add salt before tasting (group A) there were 3 subgroups: (1) 12 patients who never added salt to their food (average sodium excretion 111 mEq/24 hours); (2) 8 patients who added salt occasionally (average sodium excretion 93.5 mEq/liter); and (3) 11 patients who added salt to taste (average sodium excretion 124 mEq/liter) (Table I). For all 31 patients in group A, the average 24 hour sodium excretion level was 111 mEq/liter. The difference between the averages of groups A

TABLE II

Dietary Salt Habits of Normotensive and Hypertensive Patients

Dietary Salt Group	Normotensive				Hypertensive			
	no. of Men	no. of Women	Total	%	no. of Men	no. of Women	Total	%
Group A (salt not added before tasting)	297	348	645	78.8	229	325	554	77.3
Group B (salt added before tasting)	135	39	174	21.2	120	43	163	22.7
Total	432	387	819	100.0	349	368	717	100.0

and B (111 versus 151) was statistically significant ($P < 0.05$). There was no significant difference among the subgroups of group A or between group B and any subgroup of group A. As a result, statistical analyses were confined to the 2 main groupings, that is, group A (patients who did not add salt before tasting) and group B (patients who added salt before tasting).

Dietary salt habits of normotensive patients (Table II): There were 819 normotensive patients of whom 432 (52.7 percent) were men and 387 (47.3 percent) women. Of 432 normotensive men 69 percent, and of 387 normotensive women 90 percent were in group A. Of the total group, 174 (21.2 percent) were in group B (salt added before tasting), while 645 (78.8 percent) gave a history of lesser salt consumption and were therefore placed in group A. The proportion of male subjects was 46 percent (297 of 645) in group A and 77.6 percent (135 of 174) in group B.

Dietary salt habits of patients with essential hypertension (Table II): Of the 717 patients with essential hypertension, 349 (48.7 percent) were men and 368 (51.3 percent) were women. Of these hypertensive patients, 66 percent of the men and 88 percent of the women were in group A. Of the total, 554 (77.3 percent) were in group A, whereas 163 (22.7 percent) were in group B. The proportion of men was 41.3 percent (229 of 554) in group A and 73.6 percent (120 of 163) in group B.

There was no statistically significant difference between the hypertensive and normotensive patients with regard to dietary salt habits. There was a significantly greater percentage of men in group B than in group A ($P < 0.0001$) in both the normotensive (77.6 versus 46.0 percent) and hypertensive (73.6 versus 41.3 percent) categories.

TABLE III

Relation Between Dietary Salt Habits and Ophthalmoscopic Classification in Patients with Essential Hypertension

Dietary Salt Group	no. of Patients	Patients with Severe Retinopathy*	
		no.	%
Group A	534†		
Women	314	8	2.6‡
Men	220	6	2.7
Group B	158§		
Women	43	5	11.6‡
Men	115	7	6.1

* Keith-Wagener-Barker groups 2+, 3 or 4 (hemorrhages or exudates, or both, with or without papilledema).

† Excludes 20 patients who did not have ophthalmoscopic examinations.

‡ Difference is statistically significant (P <0.003).

§ Excludes 5 patients who did not have ophthalmoscopic examinations.

TABLE IV

Relation Between Dietary Salt Habits and Diastolic Blood Pressure in Patients with Essential Hypertension

Dietary Salt Group	no. of Patients	Patients with Diastolic Blood Pressure > 120 mm Hg	
		no.	%
Group A	554		
Women	325	56	17.2*
Men	229	52	22.7
Group B	163		
Women	43	17	39.5*
Men	120	32	26.6

* Difference is statistically significant (P <0.0006).

Severity of hypertension and dietary salt habits: The severity of hypertension in the 717 patients with essential hypertension, as estimated from retinal changes and level of diastolic blood pressure, was correlated with the dietary salt habits of these patients.

Ophthalmoscopic findings (Table III). Ophthalmoscopic examinations were reported for all but 25 patients with essential hypertension. The proportion of patients with retinal hemorrhages or exudates, or both, with or without papilledema (Keith-Wagener-Barker groups 2+, 3 and 4) was compared in the 2 categories of dietary salt intake. There was a statistically significantly greater percentage of women with severe retinopathy in group B than in group A ($P < 0.003$). There was no significant difference between the percentages of men with severe retinopathy in these 2 categories of salt consumption.

Diastolic blood pressure and dietary salt habits (Table IV). The 39.5 percent of 43 women in group B with at least 1 diastolic blood pressure measure-

TABLE V

Relation Between Dietary Salt Habits and Either or Both the Presence of Cardiomegaly and Electrocardiographic Changes of Left Ventricular Hypertrophy in Patients with Essential Hypertension

Dietary Salt Group	no. of Patients	Patients with Cardiomegaly and/or ECG Changes of LVH	
		no.	%
Group A	554		
Women	325	71	21.8
Men	229	51	22.3*
Group B	163		
Women	43	11	25.6
Men	120	47	39.2*

* Difference is statistically significant (P <0.0008).

ECG = electrocardiographic; LVH = left ventricular hypertrophy.

TABLE VI

Relation Between Dietary Salt Habits and Family History of Hypertension in Patients with Essential Hypertension

Dietary Salt Group	no. of Patients*	Patients with Family History of Hypertension	
		no.	%
Group A	381	231	60.6
Women	219	139	63.5†
Men	162	92	56.8
Group B	117	70	59.8
Women	32	26	81.3†
Men	85	44	51.8

* Patients for whom adequate information on family history was recorded.

† Difference is statistically significant (P <0.05).

ment higher than 120 mm Hg was significantly greater ($P < 0.0006$) than the 17.2 percent of 325 women in group A with this degree of elevation of diastolic blood pressure level. There was no significant difference between the percentages of men with diastolic blood pressures greater than 120 mm Hg in groups A and B.

Hypertensive heart disease (Table V). Men in group B had a significantly ($P < 0.0008$) greater prevalence (39.2 percent of 120 patients) of hypertensive heart disease (cardiomegaly or electrocardiographic changes suggestive of left ventricular hypertrophy, or both) than did the men in group A (22.3 percent of 229). There was no significant difference in the prevalence of hypertensive heart disease between women in groups A and B.

Association of family history and dietary salt habits in patients with essential hypertension: Information relative to family history was available for 498 patients with essential hypertension (Table VI). In group A, 231 of 381 patients (60.6 percent) had a family history of hypertension in at least 1 parent, sibling or grandparent. In group B, 70 of 117 patients (59.8 percent) had a family history of hypertension. There was a significantly ($P < 0.05$) greater percentage of women with a family history of hypertension in group B than in group A (81.3 percent versus 63.5 percent). There was no significant difference in this index between men in groups A and B.

In the entire group, including both normotensive and hypertensive persons (Table VII), there was a significantly ($P < 0.0005$ to $P < 0.005$) greater percentage of hypertensive persons in the group who had a family history of hypertension. Salt intake did not appear to affect this prevalence of hypertension since there was no significant difference between subjects in groups A and B in this regard.

There was no significant difference in the prevalence of a family history of vascular disease (myocardial infarction or cerebral vascular accident, or both) or diabetes mellitus between patients in group A and group B.

TABLE VII

Relation of Family History of Hypertension and Dietary Salt Habits to the Prevalence of Hypertension

Family History of Hypertension	Sex	Group A		Group B	
		no. of Patients	Hypertensive (%)	no. of Patients	Hypertensive (%)
Negative	M	153	46*	94	44†
	F	175	46‡	23	26§
Positive	M	148	62*	65	68†
	F	230	60‡	36	72§
Not stated or uncertain	M	225	30	96	36
	F	268	40	23	48
Total		1199			

* Difference is statistically significant (P <0.005); † difference is statistically significant (P <0.003); ‡ difference is statistically significant (P <0.005); § difference is statistically significant (P <0.0005).

Discussion

Dahl and Love[7] stated that the following 3 observations implicated sodium in the origin of essential hypertension: (1) the need for sodium in the production of certain forms of experimental hypertension; (2) the virtual absence of hypertension among primitive races, all of whom appear to have a low sodium diet; and (3) the amelioration of hypertension in some persons by dietary restriction of sodium. Dahl and Love[7] classified according to their habitual consumption of table salt more than 1,300 adults who reported for annual physical examinations, and correlated their observations with blood pressure determinations. They concluded that the "group with low salt intake had significantly less and the group with high salt intake significantly more hypertension than would be expected from random distribution." This finding suggested to them that the consumption of salt was an important etiologic factor in the development of essential hypertension.

Dahl[12] later reported that a direct correlation existed between average salt consumption and the prevalence of hypertension in different geographic areas and in different racial groups. Prior et al.[13] showed that between 2 Polynesian population groups the 1 with the higher average of salt consumption had a higher prevalence of hypertension.

These observations in man have counterparts in animal experiments. Meneely and colleagues[14] showed that when the salt consumption of rats was increased, the incidence and severity of elevated blood pressure also increased.

Salt ingestion and prevalence of hypertension: The present findings do not confirm the previous

observations in regard to the relation between salt ingestion and the prevalence of hypertension in man. There was no significant difference between the normotensive and hypertensive patients with regard to dietary salt habits. These findings are in agreement with the reports of Schneckloth et al.,[15] who showed that there were no differences in salt consumption among subjects with different levels of blood pressure, and of Whyte,[16] who noted similar blood pressure values in groups assumed to have differences in salt intake.

It has previously been suggested[17] that persons with hypertension have an increased appetite for salt. The fact that the hypertensive patients and the normotensive patients in our study had almost identical habits with regard to salt consumption would tend to disprove this theory. It is not known why men, whether hypertensive or normotensive, tended to consume larger amounts of salt than women.

Salt ingestion and severity of hypertension: Although this study indicates that there seems to be no relation between the prevalence of hypertension and dietary salt habits, excessive consumption of salt seemed to make hypertension more severe once it was present. Diastolic blood pressure greater than 120 mm Hg, severe hypertensive retinopathy (Keith-Wagener-Barker classifications 2+, 3 or 4), roentgenographic evidence of cardiac enlargement and electrocardiographic changes of left ventricular hypertrophy were all used as indexes of "severe hypertension." There was a significantly greater percentage of patients with severe hypertension as defined by any of these criteria in group B than in group A, thus suggesting that excessive salt ingestion seemed to influence adversely the severity of hypertension once it had developed. Among hypertensive women, excessive salt ingestion was associated with increased prevalence of diastolic blood pressure greater than 120 mm Hg and severe hypertensive retinopathy, whereas among men there was a significant relation between excessive salt ingestion and the prevalence of hypertensive heart disease as manifested by electrocardiographic findings of left ventricular hypertrophy or evidence of cardiac enlargement on chest roentgenograms, or both. No explanation is apparent for this difference between the sexes in the way excessive ingestion of sodium tended to aggravate hypertensive cardiovascular disease.

Because we were primarily interested in relating salt ingestion to prevalence of hypertension, we elected to classify patients according to their life-long dietary salt habits, even though a few were limiting salt in their diets on the advice of their family physicians when they were first examined. For those patients, severity of hypertension at the time they were examined at the Clinic was also related to previous dietary salt habits, even though the low sodium diets they had been advised to consume more recently might have modified the severity of the hypertension. It is unlikely that this would materially affect the results for the following reasons: (1) only a few patients were attempting to limit salt in their diet and these were proportionately distributed between groups A and B; (2) experience has shown that many patients do not adhere closely to a low sodium diet even though advised to do so; (3) it seems reasonable to assume that dietary sodium restriction would have a greater hypotensive effect in group B than in group A so that, if anything, the differences in severity of hypertension observed between these 2 groups would be greater, not less, if some of the patients in each group had not been on low sodium diets.

Although many of the patients in both groups were receiving antihypertensive medication (usually including a diuretic agent) at the time they were evaluated at the Cleveland Clinic, it is doubtful that therapy can explain all of the highly significant differences in severity of hypertension between these 2 groups. Again, it is plausible to assume that diuretic therapy would have a greater hypotensive effect in the patients on a high sodium diet (group B) than in patients consuming lesser quantities of sodium (group A). If this is so, therapy would tend to lessen, not increase the difference in severity of hypertension between groups A and B.

Hereditary and familial factors: It has been suggested that a genetic factor is operative in the development of hypertension and that the addition of environmental factors such as excessive salt consumption is responsible for unmasking this trait.[9] These conclusions have been supported by animal experiments with the selective inbreeding of Sprague-Dawley rats. Two strains evolved; in 1, hypertension developed upon the addition of a high salt diet, whereas the other strain remained normotensive on the same diet. It is generally accepted that hereditary factors are operative in the development of human hypertension. Thomas[18] studied the prevalence of hypertension in 2 successive generations of subjects and found that the greatest proportion of persons with hypertension always occurred among subjects who had 2 hypertensive parents. Our study showed a greater prevalence of hypertension among patients with a family history of hypertension. There was no significant difference in the prevalence of hypertension between the 2 groups of salt users.

The results of the present investigation indicate that family history of hypertension has a greater influence on the development of hypertension in an individual than a history of excessive salt consumption. However, excessive salt consumption appears to affect adversely the severity of existing hypertension.

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REPORT ON MEDICAL PROGRESS

RECENT ADVANCES IN THE BIOCHEMISTRY AND THERAPEUSIS OF POTASSIUM SALTS*

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BOSTON

POTASSIUM salts have been employed in the treatment of disease for many centuries. It seems reasonable to believe that in most cases the indications for their use were based on practical experience and were not derived by rational deductions from experimental observations. Recent investigations have enlarged our knowledge of the action of potassium salts, and at present, indications for their use are more precise and less empirical. In this review no attempt will be made to discuss all the recent data concerning the role of potassium in the animal and human organism. Only certain evidence believed to be related to the argument will be presented.

THEORETICAL AND BIOCHEMICAL CONSIDERATIONS

The inorganic salts of potassium are highly ionized in the body. Little attention need be given, therefore, to the anion; the principal effects of the presence of potassium will be assumed to be produced by the cation. There is no recognized hormone in the body that regulates potassium exchange as desoxycorticosterone regulates sodium exchange or parathormone regulates calcium exchange. It is likely that at some future time a hormone or regulator for potassium will be discovered, the lack of recognition of which offers a formidable check on pertinent investigations.

There are three known isotopes of potassium, having atomic weights of 39, 40 and 41. More than 93 per cent of the potassium in animal tissue has an atomic weight of 39,[1] nearly all the remainder consisting of that with an atomic weight of 41. The isotope with the atomic weight of 40 is responsible for all or nearly all the radioactivity of potassium. It is extremely rare, and does not appear to be more concentrated in animal tissue than elsewhere in nature. No effects of naturally occurring potassium can be attributed with certainty to its radioactivity, nor have any biologic effects been observed experimentally from irradiated potassium salts that were unlike natural potassium.[2]

Of the several satisfactory methods for the determination of serum potassium, we believe that the one revised in our laboratory[3] possesses certain advantages. The range for serum potassium in healthy men is between 3.5 and 4.5 milliequiv. per liter (14 to 18 mg. per 100 cc.) in 90 per cent of the subjects; the extreme range is from 3.4 to 4.9 milliequiv. per liter.

In this presentation the role of potassium will be discussed under three divisions: as the principal base in tissues and blood cells it is concerned with the regulation of acid-base balance of the body; as an insulin-antagonistic and epinephrine-like substance, it participates in the intermediary metabolism of carbohydrates; as an antagonist of cholin esterase and as the principal base in muscle cells, it is concerned with the transmission of the nerve impulse to the muscle fiber and with the contractility of muscle. While we do not wish to engage in polemics concerning the chemical versus the electrical theory of conduction of the nerve impulse, it appears to us that the chemical theory best explains most experimental observations.

The recognition of the participation of potassium in neuromuscular physiology is recent. To be sure, Ringer,[4] Howell[5] and others showed that large amounts of potassium in perfusion fluid exert an effect on cardiac action, but only in the last decade have investigations produced data of interest to clinicians. Brown and Feldberg[6] showed that small concentrations of potassium chloride in perfusion fluid increase the response of sympathetic ganglia to "single submaximal preganglionic volleys." They observed also that potassium stimulates the cells of normal and denervated ganglia to discharge. Conversely, Vogt[7] showed that in the dog, prolonged faradic stimulation of the preganglionic fibers of the superior cervical ganglia produced a decrease in potassium content of the ganglionic tissue. At the motor end-plates potassium has an action antagonistic to that of curare,[8] and in this action it is similar to prostigmine,[9] guanidine, ephedrine and Congo red. This suggests that

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Paper No. 9 in Studies on Myasthenia Gravis, pursued by one of the authors (R. S. S.).

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potassium has an inhibiting effect on the action of cholin esterase. Lastly, Fenn[10] has shown that during muscular contraction potassium leaves the muscle and that not until recovery does it return. These and other significant investigations constitute advances in the clinician's understanding of disease.

In an attempt to simplify this presentation, it has been assumed that the several actions of potassium are independent, although it is likely that subsequent observations will not bear this out. For example, in Addison's disease the action of potassium may not be confined to any one of the three pharmacologic effects, and in the treatment of hay fever the epinephrine-like action may be associated with a change in acid-base balance or in neuromuscular function. It is quite possible that we are misinterpreting some of the actions of potassium, but for the sake of clarity they will be discussed separately and in the order given above.

Therapeutic Considerations

Potassium salts may be prescribed in various forms for oral administration. It is our experience that an aqueous solution of potassium chloride produces less gastrointestinal distress than does the administration of the salt in gelatin capsules or in tablet form. Usually a 25 per cent solution of potassium chloride is prescribed, one teaspoonful of which contains approximately 1 gm. of the salt. The quantity to be ingested is therefore easy to calculate. One teaspoonful may be added to a wineglassful of water, milk, fruit or tomato juice and ingested with little or no gastric distress. Occasionally patients complain that they have been unable to tolerate initial daily doses totaling 6 or 8 gm. of potassium chloride. Under such circumstances smaller doses are given at first and the amount is increased daily up to the maximum desired. Those who have been instructed in this manner have been able to take the quantity prescribed without untoward effects. A more detailed description of potassium administration in patients with myasthenia gravis is given below.

Edema

An accumulation of sodium chloride and water in the interstitial spaces is the invariable state in edema, whether this arises from renal, cardiac or hepatic failure. If a hypertonic solution of potassium chloride is given, a two-fold action results. Potassium competes with sodium for body water, and if an adequate amount is not available for both, a portion of the retained sodium is displaced and subsequently excreted in the urine. Secondly, potassium competes with sodium for reabsorption by the cells of the renal tubules, so that if a large excess of potassium is present in the glomerular filtrate, it depresses the sodium reabsorption and an additional loss into the bladder urine results.

In the treatment of edema, Barker[11] found that 5 gm. of potassium chloride was an effective diuretic. Keith and Binger[12] confirmed this observation in patients and observed, in addition, a diuretic action in normal persons. Mudge and Butler,[13] however, were unable to detect any significant increase in excretion of sodium or edema fluid following the ingestion of equal amounts of potassium chloride. There is no satisfactory explanation for this failure of agreement among the various experimental observations.

Addison's Disease

The data on patients with Addison's disease appear to be more definitive. This malady is caused in part by a failure in elaboration of one or more hormones of the adrenal cortex, and involves dissipation of sodium and water and retention of potassium and urea. Some authorities hold that the increased concentration of potassium is as important in the pathogenesis of certain symptoms as is the decrease in concentration of sodium. It follows that a high potassium intake may aggravate symptoms of adrenal insufficiency; conversely, a low potassium intake may be beneficial in the treatment of this condition. A practical test, based on this observation, has been devised by Wilder and associates[14] for suspected adrenal insufficiency. Five gm. of potassium citrate is given daily for approximately three days. The patient, meanwhile, is given a diet low in sodium content. A decrease in concentration of serum sodium, an increase in output of sodium in the urine and symptoms of adrenal insufficiency follow if the presumptive diagnosis is correct. In the treatment of Addison's disease a low potassium intake (less than 2 gm. per day) has been recommended, although we have been unable to confirm its reported merits in the series of cases in which we are interested.

Recently, an entirely new concept of the value of potassium in Addison's disease has been proposed. If desoxycorticosterone acetate, a synthetic sterol similar to the sterols isolated from the adrenal cortex and useful in the treatment of Addison's disease, is given in excessive amounts it produces an increase in concentration of serum sodium, a decrease in concentration of serum potassium[15] and an exacerbation of malaise, weakness and gastrointestinal symptoms. The effects from overtreatment of patients with Addison's disease are analogous to those of patients with diabetes mellitus who have received an unnecessarily large amount of insulin. Following the assimilation of excessive

quantities of desoxycorticosterone acetate, the level of serum potassium in Addison's disease may decrease to as low as 2.5 milliequiv. per liter. Generalized paralysis of the skeletal musculature may occur, as in periodic paralysis (see below), at this level of serum potassium. For the sake of clarity, it may be reiterated that a high potassium intake is harmful in adrenal insufficiency, but beneficial in patients who have been given inadvertently an excess of the active material during treatment.

Obesity

The use of potassium salts in the treatment of obesity has been recommended by Rusk.[16] It was his experience that a high protein intake supplemented by 4 gm. or more of potassium chloride daily constituted an effective anti-obesity regimen. Undoubtedly the initial loss of weight was associated with the diuretic action of potassium salts. After this effect has passed restriction of caloric intake seems most essential.

Diabetes Mellitus

There are several reports that potassium is associated with assimilation and utilization of carbohydrates in the body. McQuarrie and his associates[17] observed in 4 diabetic children a decreased tolerance to glucose following the ingestion of 12 to 35 gm. of potassium chloride. Gellhorn and Skupa[18] demonstrated that the rate of absorption of glucose from the intestines of frogs is increased by the presence of potassium chloride. Silvette and Britton noted a loss of glycogen from the liver[19] and from the skeletal and heart muscle[20] and an increase of blood sugar following the intraperitoneal injection of potassium acetate. Furthermore, it has been known for more than a decade that a decrease of serum potassium is associated with symptoms of insulin shock. Lastly, in a susceptible person, either an injection of insulin or a high-carbohydrate meal may induce an attack of periodic paralysis with decrease of serum potassium. It may be concluded from these data that potassium has a biological action antagonistic to that of insulin. Clinically, little advantage has been taken of this effect, although McQuarrie et al. have shown that insulin hypoglycemia in diabetes mellitus may be controlled partially with potassium chloride. Further trial of potassium salts in persons difficult to regulate with insulin seems indicated.

Allergic Diseases

Another action of potassium which in many respects is opposite to that mentioned above is its epinephrine-like effect. This has been utilized in the treatment of allergic diseases. Bloom[21] reported beneficial effects in 29 patients with hay fever using 1 or 2 gm. of potassium chloride daily. Many were improved within twenty-four hours, and a few more were improved or completely relieved of symptoms within three days. Bloom recommends daily ingestion of potassium during the entire hay-fever season. Cessation of ingestion may be followed by exacerbations of symptoms. Rackemann[22] has been unable to confirm these findings using similar quantities. He has not abandoned the use of potassium salts, however, and believes that further clinical trial is indicated before making a final commitment. Patients with bronchial asthma do not benefit from potassium chloride so consistently as do those with uncomplicated hay fever.[21]

A discussion of urticaria concludes the allergic manifestations. Rusk and Kenamore[23] treated 6 patients suffering from this disease with an acid-ash diet, high in protein (which is also high in potassium) and low in sodium. In addition, from 4 to 6 gm. of potassium chloride was prescribed daily. All the patients responded to this treatment. Subsequently, a group of 20 patients were treated with similar results. These observations could not be confirmed in 8 patients by Cohen.[24]

Acid-base studies in allergic diseases are few in number, and those which are reported are not complete. Rusk and his associates[25] observed a slight increase above normal in concentrations of serum potassium during exacerbations of bronchial asthma. Clinical improvement was accompanied by a decrease in concentration. At no time was the average range for serum potassium in allergic patients as low as the average range for normals. These authors' interpretation of the higher potassium levels is similar to that advanced by us for Ménière's disease, namely, an elevation in the serum is indicative of a migration of this electrolyte from extravascular areas.

The hypothesis advanced by Bloom[21] in explanation of the beneficial effect of potassium in allergic manifestations was that it participated as an electrolyte in the acid-base balance of the blood. He assumed that the major disturbance was an altered electrolyte metabolism with involvement of the adrenal glands. This explanation is somewhat difficult to accept. One more acceptable to us is an action on the sympathetic synapses or neuromuscular junctions and restoration of vasomotor tone. This is consistent with the data and conclusions of Camp and Higgins.[26] A constriction of the bronchioles induced by histamine could be relieved as promptly and completely by potassium as by epinephrine. They conclude that one function of epinephrine is the maintenance of an optimum potassium level in the body and that changes in potassium are responsible for typical changes ascribed previously to epinephrine.

The objections raised by Engelsher[27] to the treatment of allergic diseases with potassium may be refuted in part. In a series of 64 patients with various allergic manifestations he noted epigastric pain and other intestinal disturbances in a majority. Such symptoms are more likely to develop within a day or two after beginning the regimen. Although he gave no data on the duration of treatment, it is reasonable to believe that his patients took potassium an insufficient length of time to warrant satisfactory conclusions.

Familial Periodic Paralysis

Familial periodic paralysis was one of the first neuromuscular disorders to be treated systematically with a high potassium intake.[28] This malady is characterized by spontaneous attacks of flaccid paralysis of the muscles of the extremities, with a concomitant decrease in serum potassium.[29] The concentration may be as low as 2.5 milliequiv. per liter, a level that may also be reached during paralysis from overtreatment of Addison's disease with desoxycorticosterone acetate. Paralytic seizures have been induced in susceptible persons by injections of epinephrine and of insulin and by ingestion of a high-carbohydrate meal. Each of these procedures causes a diminution in concentration of serum potassium in normal as well as in afflicted persons. The daily ingestion of 2 to 5 gm. of potassium chloride or potassium citrate appears to be effective in preventing paralytic episodes. A boy whom we have been following for more than two years has experienced a marked diminution in the number of attacks per year on such a regimen and is able to live a relatively normal life. The only time he suffers from paralytic attacks at present is after overindulgence in carbohydrates.

The pathogenesis of paralysis of the familial periodic type has not been defined. No change in cholin esterase activity of the serum has been demonstrated. The absolute level of serum potassium appears to be less of a factor in inducing an attack than is a diminution per se in concentration of this constituent. Controlled metabolic experiments have failed to demonstrate a loss of potassium from the body following water diuresis.[30] A migration of potassium from the serum into certain tissues prior to or during the paralytic stage undoubtedly occurs. It is suggested as a hypothesis that the migration is accompanied by a lowering of concentration of potassium in certain synapses of the central nervous system or at the neuromuscular junctions of affected muscles. The prevention and treatment of attacks by potassium ingestion lend weight to this hypothesis.

Myasthenia Gravis

Myasthenia gravis is another neuromuscular disorder which responds in a remarkable manner to the ingestion of potassium.[31] The mechanism of weakness in this disorder is unknown [illegible] it has been suggested that it is associated with an increase of cholin esterase activity at the neuromuscular junction and a diminution of available acetylcholine. Relief from symptoms of weakness is accompanied by restoration of the disturbed equilibrium of the chemical mediators [illegible]

Potassium chloride was first used [illegible] chusetts General Hospital in the treatment of patients with myasthenia gravis in 1936. From 5 to 10 gm. per day was prescribed in addition to prostigmine. Three patients, all severely afflicted, believed that they were improved on this regime. If 5 gm. of potassium chloride was taken simultaneously with 15 mg. of prostigmine, the efficiency of prostigmine was increased and the effect was prolonged. No diminution in the amount of prostigmine required to control the patients' symptoms, however, was observed. In 1937 potassium chloride was recommended to 3 other patients with myasthenia gravis. Benefit was admitted by only 1. The following year the use of potassium chloride was reinvestigated. At that time large amounts were given with striking benefit. The ingestion of 10 to 12 gm., three times a day, in 5 cases was followed by a significant reduction in the amount of prostigmine needed. In patients whose daily requirements were from twenty to thirty prostigmine tablets (15 mg. each) by mouth, it was possible when in the hospital to reduce the number to two or three if large amounts of potassium salts were taken.* A reduction in the cost of treatment and an opportunity to maintain a more complete state of remission were achieved. Up to the present 24 patients have taken or are taking with benefit large amounts of potassium chloride and smaller amounts of prostigmine than formerly.[32] In addition, 7 patients began the ingestion of potassium chloride but discontinued it subsequently, either because they were not helped or because of untoward symptoms. It appears to be impossible to regulate patients on potassium chloride alone. The failure of Minski[33] in the treatment of myasthenia patients with potassium chloride and prostigmine may be attributed to inadequate amounts used.

A satisfactory method for the ingestion of large amounts of potassium chloride embraces the use of

*On the outside, when one is dealing with patients who are of course much more active than a person in a hospital bed all day, no such reduction in prostigmine occurred. In these cases the amount of prostigmine was reduced from twenty to fifteen tablets, and in others from ten to seven, or approximately 20 to 40 per cent.

milk as a vehicle. We have recommended that patients prepare each day a mixture of the following:

Milk	1 quart
Cream	½ cup
Egg	1
Sugar	2 teaspoonfuls
Potassium chloride	8 teaspoonfuls (30 gm.)

The formula may be taken in three divided doses with regular meals. Other patients prefer a 25 per cent aequeous solution of potassium chloride flavored with a small amount of peppermint water. Thirty cubic centimeters of such a mixture may be taken three times a day with meals. Potassium chloride may be prescribed in 1-pound amounts of the dry crystals for the patient to prepare his own solution, or as the 25 per cent solution in large quantities.

Recently it has been observed that a combination of guanidine hydrochloride (0.0125 gm.), potassium chloride (5-10 gm.) and prostigmine (15 mg.) taken with meals gives a sustained effect for three or four hours. Only in most desperate cases is such a combination of anti-myasthenia drugs necessary. It is to be noted that potassium chloride as well as prostigmine and guanidine are potent anti-curare substances.

Ménière's Disease

The history of the use of potassium chloride in the treatment of Ménière's disease or Ménière's symptom-complex seems worth relating. Several years ago Mygind and Dederding[34] assumed that this malady was associated with a disturbance of water and salt metabolism. A waterlogged labyrinth was thought to be at fault and a dehydrating regimen was therefore indicated in treatment. Furstenberg and his associates[35] reinvestigated the problem sometime later, and showed evidence that a retention of sodium was of greater pathogenic significance in the production of symptoms than was a retention of water. They recommended a diet with a low sodium content and the ingestion of ammonium chloride. Since both regimens achieved certain success, it seemed expedient to us[36] to investigate the constituents of the blood before and after treatment. This was done in a group of 33 patients.[37] No increase in concentration of serum sodium was observed during a relapse. This appeared to exclude sodium retention as a significant pathogenic factor in production of symptoms. In 4 patients who were having severe symptoms, however, an increase in concentration of serum potassium was observed. This prompted us to consider several aspects of the regimens which have been recommended and found successful in many patients.

In a diet devised to provide for a low-sodium content, some increase in potassium usually occurs. Since a low-sodium and high-potassium diet was effective, it seemed reasonable to try a normal diet with potassium chloride added. To achieve this, approximately 6 gm. of potassium chloride in an aqueous solution was given daily. No modification of the normal diet was recommended. To date more than 50 patients have been treated in this manner. Most of those who have followed the recommendations have been benefited, although few have been relieved of all symptoms.

Those most severely affected appear to be helped most. Several patients who were having many severe attacks per month have had the incidence reduced to only a few a year. The severity and duration of each attack have been likewise diminished. All except one or two have been able to lead a relatively normal life without the fear of an unexpected incapacitating attack. Several discontinued the daily ingestion of potassium and experienced a return of symptoms after a period of a few days; return to the regimen was followed by a satisfactory alleviation of symptoms. One intelligent but skeptical patient performed this experiment three times before he was convinced of the merits of the high potassium intake. Similar satisfactory results have been reported by Chapman,[38] Graves,[39] Reese[40] and Schneider.[41] Improvement in hearing has been claimed by some patients. We are skeptical concerning this alleviation. With diminution in tinnitus some improvement in hearing would be expected. The precise action of potassium chloride in patients with this syndrome is not known. As a working hypothesis it has been assumed that an increased concentration of serum potassium is indicative of the disruption of normal chemical mediation of impulses in the eighth nerve. Ingestion of potassium may restore normal equilibrium.

Because of the benefit in Ménière's syndrome, potassium salts have been given to patients who complained of one or more symptoms of the classical syndrome, but in whom the clinical picture did not warrant a presumptive diagnosis of this malady. The symptoms complained of included vertigo, tinnitus and deafness. Since potassium chloride is a relatively harmless material, its use seems justified when other forms of therapy are ineffective. The same amounts are recommended as in the treatment of Ménière's syndrome.

Cardiac Disease

Changes in cardiac action with variation in concentration of serum potassium have been reported. Inversion of T_1 and T_2 has been noted[42] in dogs with high serum-potassium levels. In 1931 Wiggers[43] reported alleviation of ventricular fibrillation in dogs following perfusion with a solution of po-

tassium chloride. Sampson and Anderson[14] utilized this observation in human beings in the treatment of auricular and ventricular ectopic beats with tachycardia. In doses of 1 to 10 gm., potassium chloride was effective in restoring normal rhythm in 50 per cent of a small group of patients. It has been suggested that alteration of the electrocardiogram in acute coronary thrombosis[15] may be due in part to alteration in the level of potassium in the muscle.

Untoward Effects

The ill effects of excessive potassium ingestion are believed to be few. Oral administration of as much as 40 gm. per day to patients with myasthenia gravis produced no recognized untoward effects. Gastrointestinal distress usually develops before a toxic amount has been ingested. With onset of gastrointestinal distress assimilation is retarded and excessive quantities are not absorbed. The harmful effects of potassium in untreated Addison's disease have been discussed. Other conditions associated with a diminished concentration of serum sodium and increased concentration of serum potassium, such as heat cramps, acute nephritis, pemphigus and dehydration, might be aggravated. Scudder, Zwemer and Truszkowski[16] called attention to the possible toxic effects of a high serum potassium in acute intestinal obstruction. With the storage of blood and development of blood banks the concentration of the potassium in the transfusion fluid must be considered. If whole blood is stored[17] there is a slow migration of potassium from the blood cells into the plasma. When this concentration exceeds 10 milliequiv. per liter the blood is probably unsuitable for intravenous use in human beings.

Summary

Certain aspects of the role of potassium in the body are reviewed. The action of potassium appears to depend on its effect as an electrolyte, as a substance concerned with carbohydrate metabolism, as an important mediator of the nerve impulse and as a participant in muscle contraction.

In allergic manifestations, Ménière's syndrome and familial periodic paralysis, from 2 to 10 gm. of potassium chloride per day is effective. In myasthenia gravis from 20 to 40 gm. per day may be indicated. In a few conditions a low potassium intake is desirable.

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Clinical Workshop

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Pulmonary Edema Complicating Intrathecal Hypertonic Saline Injection for Intractable Pain

GALE E. THOMPSON, M.D.

The injection of large volumes of hypertonic, hypothermic saline solution into the subarachnoid space for relief of pain was first advocated by Hitchcock in 1967.[1] Unlike many other approaches to the treatment of pain, this technique rarely disturbs normal sensory or motor function. At this time the mechanism of action of the injected saline solution is not clear. Possibilities include osmotic dehydration from the hypertonic solution or a direct effect of cold on small-diameter pain fibers. Variations of the original technique have included the use of either cold or hypertonic solutions alone.

Most reported series concern patients with the intractable pain of terminal cancer. The incidence of successful results and duration of pain relief have been variable. Some patients have had dramatic relief of pain, whereas others show minimal if any change. Although the technique is used less frequently for nonmalignant conditions, Collins has reported good results in the treatment of causalgia and postherpetic neuralgia.[2]

The complications of this procedure have been relatively minor and of short duration. Various tachyarrhythmias and ectopic beats have been seen with electrocardiographic monitoring. Other common side-effects include fasciculations of face, arm and leg muscles, tachypnea, vertigo, and vomiting. One case in which transient hemiplegia followed the block has been reported.[3] The following case illustrates another serious complication of this procedure.

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REPORT OF A CASE

A 47-year-old woman was admitted to the hospital in April 1970 with a three-week history of diarrhea and rectal pain. Laparotomy disclosed an adenocarcinoma of the sigmoid colon with local extension to perirectal fat and multiple lymph node involvement. Anterior sigmoid resection was performed. Postoperatively the patient received external radiation with ^{60}Co over an eight-week period. During this time she began to complain of pain in the sacral and rectal areas. At first this was relieved by Percodan. However, because of increasing severity of pain, the patient was readmitted for trial of possible pain relief procedures. Chordotomy and posterior rhizotomy were considered unfeasible because of the midline location of the pain. A subarachnoid alcohol block was tried, with good (75 per cent) pain relief. The patient had slight weakness of the legs and some difficulty voiding following this block. However, she was able to walk and also able to control urination. A month later the pain in the rectal area recurred. Because of the motor and sensory changes which had followed the subarachnoid alcohol block, it was elected to do a block with hypertonic saline solution.

Following premedication with 15 mg morphine sulfate, the patient was lightly anesthetized with intravenous methohexital while in a sitting position. Forty ml of spinal fluid were slowly removed through a 19-gauge needle at the L3–L4 level. Forty ml of 5 per cent saline solution at nearly 0 C were injected through the same needle over a three-minute period. Before the injection was completed, the patient began to hyperventilate up to rates of 40 to 50/min, and heart rate increased from 86 to 136 beats/min. Blood pressure rose from 130/80 torr to 210/120 torr. These changes reverted toward normal over the following hour except for a persistent tachycardia of

110–120 beats/min. It was also observed that the patient had a wet and rumbling, but nonproductive, cough, and appeared cyanotic. Analysis of arterial blood gases showed *p*H 7.46, P_{CO_2} 24 torr, P_{O_2} 34 torr. Oxygen was given by nasopharyngeal catheter and skin color improved. However, over the next four hours the patient became increasingly short of breath and diaphoretic, and began coughing up moderate amounts of pink, frothy sputum. Chest x-ray revealed bilateral fluffy infiltrates. At that time, it was suspected that the cause of the pulmonary edema was some previously overlooked metastatic or infectious pulmonary process. Blood and sputum cultures were taken and supportive treatment given with oxygen and elevation of the head of the bed. Only in retrospect was it recognized that treatment should have been more vigorous. A total of 1,000 ml of 5 per cent dextrose in Ringer's lactate solution had been given intravenously during the day of the block.

The following morning the patient was greatly improved. She was smiling, complained of only minimal pain, and was eager to have breakfast. Auscultation disclosed a few moist rales at both lung bases. The patient still appeared cyanotic when breathing room air, and analysis of blood gases showed *p*H 7.49, P_{CO_2} 28 torr, P_{O_2} 44 torr. The patient showed slow improvement over the next 24 hours, with episodes of tachypnea and tachycardia on exertion. Forty-eight hours after the block, she was asymptomatic. Blood gases during breathing of room air were *p*H 7.48, P_{CO_2} 28 torr, P_{O_2} 78 torr. Chest x-ray was completely normal. In view of the rapid and complete resolution of the pulmonary problems, and in the absence of an apparent underlying cause of pulmonary edema, another etiology was sought.

Discussion

There are numerous case reports of pulmonary edema following isolated closed head injuries without trauma to any other portion of the body. Various pathophysiologic explanations have been proposed, but recent work has done much to illuminate the sequence of events. The Cushing phenomenon, first described in 1901,[4] serves as a basis for explanation. Cushing showed that any increase in intracranial pressure is followed by an increase in systemic blood pressure such that the diastolic pressure attained will exceed intracranial pressure by several torr. The exact stimulus and the site of action for this response in man are unknown, but it is obviously a protective response to ensure cerebral perfusion.

Simmons[5] has reported experimental work in chimpanzees in which intracranial pressure was raised in graded elevations by injections of saline solution into balloons previously placed in the epidural space. The consequent increases in systolic arterial pressure were accompanied by normal central venous pressures but elevated pulmonary arterial and venous pressures. At the same time, central blood volume increased by as much as 45 per cent. Similar experiments in dogs have shown that arteriovenous shunting occurs in the splanchnic and somatic beds following acute elevation of intracranial pressure.[6] Blood is mobilized centrally from the peripheral circulation and passive overload of the pulmonary bed may occur.

Luisada[7] describes three elements essential to the development of what he terms "neurogenic pulmonary edema": 1) following the acute elevation of intracranial pressure there is intense peripheral vasoconstriction, leading to systolic overload of the left ventricle; 2) diastolic overload of the left ventricle occurs following mobilization of blood from the periphery to the pulmonary bed; 3) the inordinate rise in left ventricular diastolic pressure is followed by increases in left atrial and pulmonary capillary pressures.

Hammermeister and Reichenbach[8] have suggested a more direct pathophysiologic explanation for pulmonary edema associated with intracranial disease. This does not involve blood volume shifts to the pulmonary bed, but rather emphasizes the direct myocardial damage that results from massive sympathetic discharge. They have reported a case in which pulmonary edema occurred in a patient soon after rupture of an intracranial aneurysm. Postmortem examination revealed little or no coronary disease, yet there was spotty focal necrosis of myocardial cells. Other authors have also reported direct damage to myocardial cells from exogenous vasopressors.[9]

That the pulmonary edema in the present case was associated with the hypertonicity of the injected solution is very likely. A 5 per cent saline solution will increase its volume about five times as it reaches equilibrium with body fluids. A volume of 40 ml of 5 per cent solution injected into the subarachnoid space has the potential for expanding to 200 ml as it draws water by the osmotic gradient which it produces. The final total volume of cere-

brospinal fluid would then approximate twice normal, or 300 ml, if there were no transfer of isotonic fluid out of the subarachnoid space. A volume increase of this magnitude within the bony confines of the skull and spine could lead to a marked increase in intracranial pressure and set the stage for pulmonary edema, as indicated in the experimental work cited above.

Anesthesiologists should be aware that pulmonary edema can occur following intrathecal blocks with hypertonic saline solution as well as in neurosurgical patients with head injuries. Conversations with other anesthesiologists reveal that the "wet lung" syndrome following blocks with hypertonic saline solution has been observed in other hospitals. Blood pressure must be monitored closely and enriched oxygen mixtures given. Should pulmonary edema become manifest, a potent intravenous diuretic such as furosemide is indicated. Treatment of hypertension must be tempered with an awareness that the hypertensive response may not be entirely detrimental and, in fact, it may even be beneficial when it ensures cerebral perfusion in a patient in whom the Cushing reflex is operative.

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AN EXPERIMENTAL STUDY OF SALT POISONING IN THE PIG

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INTRODUCTION

Salt poisoning is commonly diagnosed by histological examination of brain or by chloride estimation on stomach contents, body fluids or tissues (Bohosiewicz, 1957; Cox and Pitts, 1957; Garner, 1961). The recent recognition that a similar clinical and pathological picture can be produced by sodium sulphate (Dow, Lawson and Todd, 1963), however, indicates that chloride estimations alone are not enough to establish a diagnosis. Indeed Bohosiewicz (1962) suggested that in sodium chloride poisoning elevated chloride levels in the alimentary canal and tissues only justify a suspicion of poisoning and that both sodium and chloride analyses should be done. Medway and Kare (1959) estimated sodium and potassium in their experimental study and found significant changes in the concentration of each element in fluids and tissues of poisoned animals.

The experiment reported below was done to investigate the changes in chloride, sodium and potassium concentrations in the alimentary canal, body fluids and tissues in sodium chloride poisoning. Since sodium chloride and sodium sulphate can produce the same toxic syndrome under conditions of water deprivation, the effect of water deprivation alone on the concentration of the three ions was also investigated.

METHODS

Sixteen pigs were brought into the experiment when weaned and weighed 26 to 35 lb. They were divided into four groups and subjected to the following treatments.

(1) Control—normal feed, water ad lib. ($C + H_2O$).
(2) Normal feed, water restricted ($C - H_2O$).
(3) High salt, water ad lib. ($NaCl + H_2O$).
(4) High salt, water restricted ($NaCl - H_2O$).

The normal feed was a proprietary brand of pig meal containing 0·5 per cent. salt. The two high-salt groups received 2·5 g. NaCl per kg. bodyweight daily dissolved in a total of 8 oz. water. The pigs on the normal feed, water restricted regime also received the 8 oz. of water daily, but no other water. The fluids were administered individually, using a drenching gun.

Blood samples were drawn into heparin from all animals immediately before each drenching. These were immediately centrifuged and the plasma separated for analysis.

When an animal in group 4 died, one animal from each of the other three groups was killed and samples were taken for analysis from stomach and small intestine contents, urine, cerebro-spinal fluid, brain (cerebrum

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and cerebellum), liver, kidney, spleen and heart. Histopathological examination was carried out on the brain and other tissues.

Sodium and potassium were estimated in all samples by flame photometric methods. Chloride was estimated by the mercuric nitrate titration method as described by Brun (1949).

RESULTS

Clinical

The pigs in group 4 died 26, 33, 36 and 48 hours after receiving the first dose of sodium chloride and all had then received a second dose. Death was preceded by a short illness characterised by muscular tremors, rapidly progressive inco-ordination, convulsions, prostration and terminal coma. Inco-ordination was present in the pig in group 2 killed at 48 hours, but all other animals in groups 1, 2 and 3 were clinically normal at the time of slaughter.

Histology

Specific lesions in the 4 pigs in group 4 were confined to the grey matter of the cerebrum; the remainder of the brain, spinal cord and viscera appeared normal. Perineuronal vacuolation was present in sections from all cerebral lobes and was most severe in the mid-cortical laminae, particularly in the dorsal and lateral gyri. The reaction was most prominent in the sides of the affected gyri, and in the pig which survived for 48 hours intense concentration and confluence of vacuoles had resulted in broad bands of malacia in the mid-cortical laminae. Malacia was less severe in the pigs which died before 48 hours and occurred in scattered isolated foci in various gyri. Neuronal degeneration was widespread in the cortex but was most prominent in areas of intense vacuolation. Neurones in such areas were seen as shrunken, hyperchromatic structures, displaced and distorted by vacuoles, and many appeared to be suspended by their processes within vacuoles. The endothelial cells of the smaller cerebral vessels were swollen but there was never any evidence of cellular exudation of any type into the Virchow-Robin spaces or meninges.

Neuronal degeneration, perineuronal vacuolation and early focal mid-cortical laminar malacia without cellular infiltration were also present in the last pig killed in group 2. This pig showed moderate inco-ordination at the time of slaughter. Specific cerebral lesions were not detected in the 3 other pigs in this group nor in any animal in groups 1 and 3.

Chemical Analyses

Plasma. The results of the analyses are set out in Table 1. All values before dosing commenced were within or slightly below the accepted normal ranges quoted by Spector (1956). After 24 hours, chloride and sodium concentrations in both groups on restricted water intake had increased markedly, the increase being greater in

TABLE 1

CHLORIDE, SODIUM AND POTASSIUM CONCENTRATIONS IN BLOOD PLASMA (M. EQUIV./l)

	Group	*Before* (*Av. of 4*)	*After 24 hours* (*Av. of 4*)	*After 48 hours* (*One only*)
Plasma chloride	C+H_2O	101	99	101
	C−H_2O	99	113	129
	NaCl+H_2O	104	103	97
	NaCl−H_2O	100	122	155
Plasma sodium	C+H_2O	141	139	138
	C−H_2O	137	149	175
	NaCl+H_2O	146	144	141
	NaCl−H_2O	141	166	197
Plasma potassium	C+H_2O	6·2	5·9	5·8
	C−H_2O	5·1	5·0	6·2
	NaCl+H_2O	6·1	4·5	4·9
	NaCl−H_2O	6·1	4·7	4·8

the group which received additional salt. (NaCl − H_2O). No such increase occurred when water was available ad lib (NaCl + H_2O). The pigs surviving at 48 hours showed these changes even more markedly and the remaining pig in the C − H_2O group had similar plasma chloride and sodium concentrations to the pigs in the NaCl − H_2O group after 24 hours (one of which died shortly afterwards). The results for chloride and sodium in both control and poisoned pigs agree very closely with those of Cox and Pitts (1957). Medway and Kare (1959), however, found considerably higher plasma sodium levels in both control and poisoned animals than those found in this experiment.

Plasma potassium concentrations did not show any definite effect following restriction of water intake. Both groups receiving extra salt, however, showed slight reduction in average plasma potassium concentrations after 24 hours to levels just below the normal range quoted by Spector (1956). Bohosiewicz (1962) also found a slight reduction in blood potassium concentrations after NaCl administration. This is in marked contrast to the elevations in plasma potassium concentrations in poisoned pigs reported by Medway and Kare (1959) (18·0 m. eq/l in poisoned pigs, compared with 5·5 m. eq/1 in controls).

Gastro-intestinal tract. The contents of the stomach and small intestine of each group had similar levels of sodium and chloride ions, the chloride concentration being slightly higher in the stomach than the intestine in each group and the opposite being the case in respect of sodium. The sodium concentrations found in the dry matter have therefore been used to calculate the percentage of NaCl set out in Table 2. The stomach contents reflected the toxic conditions in that the (NaCl − H_2O) group showed a much higher average than the others. Individual results however varied

TABLE 2

AVERAGE NaCl CONCENTRATIONS IN STOMACH AND SMALL INTESTINE CONTENTS

NaCl percentage calculated from sodium analysis

Group	NaCl percentage	
	Stomach	*Small intestine*
$C+H_2O$	0·5	6·0
$C-H_2O$	2·9	1·9
$NaCl+H_2O$	2·3	18·6
$NaCl-H_2O$	15·0	13·7

from 4 per cent to 24 per cent. NaCl in this group whereas the range for the ($NaCl + H_2O$) group was from 0·2 to 4·8 per cent. The concentrations found in the poisoned pigs agreed with the range of 4 to 10 per cent. quoted by Garner (1961) as the concentrations usually found in accidentally poisoned animals. Small intestine contents showed quite a different picture in that both groups receiving salt had high concentrations, the group receiving water ad lib having the highest concentration of all.

Urine. Chloride and sodium concentrations in urine reflected the salt administrations, water deprivation having little effect. The highest concentrations were in the $NaCl + H_2O$ group.

Cerebro-spinal fluid. The average results for cerebrospinal fluids are set out in Table 3. The poisoned group ($NaCl - H_2O$) showed markedly higher chloride and sodium contents than the other three groups. The sodium results were similar to those found by Medway and Kare (1959), the average of 196 m. eq/l being even higher than the 179 m. eq/l found by these workers. Analysis of variance results are set out in Table 4. The effects of both salt administration and water restriction on chloride and sodium levels were highly significant and

TABLE 3

AVERAGE CHLORIDE, SODIUM AND POTASSIUM CONCENTRATIONS IN CEREBROSPINAL FLUID OF PIGS (M./EQ/l)

Group	*Cl*	*Na*	*K*
$C+H_2O$	122	140	14·2
$C-H_2O$	141	153	16·2
$NaCl+H_2O$	132	151	17·0
$NaCl-H_2O$	181	196	19·3

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TABLE 4

STATISTICAL EVALUATION OF TREATMENT EFFECTS ON THE Cl, Na AND K CONTENTS OF CEREBROSPINAL FLUID

	Salt Vs O	*Water Vs O*	*NaCl × H_2O*
Cl	>1·0%	>0·1%	>2·5%
Na	>0·1%	>0·1%	>1·0%
K	N.S.	N.S.	N.S.

the interaction of the two treatments was also significant. There was no significant effect on the potassium levels, all the cerebrospinal fluids in this experiment containing high concentrations of the element, ranging in the toxic group from 9·2 to 33·8 m. eq/l. Medway and Kare (1959) found similarly high concentrations in salt poisoned pigs, but much lower concentrations (average 5·1 m. eq/l) in control animals; and in sodium sulphate poisoning, Dow, *et al.* (1963) found both low and high concentrations in experimentally poisoned animals. The explanation of these variations in potassium level may be found in the work of Fraschini, Muller and Zanoboni (1963) who showed that in human C.S.F. the potassium concentrations may increase very rapidly after death, whereas sodium and calcium contents do not. Samples from the same subject 8, 12 and 24 hours after death had potassium concentrations of 5·8, 19·5 and 41 m. eq/l. It would seem, therefore, that factors other than sodium chloride, or sodium sulphate treatments are more important in determining C.S.F. potassium concentrations in cases of salt poisoning.

Tissues. The results of the anlaysis of the six tissues are set out in Table 5. Treatments, especially the NaCl — H_2O treatment, had much more marked effects on chloride and sodium concentrations than on potassium. In view of the decreased water content of tissues of salt-poisoned animals as compared with controls (Medway and Kare, 1959; Bohosiewicz, 1962) it is probable that the fluctuations in potassium levels are mainly the result of dehydration, since in each case the water-deprived group showed higher concentrations than the corresponding groups given water ad lib.

Considering the chloride and sodium results, it is evident that the effects of the treatments were similar in all the tissues and group averages were in all cases in the order:— NaCl — $H_2O \gg$ C — H_2O > NaCl + H_2O > C + H_2O. Increases in the average chloride concentrations in the poisoned group over the control group ranged from 60 per cent. to 115 per cent. and in sodium concentration from 45 per cent. to 100 per cent. The two intermediate groups showed much smaller increases over the controls, but it is evident that water deprivation alone had generally a greater effect on chloride and sodium concentrations than when sodium chloride was administered with an adequate supply of water.

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TABLE 5

AVERAGE CONTENTS OF CHLORIDE, SODIUM AND POTASSIUM IN TISSUES (M.EQ/KG. FRESH TISSUE)

Tissue		Treatment group			
		$C+H_2O$	$C-H_2O$	$NaCl+H_2O$	$NaCl-H_2O$
Cerebrum	Cl	47	54	50	78
	Na	53	57	58	82
	K	82	85	84	92
Cerebellum	Cl	46	52	47	78
	Na	54	58	56	79
	K	73	75	77	81
Liver	Cl	43	52	45	80
	Na	38	42	42	76
	K	70	79	77	83
Kidney	Cl	58	70	67	105
	Na	60	66	97	97
	K	63	68	65	68
Heart	Cl	42	52	47	90
	Na	38	39	40	55
	K	87	91	86	112
Spleen	Cl	51	58	56	82
	Na	40	45	43	75
	K	95	101	98	75

DISCUSSION

The results of this experiment suggest that the primary cause of the salt poisoning syndrome is water deprivation. When water was supplied ad lib. high salt intake was not dangerous. In accidental poisoning it is generally found that the precipitating factor is interruption of water supplies to pigs on a relatively high salt intake. In this experiment, however, clinical and histological evidence of salt poisoning were produced by severely restricting water intake when salt intake was normal (0·5 per cent. of ration). Increases in chloride and sodium concentrations were also produced in blood plasma, but not in the tissues. It seems obvious, therefore, that the condition is primarily one of dehydration which, in the presence of sodium ions, produces characteristic brain damage. The diagnosis of acute salt poisoning is difficult without taking into consideration the history and the gross, histological and biochemical findings. Animals surviving the condition for any length of time can be diagnosed fairly readily by either histological or biochemical examination.

The results of this experiment suggest that the most useful biochemical tests would be chloride and sodium analyses of cerebrospinal fluid, brain and possibly spleen. Analysis of stomach contents for chloride, expressed as NaCl, has been commonly used for diag-

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nostic purposes and the results in Table 2 confirm that poisoned animals generally contain more than 4 per cent. NaCl in the dry matter of the stomach. In a survey of stomach contents of normal pigs going through a bacon factory, however, Todd (1956) found that when pigs were held overnight without food before slaughter 30 per cent. were found to contain more than 4 per cent. "salt", whereas pigs slaughtered soon after arrival generally yielded values of less than 2 per cent. and none was more than 4 per cent. If the stomach contains little dry matter, therefore, hydrochloric acid secretion can seriously interfere with diagnosis by this method, and analysis of body fluids and tissues would seem preferable.

CONCLUSIONS

Salt poisoning was produced experimentally in pigs under conditions of severe water restriction and high salt intake.

Histological examination of brain tissue confirmed previous descriptions of the lesion.

Chloride, sodium and potassium analyses were made on gut contents, body tissues and fluids. The results indicated that chloride and sodium contents of cerebrospinal fluid, blood plasma, brain, liver, kidney, spleen and heart were markedly increased in fatal cases.

High salt intake had none of these effects when water was available ad lib.

Water restriction on normal salt intake produced similar clinical and histological lesions in one animal, but tissue levels of sodium and chloride were not increased.

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THE ACUTE TOXICITY OF SOME COMMON SALTS OF SODIUM, POTASSIUM AND CALCIUM TO THE COMMON BLUEGILL (Lepomis macrochirus Rafinesque)

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INTRODUCTION

The purpose of this work was to determine by controlled experiments the acute toxicity of sodium chloride, sodium sulfate, sodium nitrate, potassium chloride, potassium sulfate, potassium nitrate, calcium chloride, calcium sulfate, and calcium nitrate to the common bluegill, *Lepomis macrochirus*. From these data the relative toxicity of these salts and their anions and cations could be obtained.

Experimental studies on the toxicity of sodium, potassium, and calcium salts of chlorides, sulfates, and nitrates to fish have been few, and in many cases of little practical value. The work of Jones (1939) using Aberystwyth tap water and the three-spined stickleback is perhaps one of the better papers. He found a relationship between the solution pressures of metals and the degree of toxicity of their salts when put on a milligram per liter or on a molar concentration basis. For the metals pertinent to this paper, Jones found the order of increasing toxicity to be Na, Ca, and K on a molar concentration basis, and Ca, Na, and K on a milligram per liter basis. Garrey (1916), working with *Notropis blennius* Girard in distilled water, found that with equivalent concentrations the relative toxicity is K>Mg>Ca>Na for the chlorides. Young (1923) used perch, sunfish, and catfish in both a natural well water and a cistern water. He found no simple relationship between the degree of dissociation of the chlorides and the toxicity of Ca, Na, and K. The degree of dissociation for $CaCl_2$, NaCl, and KCl are given by Young as 81.5 per cent, 85.0 per cent, and 91.8 per cent. The toxicities of these salts were found to be for $CaCl_2$, 9,500–13,500 p.p.m.; for NaCl, 9,100–17,550 p.p.m.; and for KCl, 1,360 p.p.m., the only concentration tested, was found to be above the minimum lethal concentration. The results of Wiebe et al. (1934) on the golden shiner in aerated distilled water are of interest. These workers

found that NaCl killed the fish in a shorter length of time at 20,000 and at 15,000 p.p.m. than did $CaCl_2$ at the same concentrations. At 10,000 p.p.m., however, the $CaCl_2$ was more rapidly fatal than NaCl at that concentration. The explanation offered is a possible difference in the absorption rate of the two salts by the blood. Ellis (1940) found that goldfish in Mississippi River water were not affected by 5,000 p.p.m. NaCl, but survived only ten days or less in 10,000 p.p.m.

Phillips (1944) gives an excellent account of the physiological effects of strong NaCl solutions on brook trout. A 30-minute bath in a 3 per cent solution or a 10-minute bath in a 5 per cent solution gave an increase in blood salinity that quickly receded when the fish were placed in fresh water. A 60-minute exposure to a 3 per cent solution gave a very high chloride level that required 48 hours to return to normal. A 15-minute bath in a 5 per cent solution killed most of the fish. Those that survived and were transferred to fresh water still had a high chloride level that was apparently returning to the normal level.

Jones (1939) found that the three-spined stickleback in Aberystwyth tap water, a soft water, tolerated almost equal amounts of $NaNO_3$ and $Ca(NO_3)_2$ (0.0217 M $NaNO_3$ and 0.02 M $Ca(NO_3)_2$). With *Polycelis nigra*, a flatworm, as the test organism, Jones (1940) stated that the nitrates of certain metals were somewhat more toxic than the chlorides and, therefore, the chlorides more accurately represented metallic toxicities. In 1941 Jones gave the following order for the increasing toxicity to the flatworm of the anions pertinent to this paper: $Cl<SO_4<NO_3$. In these experiments he used the sodium salts of the anions tested. Wells (1915) found that 1,203 p.p.m. (0.012 M) KNO_3 in tap water killed bluegills in 15 days, while 869 p.p.m. (0.005 M) K_2SO_4 was lethal in four days. Jones (1939) found that 0.00127 M (127 p.p.m.) KNO_3 was the toxic threshold for the three-spined stickleback. Sanborn (1945), using a medium hard tap water and a continuous-flow apparatus, found that goldfish were not affected by 1,000 p.p.m. $NaNO_3$; they survived for four days in 2,000 p.p.m. but only 14 to 26 hours in a 4,000 p.p.m. $NaNO_3$ solution. With bluegills he found that 1,000 p.p.m. $NaNO_3$ was not lethal and in 2,000 p.p.m. the fish survived from three days to more than seven days, while at 4,000 p.p.m. they survived from 14 hours to more than seven days.

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Methods and Procedures

The test containers were five-gallon Pyrex jars with No. 12 rubber stoppers. Each was fitted with an aeration tube, 7 mm. in outside diameter, which extended the length of the jar, and a short air vent tube, which also served as an aperture through which a Tygon plastic tube was inserted for periodic withdrawal of samples. The jars were placed in a constant temperature tank (20° ± 1° C.). Compressed air at a rate of approximately 160 bubbles per minute was forced through the water over the 96-hour test period.

A constant dilution water, Chu 14, modified (Chu, 1942), was prepared from distilled water and analytical or reagent grade chemicals. The following is a list of the chemical constituents and their final concentrations:

KCl	0.02	g./l.
Na_2SiO_3	0.02	g./l.
$NaHCO_3$	0.04	g./l.
$MgSO_4 \cdot 7H_2O$	0.04	g./l.
$Ca(NO_3)_2$	0.03	g./l.
$CaCO_3$	0.01	g./l.
K_2HPO_4	0.002	g./l.
Ferric citrate-citric acid	0.004	g. Fe^{+++}/l.

In the NaCl tests, $NaHCO_3$ and Na_2SiO_3 were not added in preparing the dilution water. In the KCl tests, K_2HPO_4 and KCl were omitted from the dilution water.

Each chemical was pipetted individually in the above listed order from a concentrated stock solution (2000x), directly into five gallons of distilled water in order to prevent precipitation of the chemicals. The volume was then raised to 20.4 liters by adding distilled water. Each test container was filled and then aerated for a period of 65 hours prior to the starting of the test. All chemicals that were tested were c.p. Baker Analyzed and were added to the test jars approximately one hour before the introduction of the test fish. Any water of hydration present in the chemical was compensated for in weighing out the desired amounts.

The test organism was the common bluegill, *Lepomis macrochirus* Raf. Fish were purchased from a private fish hatchery in Maryland. All were healthy and free from any visible diseases. Prior to use they were held in large aquaria filled with Philadelphia tapwater. The test and control fish were acclimated seven days in a large aquarium filled with Chu 14, modified, and held at a temperature of 20° ± 1° C. All fish were fed daily with chopped, freshly cooked shrimp (15 min. in boiling water). The acclimated fish were never fed in the 43 hours preceding a test period nor during the

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test period. Sizes ranged from 5 cm. to 9 cm. in total length with an average weight range of 1 g. to 9 g. In accordance with the recommendation of Hart et al. (1945), no fish varied more than 50 per cent in total length from any other fish in any one test container. Ten fish were placed in each container and tests were run in duplicate for every concentration.

The criteria of the death point were the cessation of gill movement and lack of response to a mechanical stimulus for a period of five minutes. Fish that succumbed during the test period were removed at the end of every 24 hours. Those that survived were sacrificed at the end of the test period.

A control group of fish was maintained with every series of tests at the rate of 10 control fish per 100 test fish. The control fish were kept under exactly the same conditions as the test fish in all respects except for the addition of the chemical whose toxicity was to be evaluated. At no time during the course of these experiments were there any losses in the controls.

Immediately prior to the introduction of the fish and at the end of the 24-, 48-, 72-, and 96-hour test periods, the pH of the test solution was determined with a Beckman pH meter using the external electrodes. The specific resistance was measured with a conductivity dip cell (constant 0.1) and a conductivity bridge and is reported as specific conductivity in mhos at 20° C. At the end of 24, 48, 72, and 96 hours, a mortality count was taken and the dissolved oxygen was determined by the sodium azide modification of the Winkler method. Total alkalinity and hardness were determined for each test bottle at the end of the 24-hour period.

Results of Experiments

The experimental results obtained are given below in Tables 1 to 46. The following abbreviations will be used in the tables and in the discussion of results:

Alkalinity M—the alkalinity as indicated by methyl orange beyond that shown by phenolphthalein, as p.p.m. $CaCO_3$.

Alkalinity P—the alkalinity as indicated by phenolphthalein, as p.p.m. $CaCO_3$.

D. O.—dissolved oxygen, in p.p.m.

Hardness—total hardness, as p.p.m. $CaCO_3$.

p.p.m.—parts per million (milligrams per liter).

Per cent Surv.—per cent survival based on ten fish.

Sp. Cond.—specific conductivity, as mhos at 20° C.

TL_m—medial tolerance limit (concentration at which 50 per cent of organisms would be expected to survive).

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Discussion of Results

With the exception of $CaSO_4$ all the salts tested under the above experimental conditions were found to be toxic to fish. If $CaSO_4$ is toxic to fish, it was not discernible in these experiments, since a saturated solution at 20° C. (2,930 p.p.m.) produced no significant mortalities (Table 39).

The 96-hour survival curves for three sodium salts are given in Figure 1, those for the three potassium salts in Figure 2, and those for two calcium salts in Figure 3. The molar concentrations used are plotted on a logarithmic scale and the per cent survivals are plotted on an arithmetic scale. The 96-hour TL_m values for the salts tested will be found in Table 46. Except for the TL_m values of the sodium salts and for $Ca(NO_3)_2$, which were obtained experimentally, the values were estimated for the 96-hour survival curves (Figs. 2 and 3).

From these data it is evident that these salts, on a basis of their molar concentration, have the following relative toxicity: $NaCl < NaNO_3 < CaCl_2 < Na_2SO_4 < Ca(NO_3)_2 < KNO_3 < KCl < K_2SO_4$. However, if one considers the cations, the relative toxicities would be $Na < Ca < K$.

The relative toxicity of the anion on a molar basis is somewhat obscured by the toxicity of the cation with which it is associated. In the calcium and potassium salts the cation produces toxic conditions before the toxic concentration of the anion, as indicated by the sodium salts, is reached. For this reason relative toxicity of anions can only be studied in the sodium salts. In this series of studies $Cl < NO_3 < SO_4$. These findings differ from those of Jones (1941) on the flatworm, in that he found SO_4 to be slightly less toxic than NO_3 in the sodium salt form.

Summary

1. A brief account of past work dealing with the toxicity of the nine salts used in these experiments is given.

2. The sodium, potassium, and calcium salts of chloride, sulfate, and nitrate were tested under the same experimental conditions and their toxic properties evaluated.

3. A soft synthetic dilution water was used in all these experiments and the common bluegill, *Lepomis macrochirus* Raf., was the test organism.

4. The relative toxicity of the various salts was: $NaCl < NaNO_3 < CaCl_2 < Na_2SO_4 < Ca(NO_3)_2 < KNO_3 < KCl < K_2SO_4$. The relative toxicity of the cations was $Na < Ca < K$. The relative toxicity of the anions, based on the experiments with the sodium salts, was $Cl < NO_3 < SO_4$.

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Table 1. Sodium chloride (NaCl): 0.200 molar - 11,696 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	8.45	164 x 10^{-4}			7.95	162 x 10^{-4}		
24	7.32	167 x 10^{-4}	4.9	100	6.68	162 x 10^{-4}	6.4	100
48	7.55	145 x 10^{-4}	6.9	100	6.77	143 x 10^{-4}	6.6	100
72	7.75	128 x 10^{-4}	7.2	100	6.90	130 x 10^{-4}	7.2	100
96	6.94	154 x 10^{-4}	7.2	100	6.93	154 x 10^{-4}	7.2	100

* Alkalinity (M), 20.8; hardness, 51.0
** Alkalinity (M), 19.4; hardness, 52.0

Table 2. Sodium chloride (NaCl): 0.209 molar - 12,243 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	7.30	167 x 10^{-4}			7.36	166 x 10^{-4}		
24	6.74	172 x 10^{-4}	6.6	100	6.60	167 x 10^{-4}	5.6	100
48	6.62	169 x 10^{-4}	6.4	100	6.61	167 x 10^{-4}	6.1	90
72	6.64	167 x 10^{-4}	5.8	90	6.72	167 x 10^{-4}	5.9	90
96	6.80	172 x 10^{-4}	5.0	90	6.71	167 x 10^{-4}	4.8	90

* Alkalinity (M), 19.0; hardness, 50.2
** Alkalinity (M), 20.6; hardness, 49.0

Table 3. Sodium chloride (NaCl): 0.221 molar - 12,946 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	7.86	145 x 10^{-4}			7.00	167 x 10^{-4}		
24	6.54	185 x 10^{-4}	4.8	100	6.69	179 x 10^{-4}	6.5	100
48	6.50	182 x 10^{-4}	4.1	90	6.63	179 x 10^{-4}	6.1	90
72	6.52	182 x 10^{-4}	4.5	90	6.65	173 x 10^{-4}	6.0	80
96	6.60	182 x 10^{-4}	4.2	50	6.76	176 x 10^{-4}	7.9	50

* Alkalinity (M), 22.0; hardness, 51.6
** Alkalinity (M), 17.4; hardness, 48.4

Table 4. Sodium chloride (NaCl): 0.225 molar - 13,161 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	6.95	182 x 10^{-4}			8.11	182 x 10^{-4}		
24	6.60	182 x 10^{-4}	5.6	100	6.50	167 x 10^{-4}	4.3	90
48	6.67	172 x 10^{-4}	5.2	100	6.52	182 x 10^{-4}	3.0	80
72	6.88	167 x 10^{-4}	6.8	80	7.08	172 x 10^{-4}	6.1	50
96	6.72	121 x 10^{-4}	5.0	50	7.05	182 x 10^{-4}	6.1	30

* Alkalinity (M), 25.4; hardness, 41.0
** Alkalinity (M), 23.2; hardness, 51.6

Table 5. Sodium chloride (NaCl): 0.234 molar - 13,705 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	6.90	192 x 10^{-4}			6.92	189 x 10^{-4}		
24	6.45	185 x 10^{-4}	5.1	70	6.53	185 x 10^{-4}	5.5	90
48	6.50	146 x 10^{-4}	3.7	20	6.69	173 x 10^{-4}	5.7	50
72	6.80	167 x 10^{-4}	2.9	10	6.96	173 x 10^{-4}	6.2	40
96	6.89	137 x 10^{-4}	4.9	0	6.92	135 x 10^{-4}	5.0	0

* Alkalinity (M), 29.0; hardness, 42.8
** Alkalinity (M), 25.8; hardness, 39.2

Table 6. Sodium sulfate (Na_2SO_4): 0.061 molar - 8,700 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	9.04	87 x 10^{-4}			9.13	85 x 10^{-4}		
24	7.30	89 x 10^{-4}	6.0	100	7.59	89 x 10^{-4}	7.1	100
48	7.12	72 x 10^{-4}	5.1	100	7.32	72 x 10^{-4}	6.8	100
72	7.20	66 x 10^{-4}	6.9	100	7.32	60 x 10^{-4}	7.3	100
96	7.20	90 x 10^{-4}	6.2	100	7.38	90 x 10^{-4}	7.8	100

* Alkalinity (M), 54.6; hardness, 35.0
** Alkalinity (M), 54.4; hardness, 40.0

Table 7. Sodium sulfate (Na_2SO_4): 0.070 molar - 10,000 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	8.47	102 x 10^{-4}			8.31	101 x 10^{-4}		
24	7.22	103 x 10^{-4}	6.4	100	7.41	101 x 10^{-4}	7.4	100
48	7.22	102 x 10^{-4}	6.2	100	7.32	101 x 10^{-4}	8.1	90
72	7.22	101 x 10^{-4}	6.6	100	7.47	101 x 10^{-4}	7.8	90
96	7.21	102 x 10^{-4}	6.3	100	7.47	101 x 10^{-4}	8.1	90

* Alkalinity (M), 51.5; hardness, 62.0
** Alkalinity (M), 48.2; hardness, 54.8

Table 8. Sodium sulfate (Na_2SO_4): 0.081 molar - 11,500 mg./liter

Time (Hours	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	9.01	107 x 10^{-4}			9.07	107 x 10^{-4}		
24	7.37	111 x 10^{-4}	6.2	100	7.37	111 x 10^{-4}	5.3	90
48	7.13	90 x 10^{-4}	5.6	100	7.20	90 x 10^{-4}	7.3	90
72	7.30	83 x 10^{-4}	7.1	100	7.30	133 x 10^{-4}	7.4	80
96	7.39	115 x 10^{-4}	7.8	80	7.39	116 x 10^{-4}	7.9	80

* Alkalinity (M), 53.0; hardness, 37.4
** Alkalinity (M), 52.0; hardness, 35.6

Table 9. Sodium sulfate (Na_2SO_4): 0.095 molar - 13,500 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	9.18	124×10^{-4}			9.21	124×10^{-4}		
24	7.52	127×10^{-4}	6.6	60	8.05	125×10^{-4}	5.9	90
48	7.50	105×10^{-4}	7.9	40	7.07	102×10^{-4}	4.4	70
72	7.52	130×10^{-4}	8.2	40	7.42	130×10^{-4}	7.9	60
96	7.53	134×10^{-4}	8.5	40	7.51	130×10^{-4}	8.2	60

* Alkalinity (M), 56.4; hardness, 38.0
** Alkalinity (M), 51.2; hardness, 39.0

Table 10. Sodium sulfate (Na_2SO_4): 0.099 molar - 14,000 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	8.40	130×10^{-4}			8.70	133×10^{-4}		
24	7.53	133×10^{-4}	8.2	20	7.46	135×10^{-4}	7.8	40
48	7.55	137×10^{-4}	8.2	10	7.52	139×10^{-4}	8.3	30
72	7.66	---	8.4	10	7.67	---	8.3	30
96	7.62	---	8.8	10	7.63	---	8.9	30

* Alkalinity (M), 54.0; hardness, 48.0
** Alkalinity (M), 51.8; hardness, 53.0

Table 11. Sodium sulfate (Na_2SO_4): 0.102 molar - 14,500 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	8.42	143×10^{-4}			8.51	141×10^{-4}		
24	7.50	143×10^{-4}	7.0	0	7.45	141×10^{-4}	7.6	0
48	---	---	---	---	---	---	---	---
72	---	---	---	---	---	---	---	---
96	---	---	---	---	---	---	---	---

* Alkalinity (M), 46.4; hardness, 44.4
** Alkalinity (M), 44.4; hardness, 43.6

Table 12. Sodium nitrate ($NaNO_3$): 0.118 molar - 10,000 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	8.77	102×10^{-4}			8.70	102×10^{-4}		
24	7.77	102×10^{-4}	5.9	100	7.70	103×10^{-4}	5.9	100
48	7.32	102×10^{-4}	5.1	100	7.38	103×10^{-4}	4.8	100
72	7.35	103×10^{-4}	5.3	100	7.35	104×10^{-4}	4.6	100
96	7.43	104×10^{-4}	5.6	100	7.40	105×10^{-4}	5.1	100

* Alkalinity (M), 52.0; hardness, 46.0
** Alkalinity (M), 51.0; hardness, 45.0

Table 13. Sodium nitrate ($NaNO_3$): 0.135 molar - 11,500 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	8.15	111 x 10^{-4}			8.34	111 x 10^{-4}		
24	7.54	122 x 10^{-4}	4.6	100	7.63	118 x 10^{-4}	4.8	100
48	7.32	120 x 10^{-4}	4.6	100	7.38	118 x 10^{-4}	5.0	90
72	7.38	122 x 10^{-4}	5.3	80	7.32	118 x 10^{-4}	5.5	80
96	7.39	119 x 10^{-4}	5.8	80	7.30	116 x 10^{-4}	4.9	80

* Alkalinity (M), 55.0; hardness, 50.0
** Alkalinity (M), 53.0; hardness, 46.0

Table 14. Sodium nitrate ($NaNO_3$): 0.141 molar - 12,000 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	8.70	122 x 10^{-4}			8.70	122 x 10^{-4}		
24	7.75	122 x 10^{-4}	5.8	90	7.70	122 x 10^{-4}	4.1	100
48	7.27	123 x 10^{-4}	4.5	80	7.22	123 x 10^{-4}	4.3	80
72	7.38	123 x 10^{-4}	5.0	50	7.40	123 x 10^{-4}	5.0	60
96	7.53	125 x 10^{-4}	6.2	50	7.60	125 x 10^{-4}	6.3	50

* Alkalinity (M), 54.0; hardness, 48.0
** Alkalinity (M), 52.0; hardness, 47.0

Table 15. Sodium nitrate ($NaNO_3$): 0.147 molar - 12,500 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	8.52	125 x 10^{-4}			8.78	125 x 10^{-4}		
24	7.40	128 x 10^{-4}	4.2	100	7.68	127 x 10^{-4}	5.0	100
48	7.40	128 x 10^{-4}	5.3	30	7.30	127 x 10^{-4}	4.5	70
72	7.58	128 x 10^{-4}	6.2	20	7.38	127 x 10^{-4}	5.1	40
96	7.65	128 x 10^{-4}	6.6	20	7.56	127 x 10^{-4}	6.2	20

* Alkalinity (M), 56.0; hardness, 47.0
** Alkalinity (M), 54.0; hardness, 45.0

Table 16. Sodium nitrate ($NaNO_3$): 0.159 molar - 13,500 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	8.30	128 x 10^{-4}			8.32	128 x 10^{-4}		
24	7.54	137 x 10^{-4}	4.6	30	7.46	139 x 10^{-4}	4.2	70
48	7.50	139 x 10^{-4}	6.2	10	7.36	139 x 10^{-4}	5.1	30
72	7.64	141 x 10^{-4}	5.8	10	7.47	141 x 10^{-4}	5.6	20
96	7.66	139 x 10^{-4}	6.3	0	7.58	139 x 10^{-4}	6.2	0

* Alkalinity (M), 53.0; hardness, 47.0
** Alkalinity (M), 53.0; hardness, 47.0

Table 17. Potassium chloride (KCl): 0.022 molar - 1,661 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond	D.O.	% Surv.
0	8.59	25.6 x 10^{-4}			8.55	26.2 x 10^{-4}		
24	7.44	24.4 x 10^{-4}	7.7	100	7.50	24.4 x 10^{-4}	8.1	100
48	7.42	25.6 x 10^{-4}	8.0	100	7.51	25.0 x 10^{-4}	8.1	100
72	7.37	25.0 x 10^{-4}	8.0	100	7.44	25.0 x 10^{-4}	8.4	100
96	7.42	24.4 x 10^{-4}	7.8	100	7.51	26.2 x 10^{-4}	8.1	100

* Alkalinity (M), 45.0; hardness, 39.4
** Alkalinity (M), 46.0; hardness, 40.0

Table 18. Potassium chloride (KCl): 0.026 molar - 1,911 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	8.20	16.5 x 10^{-4}			9.37	32.2 x 10^{-4}		
24	7.45	28.6 x 10^{-4}	8.6	100	7.60	32.2 x 10^{-4}	6.8	100
48	7.34	26.3 x 10^{-4}	7.3	100	7.45	32.2 x 10^{-4}	7.2	100
72	7.43	25.0 x 10^{-4}	6.2	80	7.10	32.2 x 10^{-4}	3.0	100
96	7.42	27.1 x 10^{-4}	6.1	80	7.37	32.1 x 10^{-4}	7.3	100

* Alkalinity (M), 40.4, hardness, 41.4
** Alkalinity (M), 45.0; hardness, 33.4

Table 19. Potassium chloride (KCl): 0.028 molar - 2,054 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	8.50	17.9 x 10^{-4}			8.38	16.7 x 10^{-4}		
24	7.50	29.4 x 10^{-4}	7.9	90	7.58	29.4 x 10^{-4}	8.1	80
48	7.53	26.4 x 10^{-4}	8.2	30	7.53	27.8 x 10^{-4}	7.7	60
72	7.47	27.8 x 10^{-4}	7.4	20	7.49	27.1 x 10^{-4}	7.7	60
96	7.32	29.4 x 10^{-4}	6.1	20	7.53	27.8 x 10^{-4}	7.8	50

* Alkalinity (M), 42.4; hardness, 40.0
** Alkalinity (M), 43.4; hardness, 45.0

Table 20. Potassium chloride (KCl): 0.030 molar - 2,200 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	8.20				8.60			
24	7.61	10.0 x 10^{-4}	8.3	70	7.70	10.2 x 10^{-4}	9.2	60
48	7.68	25.0 x 10^{-4}	8.8	20	7.77	25.7 x 10^{-4}	8.8	30
72	7.45	25.7 x 10^{-4}	7.4	20	7.71	25.7 x 10^{-4}	7.9	20
96	7.25	26.4 x 10^{-4}	3.5	0	7.59	29.5 x 10^{-4}	6.4	0

* Alkalinity (M), 43.8; hardness, 40.4
** Alkalinity (M), 41.4; hardness, 38.4

Table 21. Potassium sulfate (K_2SO_4): 0.014 molar - 2,400 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	9.20	31.3×10^{-4}			9.20	31.3×10^{-4}		
24	7.73	32.1×10^{-4}	5.0	100	7.88	31.3×10^{-4}	5.4	100
48	7.70	32.1×10^{-4}	5.1	100	7.58	31.3×10^{-4}	5.0	100
72	7.40	32.3×10^{-4}	4.6	100	7.40	31.3×10^{-4}	4.6	100
96	7.45	32.3×10^{-4}	5.1	100	7.40	31.3×10^{-4}	5.0	100

* Alkalinity (M), 49.0; hardness, 42.0
** Alkalinity (M), 50.0; hardness, 44.0

Table 22. Potassium sulfate (K_2SO_4): 0.016 molar - 2,800 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	8.52	35.7×10^{-4}			8.75	35.7×10^{-4}		
24	7.40	34.7×10^{-4}	6.1	100	7.55	34.7×10^{-4}	5.8	100
48	7.28	36.0×10^{-4}	5.6	100	7.33	36.0×10^{-4}	5.4	100
72	7.20	36.0×10^{-4}	4.2	100	7.35	36.0×10^{-4}	4.1	100
96	7.30	35.7×10^{-4}	4.3	90	7.30	35.7×10^{-4}	4.3	90

* Alkalinity (M), 54.0; hardness, 44.0
** Alkalinity (M), 54.0; hardness, 44.0

Table 23. Potassium sulfate (K_2SO_4): 0.018 molar - 3,200 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	8.30	40.8×10^{-4}			8.40	40.8×10^{-4}		
24	7.42	40.0×10^{-4}	5.8	100	7.38	40.0×10^{-4}	4.9	100
48	7.30	40.8×10^{-4}	5.3	100	7.22	40.8×10^{-4}	4.0	90
72	7.16	40.8×10^{-4}	4.3	100	7.38	41.3×10^{-4}	5.1	70
96	7.23	40.8×10^{-4}	5.1	80	7.50	41.3×10^{-4}	5.8	60

* Alkalinity (M), 55.0; hardness, 45.0
** Alkalinity (M), 55.0; hardness, 46.0

Table 24. Potassium sulfate (K_2SO_4): 0.024 molar - 4,200 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	8.62	52.6×10^{-4}			8.88	50.5×10^{-4}		
24	7.46	52.6×10^{-4}	4.8	60	7.48	50.5×10^{-4}	4.2	60
48	7.38	52.6×10^{-4}	5.9	50	7.37	50.0×10^{-4}	5.5	60
72	7.34	53.8×10^{-4}	4.8	50	7.36	51.3×10^{-4}	5.1	40
96	7.32	52.6×10^{-4}	5.1	30	7.35	50.5×10^{-4}	5.8	30

* Alkalinity (M), 58.0; hardness, 48.0
** Alkalinity (M), 54.0; hardness, 43.0

Table 25. Potassium sulfate (K_2SO_4): 0.028 molar - 4,900 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	8.80	58.1×10^{-4}			9.00	53.1×10^{-4}		
24	8.30	58.8×10^{-4}	5.3	50	8.65	58.8×10^{-4}	5.4	10
48	7.48	58.8×10^{-4}	4.6	20	8.12	58.8×10^{-4}	4.3	10
72	7.57	58.8×10^{-4}	5.6	10	7.83	58.8×10^{-4}	4.8	10
96	7.66	58.8×10^{-4}	6.5	0	7.82	58.8×10^{-4}	5.9	10

* Alkalinity (M), 59.0; hardness, 48.0
** Alkalinity (M), 56.0; hardness, 44.0

Table 26. Potassium sulfate (K_2SO_4): 0.032 molar - 5,600 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	8.91	64.5×10^{-4}			9.00	64.5×10^{-4}		
24	7.68	64.5×10^{-4}	5.6	40	7.66	64.5×10^{-4}	5.6	40
48	7.34	65.8×10^{-4}	5.4	30	7.34	65.8×10^{-4}	5.3	30
72	7.45	65.8×10^{-4}	5.0	30	7.50	65.8×10^{-4}	5.8	30
96	7.40	66.7×10^{-4}	5.5	0	7.60	66.7×10^{-4}	7.0	0

* Alkalinity (M), 55.0; hardness, 43.0
** Alkalinity (M), 54.0; hardness, 42.0

Table 27. Potassium nitrate (KNO_3): 0.021 molar - 2,100 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	8.20	26.3×10^{-4}			8.30	26.3×10^{-4}		
24	7.63	26.3×10^{-4}	6.2	100	7.70	26.3×10^{-4}	6.2	100
48	7.52	26.3×10^{-4}	6.7	100	7.56	26.3×10^{-4}	7.0	100
72	7.50	26.3×10^{-4}	7.5	100	7.52	26.3×10^{-4}	7.3	100
96	7.48	26.3×10^{-4}	7.4	100	7.52	26.3×10^{-4}	7.4	100

* Alkalinity (M), 53.0; hardness, 47.0
** Alkalinity (M), 53.0; hardness, 49.0

Table 28. Potassium nitrate (KNO_3): 0.024 molar - 2,400 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	8.35	29.9×10^{-4}			8.30	29.9×10^{-4}		
24	7.70	29.4×10^{-4}	6.7	100	7.72	29.4×10^{-4}	6.7	100
48	7.66	29.4×10^{-4}	7.8	100	7.72	29.4×10^{-4}	7.8	100
72	7.60	29.4×10^{-4}	7.6	100	7.72	29.4×10^{-4}	8.0	100
96	7.62	29.4×10^{-4}	7.7	80	7.74	29.4×10^{-4}	8.2	80

* Alkalinity (M), 54.0; hardness, 47.0
** Alkalinity (M), 54.0; hardness, 49.0

Table 29. Potassium nitrate (KNO_3): 0.028 molar - 2,800 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	8.22	34.5×10^{-4}			8.22	34.5×10^{-4}		
24	7.74	33.9×10^{-4}	6.7	100	7.72	33.9×10^{-4}	7.1	100
48	7.60	33.9×10^{-4}	7.2	100	7.64	33.9×10^{-4}	7.4	100
72	7.48	33.9×10^{-4}	6.9	90	7.62	33.9×10^{-4}	7.7	90
96	7.47	33.6×10^{-4}	7.0	60	7.60	33.6×10^{-4}	7.8	60

* Alkalinity (M), 52.0; hardness, 46.0
** Alkalinity (M), 53.0; hardness, 47.0

Table 30. Potassium nitrate (KNO_3): 0.032 molar - 3,200 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	8.06	38.5×10^{-4}			8.20	39.1×10^{-4}		
24	7.73	38.8×10^{-4}	5.3	100	7.72	39.7×10^{-4}	5.6	100
48	7.62	38.8×10^{-4}	5.0	80	7.62	39.7×10^{-4}	4.9	90
72	7.65	38.8×10^{-4}	6.4	50	7.68	39.7×10^{-4}	5.8	70
96	7.70	38.8×10^{-4}	8.2	40	7.70	39.7×10^{-4}	8.0	40

* Alkalinity (M), 54.0; hardness, 47.0
** Alkalinity (M), 57.0; hardness, 48.0

Table 31. Potassium nitrate (KNO_3): 0.037 molar - 3,700 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	8.30	43.5×10^{-4}			8.25	43.5×10^{-4}		
24	7.78	43.5×10^{-4}	5.0	100	7.75	43.5×10^{-4}	5.0	100
48	7.70	43.5×10^{-4}	5.3	40	7.68	43.5×10^{-4}	4.8	60
72	7.78	43.5×10^{-4}	6.6	20	7.72	43.5×10^{-4}	6.0	50
96	7.80	43.5×10^{-4}	8.3	20	7.73	43.5×10^{-4}	7.8	20

* Alkalinity (M), 55.0; hardness, 46.0
** Alkalinity (M), 54.0; hardness, 46.0

Table 32. Potassium nitrate (KNO_3): 0.042 molar - 4,200 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	8.60	50.0×10^{-4}			8.55	50.0×10^{-4}		
24	7.93	48.8×10^{-4}	5.9	90	7.83	48.8×10^{-4}	6.1	80
48	7.68	49.5×10^{-4}	6.1	30	7.68	49.5×10^{-4}	5.4	30
72	7.80	49.5×10^{-4}	7.8	10	7.80	48.8×10^{-4}	7.7	20
96	7.95	49.5×10^{-4}	8.2	0	7.90	49.5×10^{-4}	8.0	0

* Alkalinity (M), 50.0; hardness, 46.0
** Alkalinity (M), 52.0; hardness, 48.0

Table 33. Calcium chloride ($CaCl_2$): 0.059 molar - 6,500 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	7.80	100 x 10^{-4}			7.80	95 x 10^{-4}		
24	7.20	100 x 10^{-4}	7.4	100	7.32	98 x 10^{-4}	8.0	100
48	7.21	96 x 10^{-4}	8.0	100	7.37	92 x 10^{-4}	8.6	100
72	7.23	100 x 10^{-4}	8.0	100	7.42	99 x 10^{-4}	9.0	100
96	7.28	100 x 10^{-4}	8.4	100	7.44	99 x 10^{-4}	8.7	100

* Alkalinity (M), 33.8; hardness, 6,[illegible]
** Alkalinity (M), 32.6; hardness, 6,[illegible]

Table 34. Calcium chloride ($CaCl_2$): 0.070 molar - 8,700 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	7.65	123 x 10^{-4}			7.70	121 x 10^{-4}		
24	7.24	123 x 10^{-4}	7.2	90	7.26	125 x 10^{-4}	7.7	100
48	7.24	120 x 10^{-4}	8.0	90	7.32	120 x 10^{-4}	8.2	90
72	7.30	123 x 10^{-4}	8.0	80	7.38	123 x 10^{-4}	8.5	80
96	7.30	123 x 10^{-4}	7.9	80	7.39	123 x 10^{-4}	8.6	80

* Alkalinity (M), 36.0; hardness, 8,370
** Alkalinity (M), 35.8; hardness, 8,400

Table 35. Calcium chloride ($CaCl_2$): 0.095 molar - 10,500 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	7.42	145 x 10^{-4}			7.42	145 x 10^{-4}		
24	7.34	145 x 10^{-4}	8.0	100	7.32	143 x 10^{-4}	7.7	90
48	7.40	147 x 10^{-4}	8.1	80	7.35	147 x 10^{-4}	8.1	80
72	7.45	147 x 10^{-4}	8.3	70	7.40	145 x 10^{-4}	8.1	60
96	7.46	143 x 10^{-4}	8.6	60	7.42	145 x 10^{-4}	8.3	60

* Alkalinity (M), 44.6; hardness, 9,600
** Alkalinity (M), 45.4; hardness, 9,500

Table 36. Calcium chloride ($CaCl_2$): 0.097 molar - 10,800 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	7.38	152 x 10^{-4}			7.39	152 x 10^{-4}		
24	7.27	147 x 10^{-4}	7.7	100	7.29	147 x 10^{-4}	7.8	100
48	7.32	154 x 10^{-4}	7.9	100	7.34	149 x 10^{-4}	8.0	90
72	7.35	149 x 10^{-4}	8.2	70	7.40	149 x 10^{-4}	8.3	60
96	7.39	147 x 10^{-4}	8.3	40	7.33	149 x 10^{-4}	8.0	40

* Alkalinity (M), 44.6; hardness, 10,030
** Alkalinity (M), 46.4; hardness, 10,070

Table 37. Calcium chloride ($CaCl_2$): 0.108 molar - 12,000 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	7.37	167 x 10^{-4}			7.38	167 x 10^{-4}		
24	7.35	167 x 10^{-4}	8.0	30	7.30	167 x 10^{-4}	7.6	40
48	7.44	167 x 10^{-4}	8.6	30	7.42	167 x 10^{-4}	8.0	20
72	7.50	167 x 10^{-4}	8.4	20	7.50	167 x 10^{-4}	8.4	10
96	7.59	167 x 10^{-4}	8.6	20	7.60	167 x 10^{-4}	8.6	10

* Alkalinity (M), 47.0; hardness, 11,450
** Alkalinity (M), 46.6; hardness, 11,480

Table 38. Calcium chloride ($CaCl_2$): 0.113 molar - 12,500 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	7.30	173 x 10^{-4}			7.32	173 x 10^{-4}		
24	7.39	173 x 10^{-4}	7.4	40	7.33	173 x 10^{-4}	7.7	20
48	7.34	170 x 10^{-4}	7.9	0	7.43	170 x 10^{-4}	8.2	0
72	---	---	---	---	---	---	---	---
96	---	---	---	---	---	---	---	---

* Alkalinity (M), 49.4; hardness, 11,960
** Alkalinity (M), 49.6; hardness, 11,810

Table 39. Calcium sulfate ($CaSO_4$): 0.022 molar - 2,980 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	7.90	19.3 x 10^{-4}			7.90	18.6 x 10^{-4}		
24	7.48	19.3 x 10^{-4}	8.3	100	7.72	19.3 x 10^{-4}	9.1	100
48	7.48	19.3 x 10^{-4}	8.1	100	7.38	19.3 x 10^{-4}	7.8	100
72	7.59	19.6 x 10^{-4}	7.8	100	7.45	19.3 x 10^{-4}	7.9	90
96	7.46	16.1 x 10^{-4}	8.0	100	7.49	16.1 x 10^{-4}	8.6	90

* Alkalinity (M), 48.4; hardness, 1,688
** Alkalinity (M), 44.2; hardness, 1,645

Table 40. Calcium nitrate ($Ca(NO_3)_2$): 0.046 molar - 7,500 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	8.30	72.5 x 10^{-4}			8.05	76.9 x 10^{-4}		
24	7.58	76.9 x 10^{-4}	5.1	100	7.42	76.9 x 10^{-4}	4.6	100
48	7.28	76.9 x 10^{-4}	5.1	100	7.34	76.9 x 10^{-4}	5.3	100
72	7.20	78.1 x 10^{-4}	5.6	100	7.40	76.9 x 10^{-4}	6.6	100
96	7.28	78.1 x 10^{-4}	5.3	100	7.40	76.9 x 10^{-4}	6.2	100

* Alkalinity (M), 46.0; hardness, 4,700
** Alkalinity (M), 46.0; hardness, 4,600

Table 41. Calcium nitrate ($Ca(NO_3)_2$): 0.053 molar - 8,700 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	7.76	71.4 x 10^{-4}			7.90	62.5 x 10^{-4}		
24	7.20	88.5 x 10^{-4}	5.1	100	7.28	88.5 x 10^{-4}	5.4	90
48	7.06	89.3 x 10^{-4}	4.2	100	7.20	89.3 x 10^{-4}	4.8	90
72	7.18	90.9 x 10^{-4}	5.4	80	7.32	90.9 x 10^{-4}	5.0	80
96	7.20	88.5 x 10^{-4}	5.4	70	7.32	88.5 x 10^{-4}	5.3	70

* Alkalinity (M), 46.0; hardness, 5,250
** Alkalinity (M), 47.0; hardness, 5,300

Table 42. Calcium nitrate ($Ca(NO_3)_2$): 0.061 molar - 10,000 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	7.98	100 x 10^{-4}			7.98	100 x 10^{-4}		
24	7.22	100 x 10^{-4}	5.0	100	7.22	100 x 10^{-4}	5.0	100
48	7.24	100 x 10^{-4}	5.6	90	7.36	100 x 10^{-4}	5.6	90
72	7.18	100 x 10^{-4}	5.3	70	7.20	100 x 10^{-4}	5.0	70
96	7.28	100 x 10^{-4}	6.0	50	7.30	100 x 10^{-4}	6.2	50

* Alkalinity (M), 44.0; hardness, 6,200
** Alkalinity (M), 45.0; hardness, 6,150

Table 43. Calcium nitrate ($Ca(NO_3)_2$): 0.070 molar - 11,500 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	7.83	92.6 x 10^{-4}			7.90	95.2 x 10^{-4}		
24	7.24	111.0 x 10^{-4}	5.0	80	7.30	111.0 x 10^{-4}	5.6	60
48	7.08	114.0 x 10^{-4}	4.6	60	7.22	114.0 x 10^{-4}	5.0	50
72	7.32	114.0 x 10^{-4}	4.8	40	7.42	114.0 x 10^{-4}	5.3	30
96	7.38	111.0 x 10^{-4}	5.0	30	7.47	111.0 x 10^{-4}	5.6	30

* Alkalinity (M), 47.0; hardness, 7,150
** Alkalinity (M), 46.0; hardness, 7,150

Table 44. Calcium nitrate ($Ca(NO_3)_2$): 0.082 molar - 13,500 mg./liter

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	8.02	128 x 10^{-4}			7.75	127 x 10^{-4}		
24	7.43	128 x 10^{-4}	4.8	40	7.32	128 x 10^{-4}	4.5	50
48	7.30	128 x 10^{-4}	5.0	20	7.32	127 x 10^{-4}	5.3	20
72	7.36	128 x 10^{-4}	5.9	0	7.48	127 x 10^{-4}	6.4	0
96	---	---	---	---	---	---	---	---

* Alkalinity (M), 44.0; hardness, 8,450
** Alkalinity (M), 45.0; hardness, 8,300

Table 45. Typical control: Chu 14, modified

Time (Hours)	Test 1*				Test 2**			
	pH	Sp. Cond.	D.O.	% Surv.	pH	Sp. Cond.	D.O.	% Surv.
0	8.24	1.44×10^{-4}			8.72	1.43×10^{-4}		
24	7.40	1.54×10^{-4}	7.9	100	7.62	1.49×10^{-4}	8.6	100
48	7.38	1.55×10^{-4}	7.8	100	7.50	1.56×10^{-4}	8.4	100
72	7.32	1.62×10^{-4}	7.2	100	7.59	1.73×10^{-4}	8.5	100
96	7.29	1.68×10^{-4}	6.2	100	7.60	1.73×10^{-4}	8.5	100

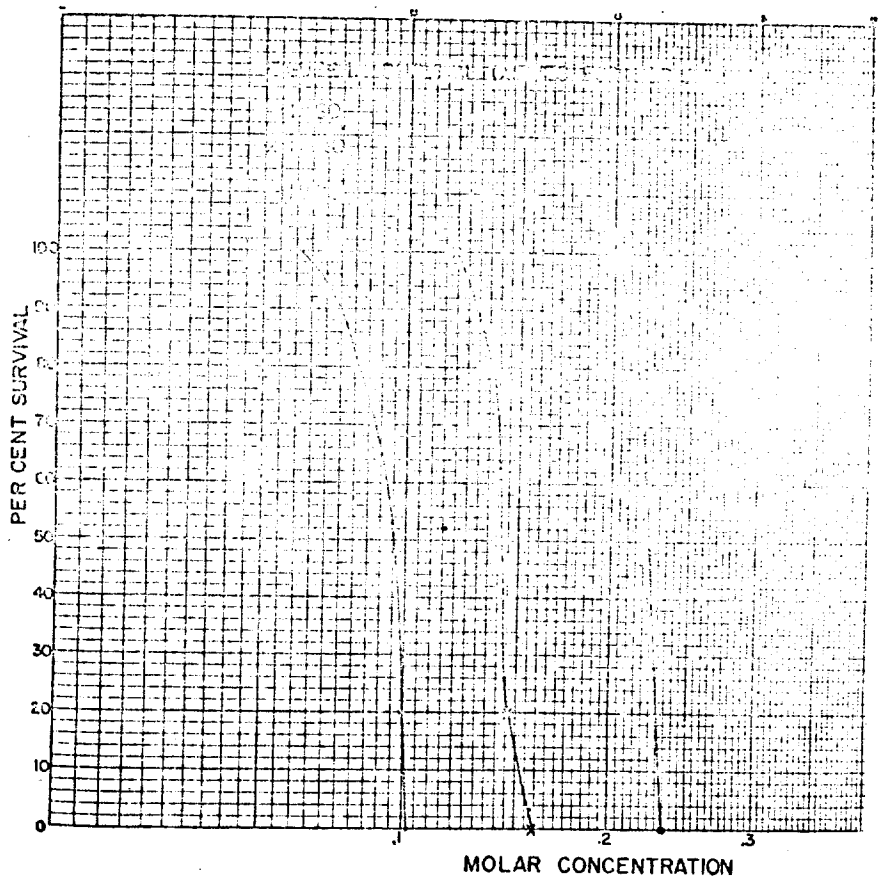
* Alkalinity (M), 36.8; hardness, 37.4
** Alkalinity (M), 37.0; hardness, 40.6

Table 46. Concentrations of salts (including both cations and anions) producing 50 per cent mortality in 96 hours

Chemical	Salt		Cation		Anion	
	mg./l.	molar concn.	mg./l.	molar concn.	mg./l.	molar concn.
NaCl	12,946	0.221	5,100	0.222	7,846	0.221
Na_2SO_4	13,500	0.095	4,372	0.095	9,128	0.095
$NaNO_3$	12,000	0.141	3,247	0.141	8,753	0.141
KCl	2,010	0.027	1,054	0.027	956	0.027
K_2SO_4	3,550	0.020	1,593	0.020	1,957	0.020
KNO_3	3,000	0.030	1,160	0.030	1,840	0.030
$CaCl_2$	10,650	0.096	3,846	0.096	6,804	0.096
$CaSO_4$	----	----	----	----	----	----
$Ca(NO_3)_2$	10,000	0.061	2,442	0.061	7,558	0.061

Errata in Table 46. In column under "Cation—molar concn." Second figure from top—for 0.095 read 0.190; fifth figure from top—for 0.020 read 0.041. In last column on right, under "Anion—molar concn." Seventh figure from top—for 0.096 read 0.192; last figure in column—for 0.061 read 0.122.

3303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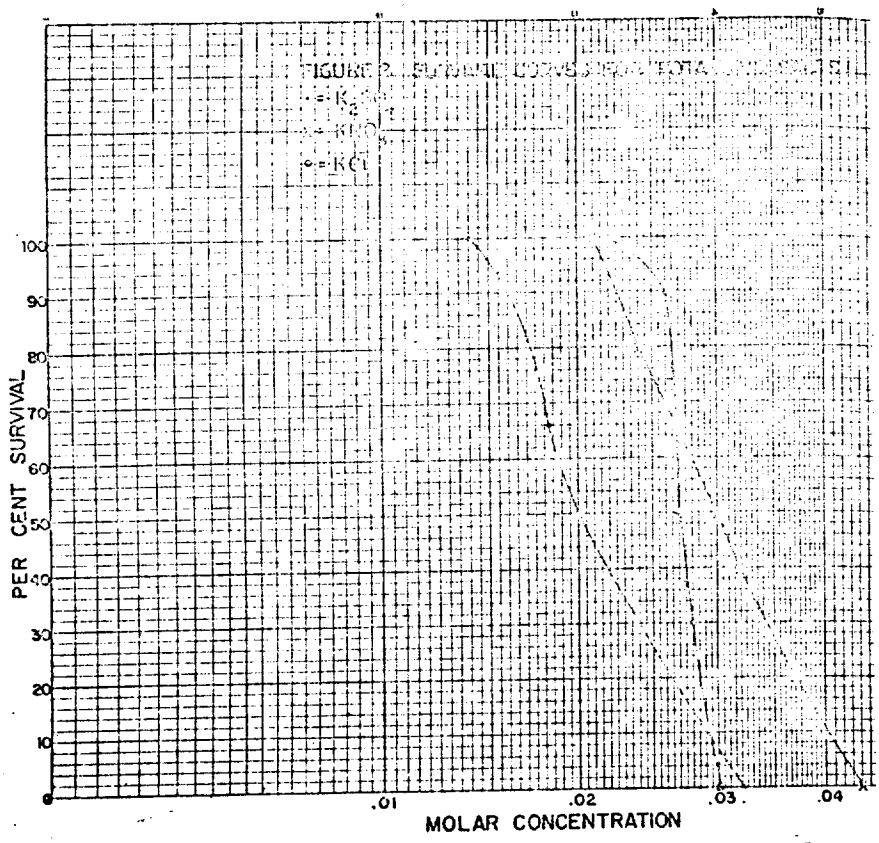
MOLAR CONCENTRATION

3303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MOLAR CONCENTRATION

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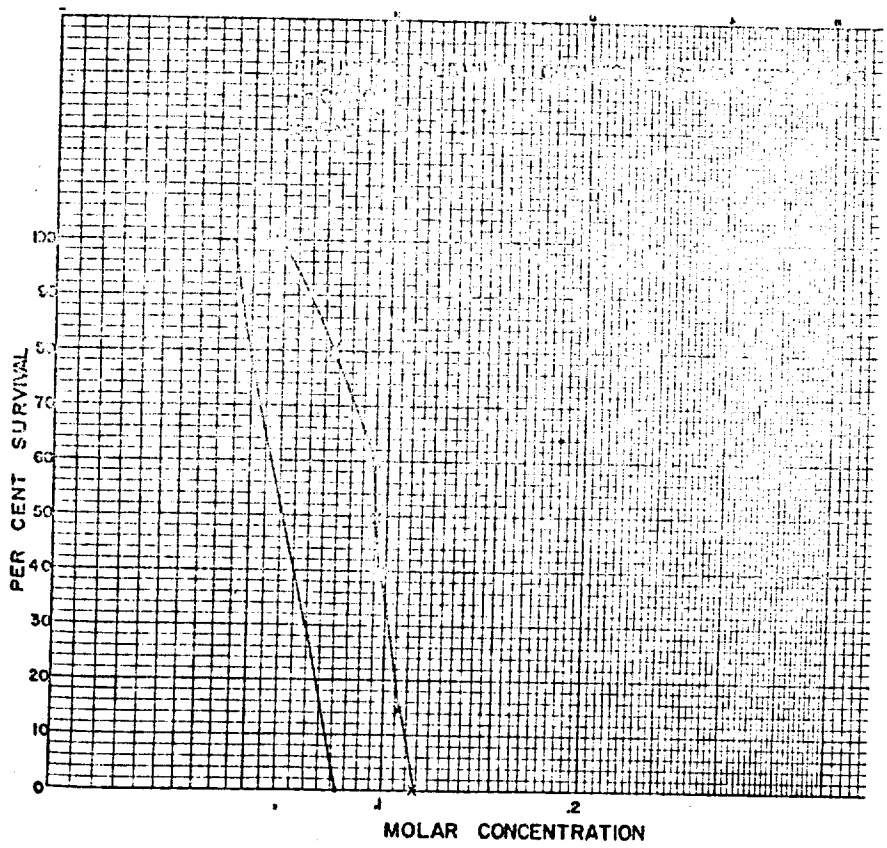
PER CENT SURVIVAL
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Plasma Potassium Curves in the Rabbit, Following Single and Repeated Injections of Potassium Chloride

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EARLIER work (1) has shown that in cats the plasma level of KCl injected intraperitoneally in sublethal doses appears to rise to a higher level and remain high for a longer time when the injection is repeated. It was thought desirable in connection with studies on shock, to repeat this work using rabbits, as being more uniform animal material and to relate the plasma levels with symptoms of toxicity.

METHODS

Male New Zealand white rabbits, bred at The Naval Medical Research Institute, weighing 750—2400 gm, were taken for the experiments.

Blood samples (0.3–0.6 ml) were taken from an ear vein before and at various times after intraperitoneal (i.p.) injection of 10% KCl. The samples were collected in 1-ml syringes containing a few grains of dry Na heparin (Connaught Laboratory), the plungers were removed and the barrels were closed by means of ⅜-inch rubber bands snapped over their long axes. The syringes were placed in 15-ml centrifuge cups, and spun for 10 minutes at 1850 rpm. The plasma and cell volumes were then read, most of the plasma was drawn off and transferred to a centrifuge tube, and centrifuging of the plasma was repeated; this was desirable in order to bring down from the plasma any remaining red blood cells with their high potassium content. Samples showing hemolysis were discarded. A measured volume of plasma, not exceeding 0.2 ml, is then taken up in a graduated 0.2-ml blood pipette, transferred to 10.1 ml of water in a 15-ml conical graduated centrifuge tube. Then 0.3 ml each of 10% Na tungstate and of ⅔ normal H_2SO_4 with 0.1 ml of 5% $AgNO_3$ are combined with water to 11 ml. The solutions are mixed, and the tubes are centrifuged.

Potassium is determined in two 5-ml portions of the centrifugate by the following modification of Truszkowski and Zwemer's argenticobaltinitrite method (2). Potassium is precipitated, and the precipitate is collected, washed, and decomposed with 0.2 N NaOH as described (2). The subsequent procedure has been modified as follows: 8-ml portions containing 5 ml of 10% acetic acid, 2 ml of 0.5% sulfphanilic acid in 30% acetic acid, and 1 ml of 0.5% α-naphthylamine in 30% acetic acid are placed in 20-ml Coleman spectrophotometer tubes graduated to 8 and 15 ml. Aliquots varying from 0.25–0.5 ml of the test material in NaOH solution are added, followed immediately by 10% acetic acid to the 15-ml mark, and the contents of the tube are mixed by inversion. The light transmission is read in a Coleman spectrophotometer, set at 490 mμ, and the K content of the sample is read from a standard transmission curve based on mg of K. The slope of this curve was determined for each operation, since it may vary, depending on slight but unavoidable differences in *a*) the exact composition of each batch of precipitating reagent, *b*) the time allowed for precipitation, *c*) the temperature at which it proceeds, and *d*) the temperature at which the precipitates are collected and washed. It is not, for this reason, possible to construct one transmissivity curve which would be applicable to all subsequent analyses. In this research, six tubes containing 0.02–0.08 mg of potassium (as standard KCl solution) were added to each series of determinations, to give a standard curve applicable to that series.

RESULTS

I. Plasma Potassium Curves After Single Injections. The results of single intraperitoneal injections of various amounts of KCl per kilogram of body weight are illustrated by the curves shown in figures 1 and 2. The animals used had been fasting for 12 hours, but had been allowed water. They were kept in an air-conditioned room during the experiments. Blood was taken from an ear vein, as long as this was possible, or from the heart in the acute or terminal stages of K poisoning, when the flow of blood from peripheral vessels is too sluggish to enable a sample to be collected within a reasonable time. The amounts taken from the heart did not exceed 0.8–1.0 ml, so that there was no question of the plasma K levels being affected by exsanguination of the animal. Terminal blood samples were taken while the heart was still beating, although breathing had ceased.

The symptoms of K poisoning in rabbits resemble those in other animals. These are flaccidity of voluntary muscle, particularly evident in the hind legs; respiratory embarrass-

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ment, leading to diminished oxygenation of the blood; and failure of peripheral circulation, causing bloodless, cold ears. Death is preceded by convulsions, and respiration fails before the heart stops beating. The time elapsing between injection and death varied from 15–30 minutes, for doses of 0.575–0.65 gm KCl/kg body weight, and this time interval did not appear to be related to the dosage level. Terminal K values/100 ml of plasma range from 58 mg (*curve 6*) to 84 mg (*curve 2*).

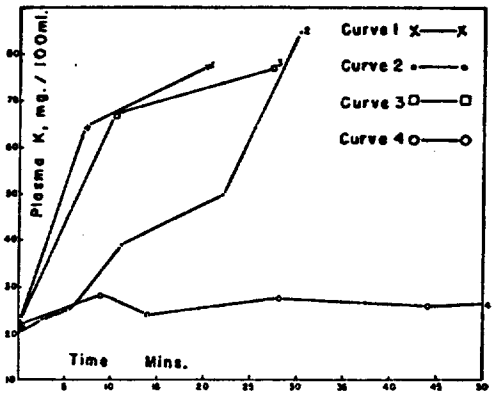

FIG. 1. Effect of single injections of 10% KCl, at different dosage levels: *Curve 1*. Dose 0.65 gm/kg body wt. The animal died 20 min. after injection. *Curve 2*. Dose 0.625 gm/kg body wt. Died 30 min. after injection. *Curve 3*. Dose 0.60 gm KCl/kg body wt. Died 27 min. after injection. *Curve 4*. Dose 0.55 gm KCl/kg body wt. Survived. Plasma K continued to rise, being 33 mg at 93 min., 32.0 mg at 166 min., and 34.7 mg/100 ml at 313 min. after the injection.

The eight curves do not provide statistically valid data for the LD_{50} for rabbits, but the curves of figure 1 show that doses of 0.6 gm KCl/kg or higher, were lethal, while 0.55 gm KCl/kg caused only a slight and transient rise in plasma K. Figure 2 shows 4 curves, for a dosage level of 0.575 gm KCl/kg. Two of these curves (*5* and *6*) rise to lethal levels, while *7* and *8* show smaller rises with survival. This dose appears to be close to the dividing line between death and survival.

The terminal plasma K values found are within the same range as reported by Tabor and Rosenthal (3) for a series of 30 rabbits given i.p. injections of 0.15 M KCl (1.12 gm KCl/kg).

II. Plasma K Levels After Additional Injections. The effect of repeating an injection (0.575 gm KCl/kg body weight) after 24 hours is illustrated by the curves of figure 3, which are on an extended time scale. *Curve 1*, figure 3, is initially the same as *curve 8* of figure 2, but is continued over a longer time. In this case, the initial injection was followed by a gradual drop in plasma K during the first 10 minutes, succeeded by a gradual rise to values considerably above the initial one. Recovery was not quite complete 24 hours later, plasma K being 25.9 mg/100 ml, as compared with 22.0 mg/100 ml initially.

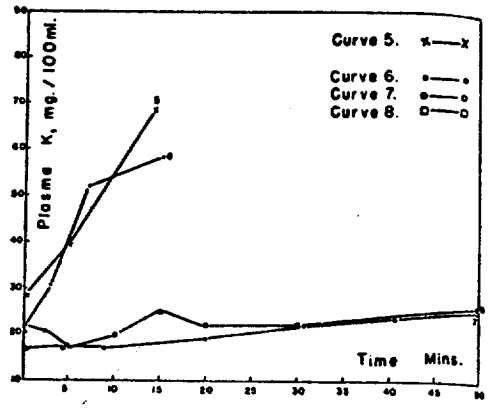

FIG. 2. Effect of a single dose of 0.575 gm KCl/kg body wt. *Curve 5*. Death 14 min. after injection. Fluid recovered from the peritoneal cavity at death contained 2.1% of KCl. *Curve 6*. Death 15 min. after injection. *Curve 7*. Survived. See also *curve 1*, fig. 3. *Curve 8*. Survived. See also *curve 1*, fig. 4.

A second injection was then given. Plasma K now rose sharply to 57.2 mg at 13 minutes, and then fell to about 50 mg/100 ml, at which level it remained during the next 50 minutes. The rabbit exhibited signs of K poisoning (limp muscles, labored breathing) during the 40 minutes after injection, but appeared to be recovered at the end of an hour.

The curves of figure 4 illustrate the effects of three intraperitoneal injections of KCl (0.575 gm/kg), repeated at 72-hour intervals.

Curve 1 is the same as *curve 7* of figure 2, but is continued over a longer time. Plasma K was apparently unchanged 5 minutes after the injection, but rose to a first maximum at 15

minutes, fell to a minimum at 20–30 minutes, and then continued to rise very gradually.

Curve 2 shows the results of a second injection 72 hours after the first one. Initial plasma K was higher than in *curve 1*, but the animal appeared to be completely recovered from the first injection. The observable symptoms of K poisoning following the second injection were, however, more intense and more prolonged than at the time of the first injection.

The rabbit appeared to be completely recovered 3 days later, and its plasma K was down to 19.8 mg/100 ml. The plasma K after the third injection rose uninterruptedly, to reach a value of 68.2 mg/100 ml at death, 34 minutes later (*curve 3*).

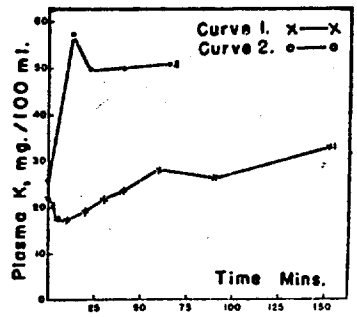

FIG. 3. Effect of repeating an injection of KCl. *Curve 1*. The rabbit was given 0.575 gm/kg body wt. intraperitoneally. *Curve 2*. Marked difference in plasma K curve when same amount is given after 24 hrs.

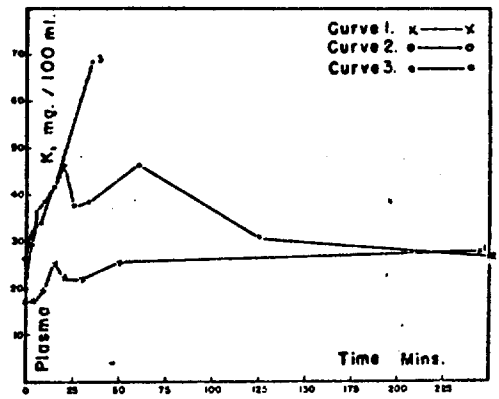

FIG. 4. Effect of repeated injections of KCl (0.575 gm/kg body wt.) at 72 hr. intervals. *Curve 1*. Same as curve 8, fig. 2, but on an extended time scale. *Curve 2*. Plasma K was 26.4 mg at 275 min. and 27.5 mg/100 ml at 400 min. after the injection. *Curve 3*. Plasma K of 68.2 mg/100 ml at death in 34 min.

DISCUSSION

It appears from *curves 7* and *8*, figure 2, that injection of sublethal doses of KCl may be followed during the next 5–15 minutes by a slight fluctuation in plasma K. A slow rise then takes place. The experimental material presented in this report is too limited to permit the drawing of any general conclusions, but it does show certain interesting features of the finer structure of K tolerance curves, which may throw some light on the problems of K regulation in animals.

The data confirm for rabbits the finding reported for cats (1) that response to injection of sublethal doses of KCl differs considerably for different individuals, and may depend on their past history, in particular with respect to previous high K loads. *Curves 1* and *2*, of figure 4, show parallel variations in the same animal at different times, although *curve 2* lies wholly above *curve 1* during the first 4 hours. In both curves K rises to a first peak after 10–15 minutes, falling after 30 minutes, and then rising to a second peak after 60 minutes.

Wilde (4) finds similarly that K rises rapidly in the plasma during intravenous injection of KCl into cats, and is quickly absorbed by the tissues, reaching a minimum level 15–20 minutes after the injection is completed. A secondary rise in blood K was observed at 40 minutes.

The following explanation of the observed fluctuations is put forward as a tentative working hypothesis.

An initial fall or absence of change in plasma K could correspond to the period of transfer of body fluids to the intraperitoneal locus of the hypertonic fluid injected. A rise would occur with the entry of K ions into the circulation. This process would be followed by entry of K ions into the cells. Whenever the velocity of cell K increment is greater than rate of entry of potassium into the circulation from the peritoneum, a fall in plasma K should result. This would continue until the cells could no longer take up potassium, and a second rise would follow. Also opposing a rise

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would be the process of renal elimination, which over a period of time could bring plasma K down to preinjection or lower level. Whether or not the animal survives the second rise will depend on the size of the dose administered, on the rate of absorption from the peritoneum, and on the rate of elimination by the kidney, if it is assumed that the K retaining capacity of the cells is saturated at this stage.

The greater plasma K rise in animals given a repeated injection might thus be ascribed to saturation of the cell, as a result of which the process of lowering plasma K by fixation within cells is blocked, and the initial rise in plasma K is therefore greater. There is also evidence that some degree of renal dysfunction may follow loading of an animal with K salts, and this factor would also affect the height of the second peak of the curves.

It is realized that the experimental evidence on which this reasoning is based is very slender, and that nothing is known with any certainty of the nature of the potassium-binding substance or substances. It seems certain, however, that fixation of K within cells requires a supply of oxygen, as well as of glucose (5), and it has also been shown that hemoglobin solutions are able to accumulate K against a concentration gradient (6).

The observation that animals appear to be progressively less able to resist repeated doses of K salts is an apparent disagreement with the finding that rats can adapt themselves to a high K intake (7) by stomach tube. It may be that the slow rate of entry of K from the alimentary canal, coupled with its fixation by liver cells, would prevent any drastic rise in plasma K, such as are recorded in this report; Thatcher's work gives no indication of development of resistance to parenterally introduced K salts.

SUMMARY

Plasma potassium time curves have been constructed for rabbits following single intraperitoneal injections of KCl. Doses of more than 0.575 gm of KCl/kg body weight were rapidly fatal, within 15–30 minutes, and terminal plasma K varied from 58–85 mg/100 ml. Sublethal doses repeated after 24 or 72 hours raise plasma K to much higher levels than did the initial injection.

The results are consistent with the view that K is rapidly taken up from the plasma into cells, where it is retained. This intracellular potassium is slowly released, so that the protoplasm remains saturated, and the cells are unable to deal efficiently with subsequent increases in plasma K.

One might conclude, from the effects of repeated injections, that the ability of an animal to cope with an abnormal increment of potassium cannot be assessed on the basis of a resting plasma K level alone. The construction of K tolerance curves would seem to be as necessary for a study of K metabolism, as a glucose tolerance curve for insight into carbohydrate metabolism.

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Blood pressures of !Kung bushmen in Northern Botswana

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About a dozen groups of people in the world do not show the rise of blood pressure with increasing age that is the average pattern in developed countries. These groups have nearly all been found in remote parts of the world (Table I). But the observations that can be made on these communities, far from medical school laboratories, are not only of anthropological interest; they may also contribute to our understanding of the nature and causes of essential hypertension. The normotensive community we describe here has not been reported hitherto in the medical literature except for our preliminary communications.[1-3]

Subjects and methods

We report here some of our investigations among bushmen of the !Kung tribe[4] in northwestern Ngamiland, Botswana, near the Aha Hills. The 800 bushmen in this area have been kept isolated by a surrounding waterless zone 60 to 100 miles wide.[5] They live as independent a life as any bushman group in existence today. Many of them still live all the year round as hunter-gatherers.[6]

I. DeVore and R. B. Lee of the Department of Social Relations, Harvard University, set up a camp next to the bushman camp near the Dobe waterhole in 1963. Since then except for the period 1965-6, the Harvard group has had one to three anthropologists living in the area, usually for periods of about 2 years. The resident scientists have learnt the !Kung language, got to know the people, and each has studied a different aspect of bushman life, taking pains, however, to disturb it as little as possible. Specialists have been brought to the camp from time to time for short visits, which have been fruitful because the visitors have been able to base their studies on the resident scientists' personal knowledge and understanding of the bushmen.

The bushmen live in camps of 20 or more people, the basis of which is an ex-

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Table I. Communities in which blood pressure does not rise with age

Ethnic or socio-economic group	Geographical location	Study
1. Africans	Kavirondo, Kenya	Donnison, 1929
2. Africans	Eastern Province, Uganda	Williams, 1941
3. Ponape Islanders	E. Caroline Islands, Micronesia	Murrill, 1949
4. Pukapuka Islanders	N. Cook Islands, Polynesia	Murphy, 1955 Hunter, 1962 Prior et al., 1968
5. Ethiopians	Ethiopia	ICNND, 1958
6. Various communities	Papua, and E. New Guinea	Whyte, 1958 Maddocks, 1965, 1967
7. Lower socio-economic group men—industrial and rural	Delhi, India	Padmavati, 1959
8. Bushmen	Central Kalahari, Botswana	Kamjner, 1960
9. Abaiang Islanders	Gilbert Islands, Micronesia	Maddocks, 1961
10. Carajas Indians	Amazon basin, N. Brazil	Lowenstein, 1961
11. Samburu, Rendille, Turkana (nomads)	Northern Frontier Province, Kenya	Shaper, 1961, 1967
12. Masai tribesmen	Tanzania	Mann et al., 1964
13. Orang Asli	West Malaysia	Burns-Cox, 1970

tended family group. Each camp is associated with a waterhole. Lee[6] has found that about 70 per cent of food by weight is vegetable, collected by the women. A variety of nuts, fruits, roots, bulbs, and leaves are available, varying with the season. The most important food is the nut of the mongongo (manketti) tree *(Ricinodendron rautenenii)* because it is abundant, keeps well, and is rich in protein and fat.[7] Meat is provided by some of the men, who hunt with poisoned arrows.

The bushmen have only a few basic possessions, which they carry with them when they move; they are therefore able to travel light. Individuals move freely between camps and the camps move about five times a year. During and after the wet season (November to March) there are pans of seasonal water out in the bush so that the bushmen can camp beside them and collect food in the surrounding area. But as the pans dry up the bushmen return to the permanent waterholes; at the end of the dry season they have to walk increasing distances to find food. Some of the bushmen obtain milk occasionally from Bantu (Herero and Tswana) pastoralists who have settled in the area in the last 45 years. A few bushmen work for the Bantu as herdsmen part of the year. The bushmen have no alcohol but they smoke tobacco. They have very little salt. There are no deposits of salt in the area and the principal means of obtaining salt is to walk to Tsumkwe, 30 miles away in South West Africa, where the Administration has established a settlement for the !Kung bushmen on the South West African side of the border.

The medical team made 3 visits to the Harvard camp; each trip included 10 days' work in the field. The first visit was made in October, 1967, at the end of the dry season, when it was hot and dry and food was scarce. The second visit was in April-May, 1968, after the rains. During that visit it was warm in the day and cool at night; there were occasional pans of standing water in the area and some of the bushmen showed signs of malaria. The third visit was made in July, 1969, halfway through the dry season. Though the nights were cold, the days were sunny and quite warm out of the wind. There was adequate food.

The bushmen were seen in family groups. Babies, pregnant women, the sick, and the aged were all included in our first two visits. A brief history was taken through an interpreter and each individual was examined with the subject lying down. At the third visit we examined only adults, for

In male bushmen systolic and diastolic pressures both showed a slight but definite downward trend with increasing age throughout adult life. In the women the line joining decade mean systolic pressures rose by about 10 mm. Hg to a higher step in the second half of life. But their diastolic pressures stayed the same until the two oldest age groups, in which there was a slight drop.

Combining the values for men and women (unweighted mean values in the third row of Table II), systolic pressures were about 3 mm. higher in people over 49 years of age while diastolic pressures fell after the age of 59. Pulse pressures were 42 to 45 mm. from ages 15 to 49 years and 52 to 53 mm. from ages 60 to 83 years. These trends are illustrated in Fig. 1, against the curve of London blood pressures taken by Hamilton and associates[9] (1954) which is typical of Western, developed communities. The mean blood pressures (diastolic + ⅓ pulse pressure) for men and women combined condense all the results into a row of single figures at the bottom of Table II. They show a remarkable constancy despite increasing age.

In the 22 bushmen who were examined on all three visits, the mean blood pressures were:

October, 1967	April-May, 1968	July, 1969
117/73	126/75	111/64

The difference cannot be explained by observer variation; nearly all were taken by A.S.T. The lower readings at the July (winter) visit were unexpected. On this occasion, subjects were examined outside in the winter sunshine and it is possible that they were more relaxed than when they were examined on their own inside the doctors' tent on the two earlier visits.

Table III shows the arm circumferences of the bushmen, arranged by sex and age. The measurements were taken midway between the right elbow and shoulder in about half the subjects whose blood pressures were recorded. The arm circumferences were at the lower end of the range in Western adults.[10,11] In both sexes they

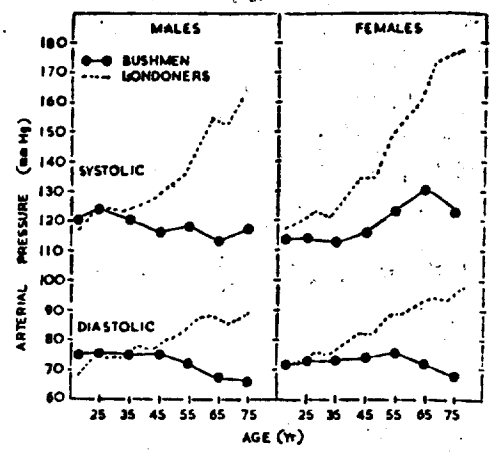

Fig. 1. Bushmen's blood pressures with age, compared with standard figures for a group from London (Hamilton and associates[9]).

were a little higher between 30 to 49 years of age and a little lower in the very young and the very old.

Almost all the bushmen examined were lean, of delicate build, and rather short. Taking all ages together, the men averaged 48 Kg. (106 lb.) in weight (unclothed) and were 161 cm. (5 ft., 3 in.) tall (without shoes). At this height the "desirable" weight for small-framed men is 53.5 to 57.2 Kg. (118 to 126 lb.) in the U.S.A.[12] The women's mean height and weight were 150 cm. (4 ft., 11 in.) tall (without shoes) and 40 Kg. (88 lb.) (unclothed), compared with a "desirable" weight range of 44.9 to 48.5 Kg. (99 to 107 lb.) for small-framed women of the same height in the United States. Unfortunately, these "desirable" weights are quoted for people wearing indoor clothes, which might weigh 1 to 2.25 Kg. The triceps skinfolds averaged 5.0 mm. in the bushmen and 9.4 mm. in the women.

To see if there were any changes in weight with age in the two sexes, weight/height ratios (metric) are shown in Table IV together with ratios calculated from average U.S. weights.[12] All bushman values were low and there was little change with age except below the age of 20 and over the age of 60 years. For comparison, the weight/height index of young American men was 38.8, and it fell to 29.5 in the same men after 6 months semi-starvation with the loss of 25 per cent of their body

Table III. Arm circumference of bushmen in cm.

Age (years)	15-19	20-29	30-39	40-49	50-59	60-69	70-83
Men	19.7	22.0	23.5	24.2	22.4	22.2	17.8
(n)	(3)	(11)	(11)	(6)	(7)	(4)	(3)
Women	18.4	20.2	21.4	21.4	19.0	21.8	16.6
(n)	(4)	(10)	(5)	(7)	(3)	(4)	(3)

*Table IV. Weight/height ratios for bushmen compared with ratios calculated from average U.S. weights**

Age (years)	15-19	20-29	30-39	40-49	50-59	60-69	70-83
Bushmen†							
Men	24.2	30.0	30.4	31.1	28.8	30.2	24.2
Women	24.0	27.5	26.4	27.0	27.1	26.1	24.7
Average U.S.[12]							
Men‡	34.0	38.7	40.4	41.3	41.6	40.7	—
Women	31.6	33.4	36.0	38.1	38.9	39.3	—

*Ratios were calculated from the following formula: $100 \times \frac{\text{weight (Kg.)}}{\text{height (cm.)}}$

†Numbers of bushmen in each cell were the same as in Table II.

‡Calculated for men in shoes 5 ft. 4 in. in height and women in shoes 5 ft. 1 in. in height.

weight, as observed in the Minnesota experiment.[13]

In the 24 hour urine specimens (Table V), the striking findings both in 1967 and 1968 were the low sodium and chloride content in the bushmen. Sodium and chloride excretions of 30 mEq. per day each would correspond to an NaCl intake of only 2 Gm. per day. The creatinine excretions look rather low in one or two of the bushman urine specimens, suggesting that the collections were incomplete. However, the bushmen were smaller and weighed less than the controls. When the creatinines are expressed per kilogram of body weight, the bushman creatinines come to 83 per cent of the controls in 1967 and 86 per cent in 1968. Potassium, nitrogen, and magnesium excretions were not lower in the bushmen than in the controls.

Discussion

There have been two previous observations of blood pressures in other groups of southern African bushmen. Bronte-Stewart and colleagues[14] mentioned mean values of 119/74 in men and mean values of 110/71 in women in northern South West Africa. Pressures were only taken in 44 subjects and old people were probably under-represented. Kaminer and Lutz[15] measured blood pressures in 99 nomadic bushmen belonging to the Central Group of tribes, living in the area south of Ghanzi, Botswana. The readings did not increase with age and none exceeded 140/90. Two complete bands of people were examined but ages could only be estimated by physical appearance.

We have confirmed these findings in a larger group of separate bushmen whose ages have been worked out with a fair degree of accuracy. There are different degrees to which populations can deviate from the standard Western blood pressure pattern exemplified by the curves for London, presented in Fig. 1. Maddocks[16] has introduced a useful classification. In the Type I pattern, mean systolic pressure

Table V. Mean values (and ranges) of 24 hour urinary sodium and other constituents in bushmen and controls

Subjects	Year	n	Creatinine (Gm./day)	Sodium (mEq./day)	Chloride (mEq./day)	Potassium (mEq./day)	Nitrogen (Gm./day)	Magnesium (mEq./day)
Bushmen	1967	6	0.84 (0.56-1.40)	31 (8-48)	30 (7-77)	70 (31-93)	10.4 (5.2-14.9)	7.4 (6.3-9.6)
	1968	4	0.98 (0.54-1.41)	29 (2-64)	36 (5-70)	103 (48-168)	10.6 (5.5-17.8)	
Controls	1967	2	1.36 (1.29 & 1.42)	212 (179 & 245)	173 (132 & 215)	68 (52 & 84)	8.7 (6.4 & 11.0)	3.2 (2.1 & 4.[illegible])
	1968	3	1.42 (1.23-1.64)	147 (96-172)	121 (62-154)	61 (43-75)	11.4 (10.1-13.1)	

rises considerably with age and diastolic pressure rises moderately. In the Type II pattern, systolic pressure rises moderately but diastolic pressure does not rise. In the Type III pattern, systolic and diastolic pressures both stay at the same low level throughout life, and in the Type IV pattern both systolic and diastolic blood pressures decline with advancing age. According to Maddocks' classification, the !Kung bushman males have a Type IV pattern and the women a Type II pattern. When the two sexes and the diastolic and systolic pressures are combined, the resulting mean blood pressures do not change with increasing age.

There are five possible explanations for the bushmen's immunity from hypertension: (1) low salt intake, (2) lack of obesity, (3) associated illnesses, (4) freedom from the stresses of civilization, and (5) high magnesium intakes. Taking them in reverse order, Verster[17] speculated that the relatively high serum magnesiums found in some central Kalahari bushmen[18] might be connected with their freedom from hypertension. However, these sera were not separated from red cells for about 2 days and neither Bernstein[18] nor we (Table V) found high urinary magnesiums in bushmen.

There is no doubt that in some communities hypertension has appeared with "acculturation."[19,20] But mental stress is only one of the changes that occur and it is the most difficult to quantify.[21] Some isolated and peaceful communities have hypertension,[22-24] although one would imagine they are not very stressful to live in.

Tropical splenomegaly[25,26] and chronic chest disease[26] have been associated with lower blood pressures than in the healthier members of the same communities. [illegible] minority of the bushmen had splenomegaly but the spleens were usually not very large and appeared after the wet season, i.e., in April, 1968, when we found blood pressures to be rather higher than at our other visits. Some of the older bushmen showed evidence of chronic bronchitis, but most of them appeared healthy on clinical examination. We would not think that chronic infections play more than a minor part in keeping blood pressures low. Maddocks and Vine[26] reached the same conclusion in New Guinea.

The bushmen have thin arms and small amounts of adipose tissue. Pickering, Roberts, and Sowry[10] calculated that to obtain the true direct blood pressure, correction should be made for arm girth. According to the table in their article, 10 mm. Hg should be added to the bushmen's indirect systolic pressures and no correction should be made to the diastolic pressures. This would not explain the absence of hypertension with increasing age, because the bushmen's arm circumference stayed about the same throughout life. More recent workers have found that the effect of arm girth per se is very small[11,27] and that adiposity has a much closer association. The bushmen have unusually small amounts of adipose tissue and (Table IV) show no "middle-aged spread." Some of the other communities in Table I remain thin throughout life.

Lastly, our findings are consistent with Dahl's hypothesis[28] that populations habitually consuming less than 5 Gm. of salt a day are relatively free of hypertension. Some more recent between-population comparisons[29] are also in keeping with this hypothesis. Even if salt intake does not

determine which individuals become hypertensive within a developed community, the bushmen appear to eat a "low salt diet" which provides little more sodium than was used to *treat* essential hypertension[30] before oral diuretics became available.

Summary

Blood pressures have been measured, and sociological, anthropometric, medical, and biochemical examinations have been made in an isolated group of !Kung bushmen in northern Botswana.

Blood pressures were taken in 152 bushmen of both sexes, aged 15 to 83 years, who were examined in family groups. Systolic and diastolic pressures declined with increasing age in the men. The women showed a small rise of systolic pressure after the menopause but their diastolic pressure decreased slightly with age. The mean blood pressure (diastolic + ⅓ pulse pressure) for men and women combined thus remained the same throughout life.

Other communities whose blood pressures do not increase with age are reviewed. Of the possible explanations in the bushmen, a low salt intake and a lack of obesity appeared to be important, with relative freedom from mental stress an additional imponderable factor.

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THE TOXICITY OF POTASSIUM CHLORIDE IN EXPERIMENTAL NEPHRITIS

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It is a common practice in the therapy of nephritis to administer certain potassium salts for their diuretic influence. On the other hand, it is well recognized that in general potassium salts are much more toxic under normal conditions, than the corresponding sodium salts. It is, therefore, quite logical to assume that, in such a disease as nephritis, the same fact should obtain, and further, that, since the rate of elimination of the salt may be considerably diminished in nephritis, potassium salts may possess an even greater toxicity than under normal circumstances.

The significance of the possible detrimental effects of potassium salts in nephritis has been shown by Smillie (1). This investigator has demonstrated that certain cases of chronic nephritis fail to excrete even sodium chloride in a normal manner. Thus, in several instances, the inclusion of 10 grams of sodium chloride in the dietary, resulted in either a failure or a diminution of excretion. When it was definitely demonstrated that a certain case was unable to eliminate normally 10 grams of sodium chloride, the patient was given potassium chloride in a single dose of 5 to 10 grams in 150 c.c. of water. In some instances this procedure resulted in an increased chloride excretion. In other cases no apparent effect could be noted. In one instance, the administration of 10 grams of potassium chloride induced weakness, abdominal distress, vomiting and precordial pain. There was marked cyanosis and a weak, irregular pulse. From the results obtained in this case, Smillie was led to investigate the matter further by animal experimentation.

[1] The data are taken from the thesis of Roy C. Ferguson presented in partial fulfillment of the requirements for the degree of M.D., Yale University, 1920.

The method of procedure adopted by Smillie was as follows: The toxicity of potassium chloride was determined in normal rabbits. This having been established, the maximal sublethal dose of potassium chloride was administered per os. A moderate nephritis was then induced by means of uranium nitrate. Under these conditions the non-protein nitrogen of the blood gradually increases, and affords a measure of the extent of renal damage. When the non-protein nitrogen of the blood had reached a sufficiently high level, a relatively small dose of potassium chloride was given. The animal usually succumbed in a short period of time. Autopsy revealed the typical lesions of uranium nephritis. In all ten experiments were carried through. A typical experiment may be cited. A rabbit received by way of the mouth 3 grams of potassium chloride, dissolved in 50 cc. of water, resulting in a rapid elimination of the salt. Uranium nephritis was then induced. When the non-protein nitrogen of the blood reached 118 mgm. per 100 cc. of blood, one gram of potassium chloride was given in 50 cc. of water. The animal died in 15 minutes. From these experiments the conclusion is drawn by Smillie that potassium salts are distinctly more toxic in certain types of nephritis than under normal conditions.

It has been shown by various investigators (1) that death does not occur in uranium nephritis until the non-protein nitrogen of the blood has reached a level of from 150 to 250 mgm. per 100 cc. of blood. Moreover, animals in this condition give definite symptoms when death is impending. In his experiments, Smillie attempted to produce moderate nephritis and then to administer potassium chloride before severe symptoms incident to the injury developed. In every instance it was found that 1 gram of potassium chloride caused immediate death in the nephritic rabbits whereas 3 grams were without influence upon normal animals.

According to Smillie death was caused by the concentration of potassium chloride in the blood, the diseased kidneys being unable to eliminate it with sufficient rapidity to prevent its toxic action upon the heart muscle.

EXPERIMENTAL

The toxicity of potassium chloride in tartrate nephritis

In the present investigation the problem of the toxicity of potassium chloride in experimental nephritis has been attacked from a slightly different viewpoint. In the first place it seemed desirable to introduce the salt by some path other than the alimentary canal inasmuch as it is well known that in the rabbit the gastro-enteric tract is usually so filled with material that the possibility exists for a very diverse rate of absorption into the blood stream of any specific substance taken by the mouth. For the purpose of obtaining more accurate measurements of toxicity, potassium chloride has been injected at a determined rate directly into the blood stream. Again, different experimental nephritides may show a variable excretion power with respect to the elimination of introduced salt. Thus in the condition induced by uranium nitrate pronounced effects are produced upon both the glomerular mechanism and upon the convoluted tubules, a fact which would predicate the probability that salt excretion would be appreciably diminished. On the other hand it has been shown by Underhill, Wells and Goldschmidt (2) that tartaric acid causes a nephritis in which the specific influence is exerted upon the convoluted tubules, the glomerular mechanism for the most part escaping serious injury. In accord with this is the observation (3) that in tartrate nephritis sodium chloride is eliminated in an apparently normal manner whereas introduced urea fails to reappear to an appreciable extent. It must therefore be quite evident that the toxicity of potassium chloride in nephritis will depend directly, in the first place, upon the portion of renal tissue damaged and secondly upon the extent of the damage to this mechanism.

Methods: Full grown rabbits were employed as experimental animals. Nephritis was induced by the subcutaneous injection of 1.0 gram of tartaric acid, per kilogram of body weight, neutralized with sodium hydroxide. Inasmuch as, with tartrate nephritis, the animal refuses food, the experiments were carried through with the rabbits in a fasting condition. As a measure

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of the extent of injury to the renal mechanism urinary analysis involving the estimation of total nitrogen and total chlorides was made upon twenty-four hour samples of urine, obtained by compression of the bladder through the abdominal wall. Previous to the administration of the tartrate, urinary analyses were obtained for a period of two days as criteria for the normal rate of elimination of the substances mentioned above. Total nitrogen was estimated by the Kjeldahl method, chlorides according to the Volhard procedure. When evidence was obtained for significant renal damage, usually on the day following the administration of the tartrate, approximately isotonic potassium chloride was introduced, without anesthesia, from a burette into the marginal ear vein at the rate of 4 cc. per minute. The protocols of the individual experiments follow.

From the results outlined in table 1 it is quite evident, that, in tartrate nephritis, total nitrogen excretion is appreciably diminished whereas that of the chlorides in general shows no such action, data which tend to confirm the observations of Underhill, Wells and Goldschmidt already cited. It is also quite apparent that the toxicity of potassium chloride when injected into the blood stream in animals suffering with tartrate nephritis is quite variable, death being produced with doses ranging from 0.23 gram to 0.43 gram per kilogram of body weight. It is possible that this variable toxicity is explicable upon the basis of the extent of injury to the renal apparatus. From the criterion of nitrogen elimination, this appears quite plausible.

The toxicity of sodium chloride in tartrate nephritis

In order to demonstrate the relative toxicity of potassium and sodium chloride in tartrate nephritis, rabbits were maintained under the conditions cited above, except that sodium chloride was injected instead of potassium chloride. The results of two such experiments may be found in tables 1 and 2.

It is quite apparent from the data in table 2 that the intravenous injection of large quantities of sodium chloride is without appreciable detrimental influence upon rabbits with tartrate nephritis.

TABLE 1

The toxicity of potassium chloride in tartrate nephritis

DATE	URINE					REMARKS
	Volume	Specific gravity	Reaction to litmus	Total nitrogen	Chlorides as NaCl	
1919	*cc.*			*grams*	*grams*	
						Rabbit of 1.7 kilos body weight
October 28						No analysis
October 29	64	1.006	Acid	0.793	0.03	Water intake, 50 cc.
October 30	3		Acid	0.006	0.015	Injection of 1.7 grams tartaric acid subcutaneously
						Water intake, 45 cc.
						Injection of 45 cc. 0.9 per cent KCl in twelve minutes caused death = 0.23 gram KCl per kilo body weight
						Fasting rabbit of 1.6 kilos body weight
November 4	53	1.025	Acid		0.04	No water taken
November 5	23		Acid	0.800	0.04	No water taken
November 6	18		Acid	0.090	0.09	Injection of 1.6 grams tartaric acid subcutaneously
						No water taken
						Injection of 78 cc. 0.9 per cent KCl in twenty-one minutes caused death = 0.43 gram KCl per kilo body weight
						Fasting rabbit of 1.9 kilos body weight
November 11	69	1.023	Alkaline	0.710	0.24	
November 12	76	1.020	Acid	0.970	0.10	No water taken
November 13	25		Acid	0.110	0.15	Injection of 1.9 grams tartaric acid subcutaneously
						Water intake, 120 cc.
						Injection of 67 cc. 0.9 per cent KCl caused death in seventeen minutes = 0.31 gram per kilo body weight

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TABLE 2

The toxicity of sodium chloride in tartrate nephritis

DATE	URINE					REMARKS
	Volume	Specific gravity	Reaction to litmus	Total nitrogen	Chlorides as NaCl	
1919	*cc.*			*grams*	*grams*	
						Fasting rabbit of 1.4 kilos body weight
December 2	71	1.018	Acid	0.79	0.24	No water taken
December 3	72	1.025	Acid	1.32	0.14	No water taken
December 4	7		Alkaline	0.06	0.04	Water intake, 130 cc.
						Injection of 1.4 grams tartaric acid subcutaneously
						Injection of 140 cc. 0.9 per cent NaCl in thirty-five minutes with no detrimental influence = 0.9 gram NaCl per kilo body weight
						No water taken
December 5	Few				0.03	
1920						Fasting rabbit of 1.8 kilos body weight
February 3	20		Acid	0.47	0.06	Water intake, 168 cc.
February 4	46		Acid	0.93	0.04	Water intake, 100 cc.
February 5	4		Acid	0.02	0.015	No water taken
						Injection of 1.8 grams tartaric acid subcutaneously
						Injection of 150 cc. of 0.9 per cent NaCl in thirty-eight minutes with no detrimental influence = 0.75 gram NaCl per kilo body weight
February 6	7		Acid	0.03	0.05	

It may therefore be concluded that in tartrate nephritis potassium chloride is much more toxic than sodium chloride even though the latter is not excreted to a marked degree.

The toxicity of potassium chloride in normal rabbits

As a control of the results of the toxicity of potassium chloride in tartrate nephritis, experiments have been carried through with normal rabbits in which the toxicity of potassium chloride has been determined. In these experiments the technique has been identical with that of the above detailed trials, except that nephritis was not induced. The toxicity of potassium chloride in normal rabbits in a fasting condition may be seen from the results in table 3.

From the results of the three experiments with normal fasting rabbits detailed above it is quite apparent that potassium chloride is just as toxic under these conditions as it is in tartrate nephritis. It is therefore quite evident that the production of tartrate nephritis does not render an animal more susceptible to potassium chloride.

There is thus created a discrepancy between the results of Smillie and those herein reported. The most probable explanation to account for this divergence is in the different lesions produced by the two substances, namely, uranium nitrate (Smillie) and tartrate. The former usually causes an involvement of both the glomerulus and the convoluted tubules, whereas with tartrate the principal action is upon the tubules. On the other hand, if one may use the experiments with sodium chloride as a criterion, this view is somewhat discounted since the damage to the kidneys in these experiments was apparently so great that little or none of the injected chloride was eliminated. It is therefore probable that in the potassium chloride experiments, little opportunity was afforded for the prompt elimination of the salt. Nevertheless, in the experiments with normal fasting rabbits there was no obstacle to rapid excretion of the salt. Potassium chloride however was just as toxic, as it was after nephritis had been induced. The only adequate explanation for the discrepancy in results must therefore reside in the manner of introduction of the salt—oral administration versus intravenous injection.

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TABLE 3

The toxicity of potassium chloride in normal rabbits

DATE	URINE					REMARKS
	Volume	Specific gravity	Reaction to litmus	Total nitrogen	Chlorides as NaCl	
1930	*cc.*			*grams*	*grams*	
						Fasting rabbit of 1.5 kilos body weight
February 10	17		Acid	0.52	0.07	Water intake, 45 cc.
February 11	69	1.030	Acid	1.58	0.08	Water intake, 100 cc.
February 12	95	1.033	Acid	2.32	0.06	Water intake, 52 cc.
						Injection of 40 cc. 0.9 per cent KCl in ten minutes caused death = 0.24 gram KCl per kilo body weight
						Fasting rabbit of 2.4 kilos body weight
February 16	49		Acid	0.74	0.10	Water intake, 35 cc.
February 17	91	1.040	Acid	1.05	0.10	No water intake
February 18	50		Acid	1.07	0.10	No water intake
						Injection of 90 cc. 0.9 per cent KCl in twenty-three minutes caused death = 0.33 gram per kilo body weight
						Fasting rabbit of 1.9 kilos body weight
February 24	70	1.040	Acid	0.44	0.14	No water intake
February 25	75	1.034	Acid	0.95	0.16	Water intake, 60 cc.
February 26	63	1.045	Acid	0.82	0.12	Water intake, 46 cc.
						Injection of 66 cc. of 0.9 per cent KCl caused death in sixteen minutes = 0.31 gram KCl per kilo body weight

CONCLUSIONS

Potassium chloride intravenously administered to rabbits with tartrate nephritis is much more toxic than sodium chloride introduced under similar experimental conditions.

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Potassium chloride intravenously injected is no more toxi to rabbits with tartrate nephritis than it is to normal animal

The induction of a nephritic condition in rabbits by tartrat therefore, does not render the animal more susceptible to pota sium chloride introduced directly into the blood stream.

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Medical Memoranda

Fatal Hypernatraemia after a Saline Emetic

Hypertonic saline can be used to provoke vomiting in cases of acute poisoning in attempted suicide.[1] In certain circumstances this may lead to dangerous side-effects as illustrated by the following report.

CASE REPORT

A 74-year-old man was admitted to hospital, having taken 60 mg. of perphenazine ("fentazin") and 750 mg. of imipramine ("tofranil"). This quantity had been intended to represent a ten-day supply. He had been prescribed these tablets by his general practitioner the previous day for what was considered to be mild depression. Apart from having had a transvesical prostatectomy for benign prostatic enlargement in 1957 there was no relevant history of organic disease.

On examination he appeared to be well nourished. He could respond to simple questions and obey simple commands, and could hold a tumbler and drink on request. Agitation and a coarse Parkinsonian tremor of the hands were conspicuous.

The pulse rate was 84/min., regular, and blood-pressure 115/80 mm. Hg (a recording the previous day by his general practitioner gave 170/90 mm. Hg). Crepitations were present at both lung bases. The pupils reacted sluggishly to direct light; there was no neck stiffness, and Kernig's sign was negative. The patient was able to move all limbs equally. Tendon jerks in upper and lower limbs were brisk. Clonus was absent but both plantar responses were extensor.

An attempt to pass a stomach-tube was unsuccessful because of resistance by the patient. To provoke vomiting a tablespoonful of NaCl (30 g.) in 300 ml. of warm water was given and the patient drank this but no vomiting occurred. The dose was repeated, and only when he lay flat did he vomit, approximately 150 ml. being recovered. There was no retching. The vomit was discarded.

An intravenous infusion of NaCl 0.18% in dextrose 4.3% for the purpose of blood lavage,[2] and a course of penicillin, were begun. Urethral catheterization was required 10 hours after admission for acute retention of urine. The urine output for the first 24 hours was 2,100 ml. The patient's condition was unchanged for approximately 20 hours. From this time his condition deteriorated. A tachycardia with frequent ventricular ectopic beats accompanied signs of increasing pulmonary congestion, raised jugulo-venous pressure, and the development of sacral oedema. The systolic blood-pressure remained around 115 mm. Hg until shortly before death. Serial biochemical estimations are shown in the accompanying Table.

	Na (mEq l.)	Cl (mEq l.)	K (mEq l.)	Bic. (mEq l.)	Urea/mg. 100 ml.
On admission	143	120	4·3	26	37
24 hours after admission ..	174	152	3·7	25	45
27 hours after admission ..	172	152	3·7	25	45

The patient died 32 hours after admission.

The post-mortem findings confirmed the clinical diagnosis of pulmonary oedema. There were lesser degrees of oedema in other organs.

COMMENT

The cause of death in this patient was pulmonary oedema secondary to hypernatraemia, which in turn was caused by the absorption of a large quantity of hypertonic saline used in an attempt to produce vomiting. This attempt failed because the patient had taken an overdose of perphenazine, a chlorpromazine derivative, which is a potent anti-emetic. The Parkinsonian state[3] and hypotension[4] are well-recognized side-effects of phenothiazine drugs.

Imipramine has no anti-emetic effect and may even cause nausea and vomiting as a side-effect.[5] It can cause a Parkinsonian tremor.[6]

The anti-emetic effect of chlorpromazine is well documented[7] and according to Wang[8] perphenazine is 16.6 times more potent as an anti-emetic than the former drug and is more widespread in its anti-emetic action.

The rapid increase in levels of serum sodium and chloride could not be explained by the 10.8 g. of NaCl present in 6 litres of 4.3% dextrose and 0.18% NaCl which the patient received intravenously.

The total intake of NaCl was approximately 70.8 g. (60 g. orally and 10.8 g. intravenously). This is equal to 1,180 mEq Na.

Assuming a total body water of 50 litres, the expected rise in serum sodium would be 23.6 mEq. The observed rise, as the Table shows, was 30 mEq. In view of the possible loss of sodium in the vomit it is suggested that a complex interplay of osmotic forces of sodium in the gut and in the circulation must account for the observed rise in serum sodium.

Hypertonic saline—1 oz. (31 g.) of NaCl to 8 fl. oz. (227 ml.)—is listed in "Drugs of Special Value" section in the H.M.S.O. (1962) publication.[1] That this treatment carries a considerable risk where the new anti-emetic drugs are concerned is illustrated by this case report.

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Foetal Death after Pethidine and Promazine

Encouraged by reports on the value of promazine ("sparine") in obstetrics (Donald, 1959; MacVicar and Murray, 1960; etc.), we have made increasing use of this drug in the maternity department at St. George's Hospital over the past two years. During the five months preceding the admission of the case described below, 160 patients had received one or more injections of promazine 50 mg. with pethidine 150 mg. intramuscularly during the first stage of labour. No ill effects to any mother or child had been observed, and it was our general impression that labour was considerably less of an ordeal for a patient under the influence of this combination of drugs than with pethidine alone. Apart from intramuscular sedation in labour we have also successfully used an intravenous mixture of pethidine, promazine, and levallorphan on many

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THE EFFECT OF AGE ON THE INCIDENCE OF SODIUM CHLORIDE-INDUCED TESTICULAR CYSTS IN CHICKS

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Abstract—1. Chicks can develop cystic testes when fed diets containing high levels of sodium.

2. Cysts developed within 1 week of feeding a diet containing 1·13 per cent sodium.

3. The greatest occurrence of cysts was during the second and third weeks of life.

4. Chicks fed a normal diet for the first 3 weeks of life did not develop cysts when subsequently transferred to the high sodium diet.

INTRODUCTION

It has recently been shown by Siller *et al.* (1972) that the inclusion of high levels of sodium in chick diets can cause testicular cysts, in addition to the cardiovascular lesions which are the usual manifestations of sodium toxicity. Further investigation by Dewar *et al.* (1972) showed that these cystic lesions could be induced, especially in broiler chicks, by sodium levels below those required to produce the other signs of toxicity. It was suggested, in fact, that the occurrence of these testicular lesions defined a new criterion for the assessment of sodium toxicity in the chick. In the course of these experiments evidence was obtained that as chicks grew older they became less sensitive to dietary sodium. The following experiment was therefore conducted to determine the age at which the chick testis is most sensitive to dietary sodium and the duration of exposure to an elevated sodium level which is required to produce the lesions.

MATERIALS AND METHODS

Experimental plan and husbandry

Groups of day-old male broiler chicks (*Gallus domesticus*) were weighed, wing-banded and housed in compartments of electrically heated brooders. They were fed the Poultry Research Centre chick mash (Bolton, 1967)—sodium content 0·13 per cent—for 1, 2 or 3 weeks, then transferred to a diet—sodium content 1·13 per cent—consisting of the chick mash supplemented with sodium chloride for a further 1, 2 or 3 weeks. The latter diet was also fed to groups of birds from day-old. After being subjected to these dietary treatments, which are shown in Table 1, the birds were killed and their testes were examined.

Chemical and histological methods

Sodium contents of diets were determined by atomic absorption spectrophotometry of hydrochloric acid extracts of the ash remaining after ignition at 450°C.

Unless at least one testis was found to be grossly cystic, both testes from each bird were fixed in formalin and embedded in paraffin wax. Sagittal sections were cut from each organ and stained with haematoxylin and eosin. Dilatation of the seminiferous tubules was recognized by the criteria described by Siller *et al.* (1972).

RESULTS

The results are summarized in Table 1. The number of birds shown in each group represents those alive at the end of the experimental period; mortality from intercurrent disease or sodium toxicity was generally low. Birds showing macroscopic evidence of testicular cysts are shown separately from those in which lesions

TABLE 1—THE INCIDENCE OF CYSTIC LESIONS IN CHICKS FED 0·13 AND 1·13 per cent SODIUM FOR DIFFERENT LENGTHS OF TIME

No. of weeks (0·13 per cent sodium)	No. of weeks (1·13 per cent sodium)	No. of birds	Age at examination (weeks)	No. of birds having cystic testes		Total incidence (%)
				Macro.*	Histo.†	
0	1	44	1	2	0	4·5
0	2	26	2	4	3	27
0	3	43	3	13	5	42
1	1	32	2	1	5	19
1	2	36	3	3	7	28
1	3	31	4	4	5	29
2	1	26	3	0	1	4
2	2	29	4	0	4	13
2	3	24	5	1	0	4
3	3	33	6	0	0	0

* Macroscopically discernible.
† Recognizable only by histological examination.

were found only on histological examination. The total incidence is the combined occurrence of both macroscopic and microscopic cysts, but since serial sections would have been necessary to ensure that no early cysts had been overlooked, this may be an underestimate.

The data indicate that the testes of birds older than 3 weeks were not affected by subsequent supplementation of the diet with 1 per cent of sodium. Although birds fed 1·13 per cent sodium from day-old were able to develop cystic lesions within 1 week, cysts developed mainly during the second and third weeks of life. When chicks which had been fed a normal diet for their first week were transferred to the sodium-supplemented diet, there was a considerable incidence of cystic formation during both of the subsequent 2 weeks. There was, however, little

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increase in the incidence of cysts when feeding the high sodium diet continued during the fourth week. The testes of birds which had been fed a normal diet until 2 weeks of age were found to contain lesions 1 week after the birds had been transferred to the high sodium diet. The incidence of cysts in these birds increased when the high sodium diet was fed for 2 weeks but decreased when it was fed for 3 weeks. Since no evidence has been obtained to date to suggest that cysts can regress in chicks of this age it is probable that this discrepancy is a result of the comparatively small sample size.

DISCUSSION

The results confirm the earlier findings of Dewar *et al.* (1972) that the chick testis is very sensitive to dietary sodium, and show that this sensitivity varies considerably with age. Macroscopically discernible cysts were formed within 1 week of feeding a high sodium diet, even during the first week of life. Although some cysts developed during the first week, the incidence of cystic lesions was greatest during the second and third weeks of life; it was further increased during this period if a high sodium diet had been fed from day-old. After 3 weeks of age the chick testis was apparently unaffected by the level of 1·13 per cent sodium investigated.

The mechanism by which the cystic dilatation of the seminiferous tubules develops is not understood. Siller *et al.* (1972) have suggested that the excessive dietary salt may cause the premature secretion of fluid by the tubular epithelium in some way as yet obscure and that this fluid does not escape through the rete testis to be absorbed in the ductuli efferentes. One may perhaps postulate that, whatever the mechanism by which fluid enters the seminiferous tubules, incomplete patency of the efferent duct system could account for its retention and the subsequent development of cystic dilation of the tubules in chicks below 3 weeks of age. If that were so it would imply that, in the fowl, continuity is established by the age of about 3 weeks. However, the time of complete patency, which in the rat coincides with the onset of spermatogenesis (Roosen-Runge, 1961), is not known in the fowl. Although further investigation into the pathogenesis of testicular cysts is required, the lack of fluid drainage is believed to be of importance, since it is well known that in mammals the prevention of fluid absorption by ligation of the excurrent ducts above the ductuli efferentes (Tuck *et al.*, 1971) or chemical interference (Mason *et al.*, 1951) results in testicular enlargement due to accumulated fluid and distension of the seminiferous tubules.

A difference in the secretory response of the immature seminiferous tubules of the chicks in the present study is probably not the basic explanation for the age disparity because it is unlikely that at 6 weeks, when the testes appear to be refractive to excess dietary sodium, the tubules are less able to secrete than at 3 weeks. It is more probable, but still requires confirmation, that the difference is renal in origin. It may well be that the kidneys of very young birds, up to 3 weeks of age, are unable to cope effectively with the excess intake of salt and it is well known that

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young birds are more prone to oedema and ascites under conditions of salt loading (Krakower & Goettsch, 1945).

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Key Word Index—Age dependence; chick; cyst; efferent duct; rete testis; seminiferous tubule; sodium; testis; *Gallus domesticus*.

THE TOXICITY OF ORALLY ADMINISTERED POTASSIUM SALTS IN RENAL INSUFFICIENCY [1]

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Smillie (1) in 1915 reported the sudden collapse of a patient with nephritis seven hours after the oral administration of 10 grams of potassium chloride. He attributed this collapse to an excessive accumulation of potassium in the body owing to impaired excretion. The patient ultimately recovered. Smillie also found that sudden death commonly followed the oral administration of potassium salts to rabbits with uranium nephritis. Similar amounts were without effect in control animals. He reported no chemical determinations of potassium in the serum or urine. Smillie's observations have frequently been cited as indications of the possibility of potassium poisoning in renal insufficiency. However, the complex extrarenal changes which occur in uranium nephritis and the peculiarly specialized character of the rabbit kidney make it improper to generalize from these experiments concerning oral potassium poisoning. There is, on the other hand, reason for minimizing the danger of toxicity from potassium in human nephritis. High concentrations of potassium in the serum are rare even in the most advanced nephritis (2), although these patients may ingest considerable amounts of meat and other foods containing potassium, and although they must continually be liberating potassium from the breakdown of tissue.

In the present study an attempt is made to ascertain whether humans or animals can be poisoned by oral administration of potassium. Potassium salts were administered to human subjects with and without renal impairment, and the concentrations of potassium in serum and urine followed at intervals thereafter. In another type of experiment, dogs whose ureters had been tied received potassium salts by stomach tube. Frequent electrocardiographic observations and chemical determinations of potassium in serum were used for the detection and estimation of toxic effects of potassium in these animals (10).

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METHODS

(A) General

The method of conducting the observations in fasting human subjects has been described elsewhere (3). Samples of urine and of serum were obtained at intervals before and after the ingestion of potassium salts. Urine specimens were collected without catheterization. Usually considerable water was given during the course of the experiment. Potassium salts were administered either in capsules, followed by water, or dissolved in tomato juice. Urea clearances were determined in all experiments, and in some, sucrose clearances were measured after preliminary intravenous injections of sucrose. Both sucrose and urea clearances vary with the glomerular filtration rate and in renal disease are reduced in about the same proportion as the filtration rate (3, 4). Accordingly, they may both be used as relative measures of glomerular filtration. Potassium was determined as the chloroplatinate by the method of Hald (5). Clearances were calculated by the general method previously described (3).

For the animal experiments nine dogs were employed. All experiments were carried out under nembutal anesthesia (40 mgm. per kgm.). A tracheal cannula was inserted. Urinary excretion was suppressed by ligation of the ureters. Potassium chloride in isotonic or hypertonic solution was introduced by tube into the stomach or duodenum. Serial electrocardiograms from lead II were taken and samples of blood for potassium analysis were obtained by direct puncture of the heart at the termination of the experiment.

(B) Electrocardiographic changes as criteria of potassium poisoning

As the concentration of potassium in the serum rises, the following sequence of electrocardiographic changes is observed (6): (1) increased amplitude of the T wave, (2) depression of the ST segment with development of a diphasic T wave, (3) disappearance of the P wave, (4) intraventricular block, and (5) cardiac arrest at concentrations of serum potassium of 12 to 20 m.eq. per liter. These changes, save for the last, are reversible. No one of them is, of course, characteristic; but when

TABLE I

Fasting excretion and clearance of potassium; no salts of potassium ingested

Number	Diagnosis	Serum potassium		Urine potassium		Clearances		
		Time †	Concentration	Time	Excretion rate	Potassium	Sucrose	Urea
		minutes	*m. eq. per liter*	*minutes*	*m. eq. per minute ×10⁰*	*cc. per minute*	*cc. per minute*	*cc. per minute*
1	Arthritis			0–33	50.2	10.0*	143	40
				33–65	53.2	10.6*	122	76
				65–93	35.5	7.1*	122	60
2	Diabetes	45	5.00	0–42	53.9	10.8	111	42
				42–95	70.9	14.2	110	40
		128	4.60	95–128	43.4	9.0	108	41
3	Hyperthyroidism	112	5.15	6–71	47.3	9.2	170	67
				71–115	36.9	7.4	126	55
4	Nephrosclerosis	8	5.00	0–37	42.8	8.6	92	47
				37–81	46.9	9.4	82	36
		133	5.00	81–129	32.8	6.6	68	30
5	Pyelonephritis	5	5.40	0–36	50.3	9.7	16	9
				36–92	46.0	8.9	18	9
		144	5.65	92–135	40.1	7.1	15	8

* Assuming serum potassium of 5.0 m.eq. per liter.
† Time from beginning of experiment.

found in sequence they are sufficiently specific to serve as criteria by which the presence and degree of cardiac potassium poisoning may be detected.

RESULTS

(*A*) *Observations on human subjects*

Five experiments, with fasting human subjects, are summarized in Table I. In Subjects 1, 2 and 3 glomerular filtration, as measured by the sucrose and urea clearances (3, 4), was normal; in Subject 4 somewhat reduced, and in Subject 5 severely impaired. In all five, however, the fasting rate of excretion of potassium was approximately the same, averaging about 0.05 m. eq. per minute,[2] and varying over the extreme range of 0.03 to 0.07 m. eq. per minute. The excretion rate was no lower in Subject 5 than in the others. Since the serum concentration was normal in all experiments, this means that fasting potassium clearance was usually about the same in all subjects, whether or not glomerular filtration was reduced. In magnitude it was about 10 cc. per minute; this was about one-twelfth or one-fifteenth of the average glomerular filtration rate in normal subjects (7), but amounted to nearly half the glomerular filtration in Subject 5. This means that in the subjects with impaired function a normal fasting excretion rate of potassium was maintained by a great decrease in the usual proportion of potassium per unit of filtrate reabsorbed by the tubules.

The response of two subjects with normal renal function to the ingestion of potassium salts is recorded in Table II A. In the first subject (1a) the rate of excretion of potassium increased over fivefold. The serum concentration rose but slightly, so that the clearance of potassium rose to an almost equal extent. In the second subject (2a) the increase is partially masked by an abnormally high fasting excretion rate of potassium, due to some unknown cause. Here too, however, an excretion rate many times the normal fasting value continued for some hours after ingestion and (since the serum concentration rose but little) was associated with a marked elevation of the potassium clearance. In spite of this increased excretion rate, elimination of the ingested potassium was slow. In Experiment 1a only about one-third was eliminated in two hours, while in Experiment 2b only about three-fourths had been recovered from the urine in five hours.

The course following the administration of potassium salts in five subjects (1b, 2b, 3b, 4b, 5b) exhibiting varying degrees of renal impairment is outlined in Table II B. In these subjects, too, the rate of excretion increased definitely following the ingestion of potassium, but not as much as it did in the normal subjects of Table II A. The maximal increase in potassium concentration in the serum was about the same in nephritic as in normal subjects but the elevated concentration persisted for a longer period in the more severe nephritics. Since the initial level was a little higher in some of the nephritic subjects, the final total concentration attained was sometimes a trifle higher. However, the concentration never exceeded 8 m. eq. per liter with doses of the size here given. Attempts to administer potassium salts in amounts greater than these were unsuccessful, since cramp-like epigastric pains followed by nausea and vomiting regularly intervened. In

[2] The average fasting excretion rate of 0.05 m.eq. per minute corresponds to 2.9 grams of potassium daily. On the assumption that in the breakdown of tissue 1 gram of potassium is released for every 2.5 grams of nitrogen (2), this corresponds to $2.9 \times 2.5 \times 6.25 = 45$ grams of protein broken down daily. This is a reasonable figure for tissue catabolism.

TABLE II

(A) Subjects without renal impairment; response to ingestion of potassium salts

Number	Diagnosis	Potassium ingested		Serum potassium		Urine potassium			Total amount excreted	Clearances		
		Time	Amount	Time	Concentration	Time	Excretion rate	Amount excreted		Potassium	Sucrose	Urea
		*minutes**	*m. eq.*	*minutes**	*m. eq. per liter*	*minutes**	*m. eq. per minute ×10³*	*m. eq.†*	*m. eq.*	*cc. per minute*	*cc. per minute*	*cc. per minute*
1A	Hypertension	0	67	3	4.75	0–42	45.6	1.9		8.7	139	65
				68	5.75	42–80	244.0	9.3		42.3	208	94
				127	5.50	80–124	238.0	10.5		42.8	207	103
									21.7			
2A	Hodgkin's disease	83–143	100	0	4.80	0–53	280.0	(14.8)		58.0		157
						53–80	143.8	(3.9)		30.1		91
				150	7.65	80–125	224.0	10.1		29.3		95
				248	6.25	125–240	345.0	39.7		50.5		61
				376	5.00	240–375	238.2	32.1		42.2		56
									81.9			

(B) Subjects with renal impairment; response to ingestion of potassium chloride

1B	Nephrosclerosis	134	67	0	4.90	0–58	22.4	(1.3)		4.6		
				67	5.00	58–140	63.5	5.2		12.1	71	56
				162	5.45	140–208	64.0	4.4		10.2	49	32
				211	6.25	208–270	61.5	3.8		9.8	71	29
									13.4			
2B	Acute nephritis	105–115	67‡	0		0–95	45.2	(4.2)		9.0		20
				207	6.25	95–202	79.3	8.5		14.1		22
				246	6.50	202–240	114.3	4.4		18.0	36	26
				296	5.90	240–270	74.1	2.2		11.4	25	16
						270–292	68.9	1.5		11.3	23	15
									16.6			
3B	Acute nephritis	79–146	107	0	6.35	0–42	54.7	(2.3)		8.6		9
				79	6.95	42–79	48.9	(1.8)		7.9		10
				196	7.45	79–122	66.2	2.9		10.0		13
				232	7.65	122–189	83.7	5.6		11.6		9
				300	7.70	189–227	104.5	4.0		13.9		8
				409	7.95	227–297	87.0	6.1		11.3		6
						297–350	114.0	6.0		14.9		12
						350–406	89.9	5.0		11.5		7
									29.6			
4B	Chronic nephritis	106–146	134	0	4.90	0–48	32.5	(1.6)		8.6		9
						48–104	— §	2.2 estimated				
						104–135	49.0	1.5		9.5		8
				180	6.75	135–174	73.7	2.9		12.5		9
						174–251	— §	6.2 estimated				
				357	7.35	251–301	84.0	4.3		12.2		9
						301–357	93.3	5.2		12.9		11
									22.3			
5B	Chronic nephritis	156–241	94	84	5.00	0–90	41.3	(3.7)		8.3		17
				150	6.05	90–155	75.8	(4.9)		13.8		23
				238	6.70	155–234	99.2	7.8		15.6		24
				326	6.95	234–322	110.3	9.8		16.2		19
				404	7.50	322–401	112.0	8.8		15.5		18
									26.4			

* Time from beginning of experiment.
† Figures in parentheses are not included in the summation of the amount excreted.
‡ Vomited.
§ Collection incomplete.

Experiment 3b "collapse," similar to that described by Smillie, occurred. The patient became very pale, sweated profusely, the pulse became rapid, the blood pressure fell, and he lost consciousness for an instant. He then vomited a small amount of fluid after which the symptoms disappeared completely and he felt entirely normal. The serum potassium was 7.70 m. eq. per liter at the time of the attack, and continued to rise to 7.95 later, although the patient had recovered completely. This makes it unlikely that the episode was due to cardiac potassium poisoning. It appeared rather to have been a transient reflex vasodilatation in a subject with acute arterial disease, perhaps induced by gastro-intestinal irritation, *i.e.*, a vagotonic associate of nausea and vomiting.

Since the increase in the rate of excretion of potassium is less than that in normal subjects, elimination is correspondingly delayed, so that even after five and six hours only one-quarter or less of the ingested potassium had been eliminated. Nevertheless, as has been observed, the serum concentration is only moderately elevated. The reason for this presumably lies in the mode of distribution of potassium in the body. Also, in these cases its absorption may have been greatly delayed.

The increase in concentration of potassium in the serum following the ingestion (8) or injection (9, 10) of potassium salts is only about one-third as great as that which would be expected were its distribution purely extracellular. In other words, about two-thirds of the absorbed potassium leaves the extracellular fluid. This amount can be accounted for on the assumption that a uniform rise of potassium concentration of the same magnitude as that in serum water occurs throughout the cellular water. However, segregation of some or all of this potassium in some particular tissue is equally consistent with the facts. Hahn, Hevesy and Rebbe (11), using a radioactive isotope of potassium, have demonstrated an absence of diffusion equilibrium between cellular and extracellular potassium. This indicates that potassium ions exchange relatively slowly across the cell membranes of the body, and so perhaps makes the hypothesis of uniform distribution in all body water somewhat less probable. The interpretation of their experiments, in which the concentration of radioactive potassium in various tissues was determined, is not entirely clear; possibly some special accumulation in bones took place. Whatever the mechanism, it is certain that the body does have the capacity to store at least two parts of potassium elsewhere for each part that accumulates in the extracellular fluid. This means that, with respect to potassium, the body has a buffer capacity by which increases in the concentration of this element in the serum, after ingestion of its salts, are minimized. The quantitative importance of this buffer mechanism may best be seen by considering a typical example. This has been done graphically in Figure 1, using data of Experiment

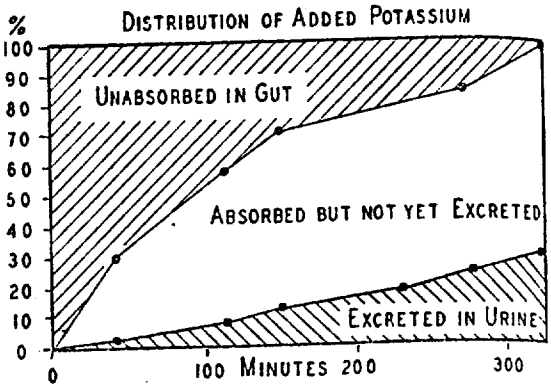

FIG. 1. PROPORTIONS OF THE INGESTED POTASSIUM (A) STILL UNABSORBED FROM GUT, (B) ABSORBED AND PRESENT IN THE BODY AND (C) EXCRETED IN THE URINE

The amount present in the urine was determined by chemical analysis. That present in the body was calculated on the assumption that one-third of the ingested potassium was present in the extracellular fluid. The increase in concentration of potassium in the serum water was multiplied by the volume of the extracellular fluid (assumed to be 15 liters), and this figure in turn multiplied by three to obtain the total amount distributed in the body. The amount still present in the gut was calculated by difference. Since the factor three is based largely on experiments with intravenous injections in dogs and in a very few human subjects, it is quite an approximate one. Therefore, these calculated values are only approximate, but serve to illustrate the several components in the system. From the data of Experiment 3b, Table II B.

3b of Table II B. Absorption from the gut is slow, being complete only after some five hours. Excretion in the urine is also very gradual. At the end of five hours the greater part of the potassium can be accounted for within the body. Be-

cause a large proportion finds a temporary repository outside the extracellular fluid, the increase in the concentration of potassium in serum water is only about one-third as great as that of a substance limited to an extracellular distribution.

Although excretion rate is not as greatly elevated in patients with renal insufficiency as in normal subjects, it increases sufficiently to cause the clearance of potassium to rise. This enables the excess potassium to be eliminated without a great change in the potassium of serum and, although elimination is slow, it is sufficiently increased above normal so that the ingested potassium could be eliminated within twenty-four hours.

Discussion of the observations on human subjects

The experiments with human subjects which have just been described indicate the reasons for the rarity of potassium poisoning in human nephritis. In summary they are as follows:

(*1*) The fasting clearance of potassium is not reduced in renal disease, so that the basal excretion of potassium continues without increase in potassium in the serum.

(*2*) The distribution of the potassium ion beyond the confines of the extracellular fluid provides a temporary storage site for large amounts of potassium without much increase in serum concentration.

(*3*) The relatively slow gastro-intestinal absorption of potassium prevents transient high concentrations of potassium in the serum.

(*4*) Even with the most severe renal disease the clearance of potassium rises significantly above the fasting level following the ingestion of potassium salts. The associated increase in excretion rate permits the extra ingested potassium to be eliminated after some hours.

(*5*) It is difficult to administer amounts of potassium too great to be cared for by the storage and excretory capacities without inducing nausea and vomiting.

However, the possibility that potassium poisoning may under some circumstances occur in renal insufficiency has not been excluded. Since experiments designed to test this possibility are obviously too dangerous to attempt with human subjects, resort was had to animal experimentation.

(*B*) *Experiments with dogs*

(*1*) Control experiments.

Potassium salts were introduced directly into the duodenum of two normal anesthetized dogs in order to insure the most rapid possible absorption. In spite of this, the potassium of serum rose but slightly after four to ten hours, and only the earlier electrocardiographic changes appeared. Rapid absorption from the gastro-intestinal tract certainly took place under these conditions, as was apparent in experiments similar in every respect save that the ureters were obstructed. Failure to produce a notable accumulation of potassium in the body fluids appears, therefore, to have been due to rapid excretion rather than faulty absorption. Evidently, the rate at which potassium may normally be excreted by the kidneys easily equals the maximal rate of absorption from the gastro-intestinal tract. It is therefore reasonable to conclude that fatal poisoning from oral ingestion of potassium cannot occur in the presence of normal renal function.

(*2*) Experiments with dogs with ligated ureters.

(*a*) Delay in absorption of potassium salts introduced into the stomach.

When solutions of potassium chloride were introduced into the stomach by tube, electrocardiographic or chemical evidences of potassium poisoning usually failed to develop for an hour or more. Pressure on the abdomen forced back through the stomach tube as much fluid as had been administered, indicating that the absence of potassium effects was due to failure of absorption. The cause for this was at once apparent on opening the abdomen. The pylorus was so tightly contracted that even with vigorous manual manipulation it was impossible to force the stomach contents past it into the duodenum.

The fluid was permitted to remain in the stomach of three animals. One animal showed the first electrocardiographic signs of potassium poisoning after thirty-five minutes, and died after an hour. The other two still survived at the end of ten hours, but with unmistakable electrocardiographic changes characteristic of pre-terminal potassium poisoning and with concentrations of potassium in the serum of 10.1 and 10.9 m. eq. per liter, respectively. It seems probable that the

long survival was due to cessation of absorption of potassium due to circulatory failure after the potassium concentration had risen to a level nearly, but not quite, sufficient to produce death. There is other evidence that such circulatory failure does occur (12).

(*b*) Duodenal administration of potassium.

Potassium chloride was administered by means of a tube passed through the pylorus via a gastrostomy in order to avoid delay in absorption due to pylorospasm. In three experiments this was done after a previous unsuccessful attempt

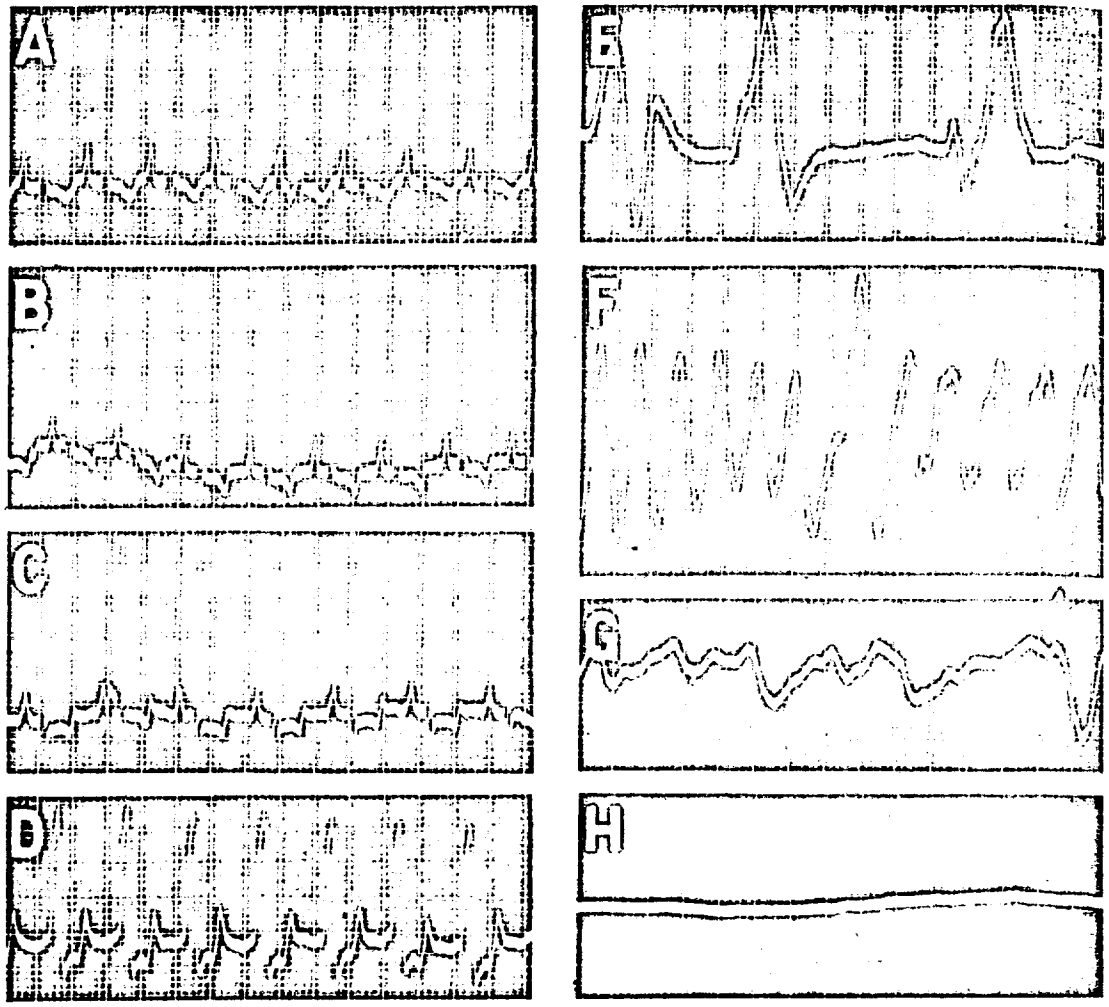

FIG. 2. SERIAL ELECTROCARDIOGRAMS OBTAINED IN A DOG WITH LIGATED URETERS FOLLOWING THE ADMINISTRATION OF TWICE ISOTONIC KCl SOLUTION BY TUBE INTO THE DUODENUM

All records from lead II. Dog, 7.5 kgm.

(A) Control.

(B) No change seventeen minutes after 1 liter of solution before ureters were tied.

(C) Depression of ST segment and beginning diphasic T wave fifteen minutes after ligation of ureters and administration of another liter of KCl.

(D) Disappearance of P waves, slight intraventricular block, marked ST segment depression, diphasic T wave thirty-five minutes after ureteral ligation.

(E) Marked intraventricular block and some slowing fifty minutes after ligation.

(F) and (G) Sudden ventricular flutter, followed within two minutes by coarse ventricular fibrillation.

(H) Arrest two minutes later. Concentration of potassium in serum 13.0.

to bring about absorption by introduction into the stomach, while in three others no such prior attempt had been made. Prompt and vigorous purgation occurred in all three experiments, and much of the solution administered was quickly lost in this way. All six animals died within a few minutes to an hour, depending on the rate at which the potassium chloride was administered. That death was due to cardiac potassium poisoning appears certain from the characteristic sequence of electrocardiographic changes in each instance (Figure 2, A to E), and from the high concentrations of potassium in the blood just before death. These were 9.8, 10.8, 12.4, 13.0, 13.0, 14.4 m. eq. per liter, respectively, and fall in or just below the range associated with cardiac arrest from intravenous potassium (6).

In a few of these experiments exitus did not occur following cardiac slowing and arrest. Instead, the rate was maintained for some time in the face of increasing intraventricular block until suddenly ventricular flutter and coarse fibrillation appeared (Figure 2, F, G). It seems reasonable to suppose that in these experiments circulatory failure occurred as the result of intraventricular block, delaying further absorption of potassium so that the final stages of slowing and arrest did not appear.

DISCUSSION

Evidently, it is possible for fatal poisoning from oral potassium to occur under certain experimental conditions. The essential conditions are suppression of urinary secretion and direct introduction of the potassium solution into the duodenum. In the dog, at least, this second condition is difficult of attainment after ordinary oral administration owing to pylorospasm. The relatively slow absorption of potassium and the severe epigastric pain, cramps and vomiting observed in human subjects after potassium salts are administered strongly suggest that in man, too, pylorospasm may occur. No pylorospasm occurred in control experiments in which water or normal saline was introduced into the stomach of a dog with ligated ureters. This spasm after potassium chloride is therefore not simply a response of the pylorus of such an animal to the ingestion of any fluid. However, there is no evidence that the production of pylorospasm is a unique property of the potassium ion, as other irritant salts were not tested.

The animal experiments, therefore, indicate that, while oral potassium poisoning may occur, the conditions necessary for its production are not likely to occur in human subjects with nephritis. In a deeply anesthetized animal with complete anuria, enough potassium may eventually pass the pyloric sphincter to cause death. In man, comparable doses are, of course, not given; and even with smaller amounts vomiting acts to remove a great proportion of the material administered. Moreover, a distinction must be drawn between the situation in nephritis and that in complete anuria. In the nephritic patient renal function is rarely completely suppressed. The rate of excretion of potassium, though reduced, is sufficient to eliminate that coming from tissue breakdown, as well as a certain excess. Consequently, the small amounts passing the sphincter and absorbed from the duodenum can be gradually eliminated, while the distribution in the body minimizes the transient increase in concentration of circulating potassium. On the other hand, in complete anuria (such as that following mercury poisoning), there is obviously much more possibility of potassium poisoning, since the delays of absorption and the wide distribution are of temporary value only. Indeed, in animals with complete urinary obstruction, gradual elevation of serum potassium due to tissue breakdown is the rule (13). The danger of giving even small amounts of potassium by mouth under such circumstances is self-evident.

CONCLUSION

1. Cardiac death due to potassium absorbed from the gastro-intestinal tract can be produced experimentally in dogs whose ureters have been tied.

2. Direct introduction of potassium salts into the stomach induces spasm of the pylorus, preventing absorption and delaying death. Rapid death occurs when potassium salts are introduced directly into the duodenum of animals with ligated ureters.

3. It is unlikely that the conditions necessary for fatal poisoning by oral potassium administra-

tion can occur in patients with nephritis so long as urine is being excreted. The relatively slow absorption, the vomiting when large doses are given, the mode of distribution in the body and, most important of all, the continued ability of the severely damaged nephritic kidney to excrete potassium all combine to make such poisoning very difficult to bring about.

4. Poisoning from oral administration of potassium is a more distinct possibility with complete anuria from any cause.

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POTASSIUM CHLORIDE AND INTESTINAL ULCERATION

SIR,—You drew attention (July 3) to intestinal ulceration produced by enteric-coated potassium chloride. More than 300 cases have already been identified.[1] The precipitating factor appears to be the rapid release of potassium chloride over a short segment of intestine.[2,3] This danger led you to recommend the withdrawal of potassium-chloride tablets and their replacement by effervescent potassium preparations. This recommendation, however, overlooks the fact that the chloride anion may be as important in therapy as the potassium.[4-6] There is a need therefore for a potassium-chloride formulation which is well tolerated by the stomach and the small gut.

The tablet which is the subject of the present study ('Slow-K', Ciba) has been formulated to meet this requirement. The rate of solubility of potassium chloride is reduced by partially coating the crystals with an inert insoluble wax. The tablets are then prepared from the mixture and are sugar-coated, not enteric-coated. Unlike conventional tablets they do not disintegrate in fluids; the potassium is gradually dissolved out leaving the wax as a spongy matrix.

TABLE I

Case no.	Dose of potassium chloride		Duration of therapy (days)	Mean control potassium excretion (A) (mEq. per day)	Mean test potassium excretion (B) (mEq. per day)	B-A (mEq. per day)
	mEq. per day	g.				
1	161	12·0	45	15·6	14·0	−1·6
2	72	5·4	30	12·0	9·8	−2·2
3	97	7·2	27	12·7	15·6	+2·9
4	161	12·0	24	9·7	10·3	+0·6
5	97	7·2	21	16·0	13·0	−3·0
6	97	7·2	9	7·0	9·4	+2·4

In simulated intestinal juice (*British Pharmacopœia 1958*) these tablets release their potassium chloride progressively over a period of some four hours, whereas conventional enteric-coated tablets are virtually totally dissolved in fifteen minutes.[7] It was necessary to determine whether the potassium chloride in slow-K tablets is fully absorbed from the gut or not. Metabolic studies to determine this point have been carried out in 6 patients (see accompanying table). The patients received a constant food intake and after nine to twelve days the tablets, each containing 600 mg. potassium chloride, were started. They were given in divided doses 4–5 times a day without special reference to meals. Three-day fæcal and daily urinary collections were made and the excreta analysed for potassium. The results show complete absorption of the potassium supplement. In a previous test of four commercially available enteric-coated potassium-chloride tablets,[8] only one formulation was satisfactorily absorbed.

During the present studies none of the patients complained of gastrointestinal symptoms, and occult-blood tests on the stools were negative. During the past six months 20 patients requiring additional potassium have been given this in the form of slow-K. No side-effects have been noticed, and repeated occult-blood tests have been negative.

Only time and the study of very large numbers of patients can tell whether slow-K tablets are free from the danger of producing intestinal ulceration. On theoretical grounds they should be safe, and these studies show that they are effective as a potassium-chloride supplement.

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THE CHRONIC TOXICITY OF SALT (SODIUM CHLORIDE)

THE 1956 JEROME COCHRAN LECTURE

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Nashville, Tennessee

Sodium chloride (common table salt) is one of the oldest known but least understood of our essential nutrients. This paradoxical statement is rather startling but nevertheless easily documented. Knowledge of its first use is lost in antiquity. Much of its chemical nature and properties has been known for centuries and only the development of nuclear physics in recent years has added anything of importance to this aspect. Compared to it, such nutrients as thiamin and vitamin B_{12} are the merest parvenus.

It is customary to describe and categorize nutrients by such characteristics as their requirement, their action, the effects of excessive intake and of their inbalance with related nutrients. Much is known of this in respect to vitamins, protein, calories and other nutrients. Much is known of this with respect to sodium chloride also but much is not known. For example, the quantitative requirement of sodium chloride for growth and maintenance of health in man and other animals has not been ascertained. Its action on certain tissues and cells is obscure and the harmful effects, if any, in moderate excess are uncertain. That these things remain unknown for such a well-known and common nutrient as salt is the basis of the paradox.

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I have said that knowledge and use of salt goes back to antiquity. It is also true that as far back as these earliest times there were areas of the human world where salt was not used. The social, religious, economic, ethnic and political aspects of the use of salt are well recorded in history. Meneely has summarized them well in a recent editorial.[1] The great craving for salt by salt-using peoples is shown by the fact that wars have been fought for sources of it, people have been executed for smuggling it, and men, women and children sold into slavery for it. There exists therefore the need of explanation for the existence of these two dietary or nutritional situations regarding the consumption of salt.

The explanation probably lies in the distinction between salt in the diet and salt added to the diet. The evidence seems clear that herbivores require salt in their diet. As is well known, herbivorous animals will travel far to reach salt licks which play a large part in their ecology. Carnivores, on the other hand, do not frequent salt licks and presumably acquire the salt they need from their natural diet, i. e., the flesh of other animals. Man for hundreds of thousands of years, from his first emergence as a human being to the food-producing or early agricultural age, was probably primarily a carnivore. As such he required no salt added to his food. With the change from a predominantly meat-eating animal to a large consumer of cereals and vegetables there came the need for additional salt.

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As Meneely has pointed out, this was apparently not due to the smaller actual salt content of the non-meat diet but to the greater potassium content of the cereal and vegetable diet. There is another factor, however, affecting the addition of salt to the food. As man developed a craving and need for salt with his change to a more cereal and fruit diet, the preservative properties of salt were discovered. This resulted in the development of a large variety of salted foods which, in turn, led to a taste for salt and an addiction which today is reflected in the presence of added salt (sometimes in large amounts) in our food and on nearly every dining table, public and private.

Thus it becomes a matter of interest and importance to determine the human need for salt as contrasted with his desire for it, its relation to potassium intake and its possible toxicity or injuriousness, particularly on the basis of long continued intake.

Knowledge regarding the relation of salt to disease has been available for many years. Its relation to edema and to congestive heart failure has been recognized for a long time, even if not fully understood, and most recently acknowledged in the employment of low sodium diets in treatment of these conditions. Allen[2] considered it important in hypertension, and the rice diet of Kempner[3] is an example of the practical application of such theories. Selye[4] was able to produce lesions in chicks, fed large amounts of salt, similar to those found in nephrosclerosis in man. Sapirstein[5] produced renal lesions and hypertension in rats by providing only salted drinking water. There is a large literature on salt as an electrolyte in abnormal states, in animals as well as man. The minimal sodium chloride requirements for some laboratory animals has been determined and the need for salt for the growth of farm animals has been well documented. However, despite this knowledge there is little known as to the actual requirements for man, or what, if any, constitutes a toxic level.

With this situation in mind the Vanderbilt-Thayer group became interested in investigating further the possible toxic effects of salt. Most of the previous animal experimental work has been done with birds because the more primitive renal glomerulus of fowl is more susceptible to injury by sodium chloride. Working with rats, Campbell[6] had found little or no effect on growth, health and reproduction on diets containing up to 5.06 per cent sodium chloride. At the higher levels some evidence of renal damage was found.

The Vanderbilt-Thayer group studied the effect on rats of diets containing seven levels of sodium chloride.[7-25] The basic No. 1 diet was a synthetic or natural fully-adequate diet, with a low sodium chloride content, 0.01 per cent (Fig. 1). Diets II to VII contained 0.15, 2.8, 5.6, 7.0, 8.4, & 9.8 per cent NaCl, amounts ranging from what might be considered "normal" or usual for rats to as high as 9.8 per cent sodium chloride (Fig. 2). The rats were provided with demineralized water and the diet *ab libitum*, and were weighed and carefully examined weekly.

Ration I (Low-Na)

Casein, Vitamin-Test	25.1%
Cane Sugar	51.8%
Shortening, All-Vegetable	20.0%
Mineral Mixture	2.9%
Vitamins	0.2%

Fig. 1. Composition of basic purified rat ration.

DIETARY SODIUM CHLORIDE

Ration	% NaCl
I (Low-Na)	0.01
II (Control)	0.15
III	2.8
IV	5.6
V	7.0
VI	8.4
VII	9.8

Fig. 2. Sodium chloride content of 7 experimental rations.

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All seven groups of rats consumed the rations, grew and gained weight. The group on the low salt diet exhibited some anorexia, salt hunger and grew less rapidly. Growth on the control diet (0.15%) paralleled growth on usual standard rat diets.

All in all the rats remained in excellent health during the twenty weeks of the first part of the experiment. Only 17 of a total of 193 died or were sacrificed during the initial 20 weeks; 9 deaths were attributable to dietary stress, less than 5 per cent of the total number or 10 per cent of those on these high salt diets. Six died of causes not related to the diet. These on the higher rations of salt developed a marked polydypsia (Fig. 3) and polyuria, a well known phenomenon of high salt intake with unlimited water. However, beginning in the second month, some of those on the highest level of salt intake began to show rapidly developing, massive edema (Figs. 4 & 5). Altogether some 15 per cent of the 90 rats on these higher salt diets exhibited this syndrome (Fig. 6). Studies with the radioactive isotope of sodium showed the volume of extra-cellular water to be as great as twice normal in some of the edematous animals. The edematous animals, at necropsy, showed gross edema of the tissues, diminished total serum proteins, severe anemia and azotemia. Blood pressures were elevated in most of the edematous rats tested. Histologic examination of the tissues of these animals showed striking lesions in the kidneys with the glomerulus most severely affected, though the whole nephron was involved. The glomerular tufts were enlarged, the endothelial and epithelial cells swollen, the cytoplasm vacuolated, and the

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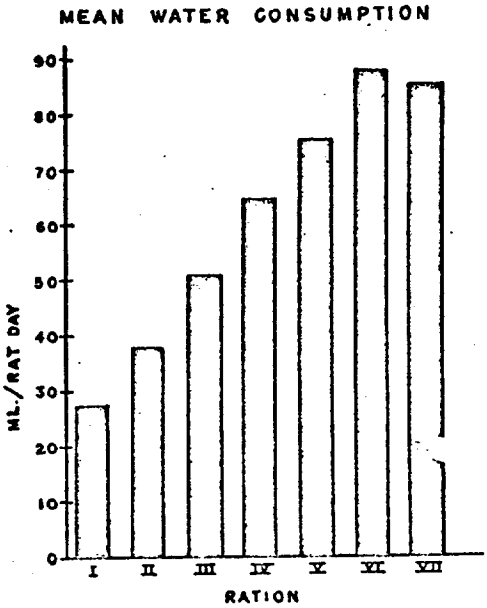

Fig. 3. Mean water consumption by ration first 20 weeks.

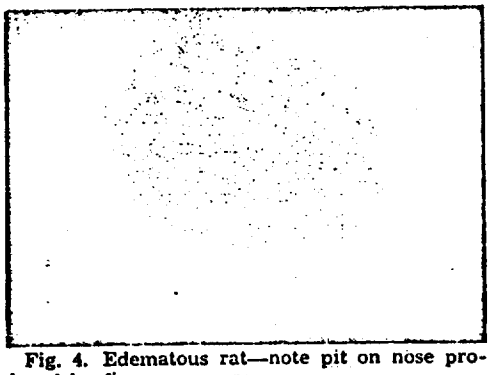

Fig. 4. Edematous rat—note pit on nose produced by finger pressure.

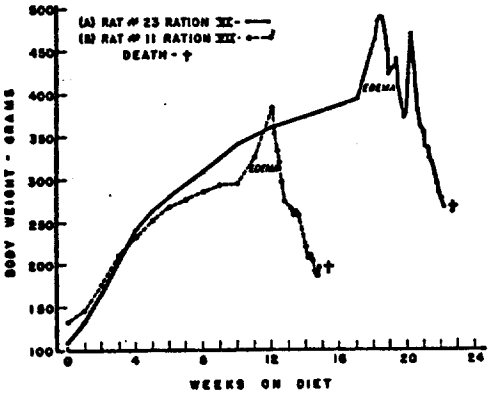

Fig. 5. Weight curves of 2 edematous rats.

CLINICAL FEATURES OF A SYNDROME OCCURRING IN 15 PERCENT OF RATS EATING FROM 7.0 TO 9.8% NaCl IN THE DIET

Time of Occurrence: 2 to 6 Months

- Edema
- Hypertension
- Anemia
- Lipemia
- Hypoproteinemia
- Azotemia

Fig. 6. Clinical picture resembling nephrosis.

nuclei misshapen and pyknotic. The basement membranes were swollen and degenerated. There were large amounts of lipid in the glomerular tufts. The straight and convoluted tubules were dilated and contained precipitate of protein. The lining epithelium was pale and granular, with lipid material in the cytoplasm. The renal arterioles and some small arteries were affected, with lipid vacuoles in the smooth muscle cells some of which were necrotic. In many arterioles the elastic lamina was swollen and frayed, the lumen narrowed or even occluded. Extrarenal arteries were only occasionally involved. However, in rats on the higher salt rations (7.0, 8.4 & 9.8% NaCl) who survived longer and did not become edematous the kidney showed the same or greater changes, and other visceral arterial lesions were fairly constant, especially in the heart, pancreas, testes and gastro-intestinal tract. In those with heart lesions there were areas of scarring.

Reliable blood pressure determinations were obtained beginning with the ninth month. Nearly all the rats on the diets containing the three higher levels of salt (7.0, 8.4 & 9.8% NaCl), and most of these eating 5.6% NaCl, exhibited hypertension (Fig. 7). Significant elevation was found in some of the group on the lowest increased salt diet (III 2.8%). After twelve months the elevation in systolic blood pressure was proved to be in direct linear relation to the concentration of salt in the diets (Fig. 8).

Some of the rats in groups III and IV which were sacrificed failed to show histologic lesions by ordinary examination despite significant elevation of blood pressure. On the other hand, rats from groups V, VI and VII, (7.0, 8.4 and 9.8%) which were sacrificed exhibited lesions resembling those described in the edematous rats but were less severe and less uniform. The involve-

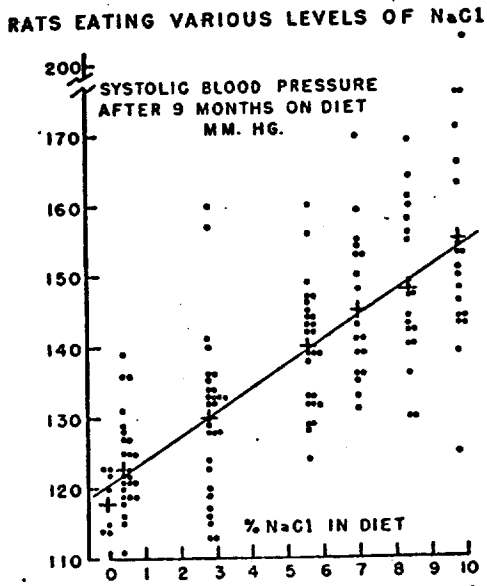

Fig. 7. Systolic blood pressure after 9 months.

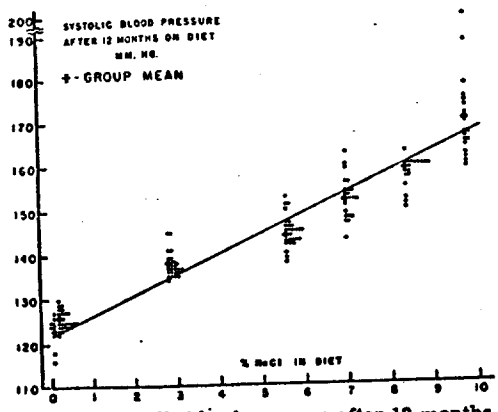

Fig. 8. Systolic blood pressure after 12 months.

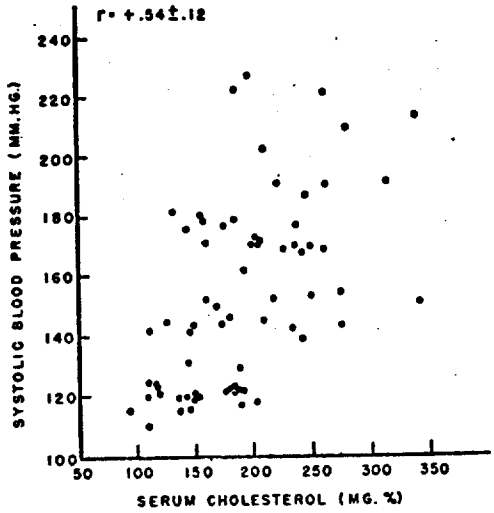

Fig. 9. Correlation of systolic blood pressure and serum cholesterol.

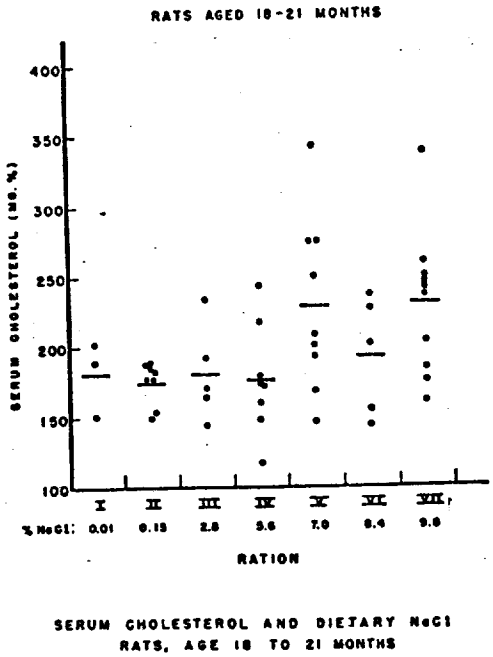

Fig. 10. Relation of serum cholesterol to per cent of NaCl in ration.

ment of the nephrons was spotty and changes in the arterioles when present were usually only hypertrophy of the media. However, some of these rats sacrificed some months later, when they were apparently declining before death, showed lesions equal to those in the edematous group.

Some of the animals in the later months developed dyspnea, edema, enlargement of the heart and elevated venous pressure, resembling congestive heart failure.

Serum cholesterol determinations were made on a few rats in each ration group. Hypercholesterolemia correlated positively with elevations in systolic blood pressure (Fig. 9) and was associated with increased dietary NaCl (Fig. 10).

Electrocardiograms were taken on all survivors after nineteen months on the experimental regimen. Some 80 per cent of the rats eating 9.8% NaCl exhibited abnor-

malities (Fig. 11). The incidence of specific abnormalities (Fig. 12) was also associated with the amount of salt in the ration. Selected tracings are shown for animals with moderate hypertension (Fig. 13) and severe hypertension (Figs. 14 & 15).

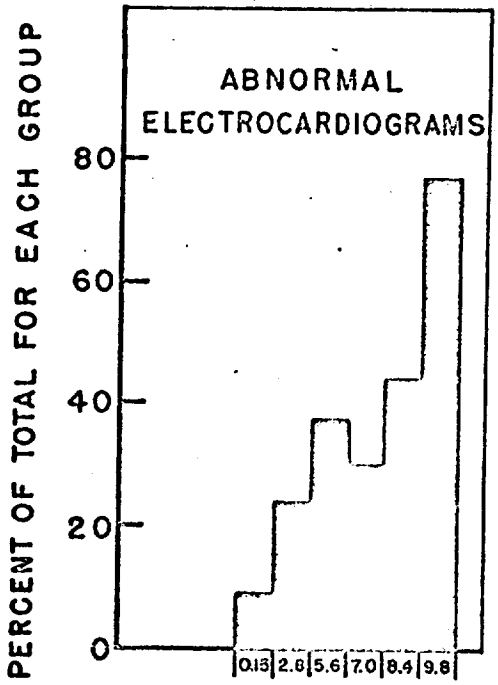

Fig. 11. Relation of incidence of ECG abnormalities and per cent of NaCl in ration.

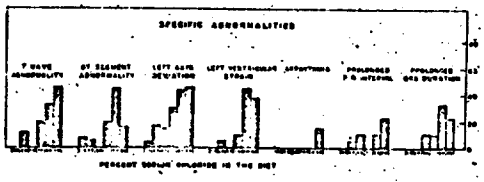

Fig. 12. Relation of specific of ECG abnormalities and per cent of NaCl in ration.

The mean maximum body weight achieved was related to the amount of salt in the diet, decreasing significantly (Po. 01) at the lowest level (0.01% NaCl) as well as at all of the levels above the control group. An exceptionally obese rat (Fig. 16) in the control group at an intake level of 0.15% NaCl achieved a maximum weight of 1008 grams at which time his systolic blood pressure, serum cholesterol and electrocardiogram were normal. Obesity was observed in a few rats at all levels up to and including 5.6% NaCl. It was not seen in rats eating 7.0% or more of NaCl in the diet. A surprising incidental finding was the negative correlation between the maximum body-weight attained and the systolic blood pressure at maximum weight (Fig. 17).

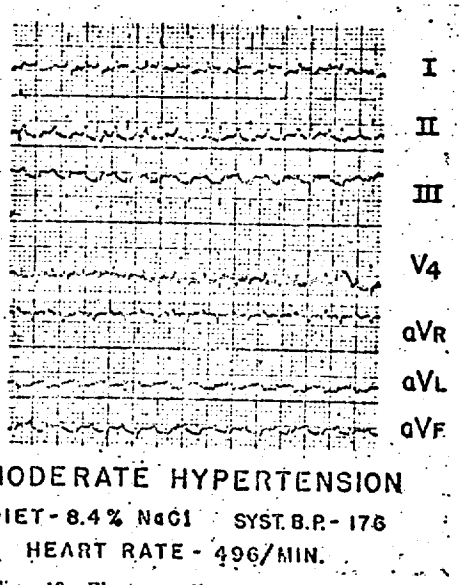

Fig. 13. Electrocardiogram of rat after 19 months on 8.4% NaCl.

The adverse influence of different levels of added salt upon survival is indicated in Fig. 18. Two additional groups of rats from a later experiment eating 21.0 and 14.0% NaCl are included on the survival curve. All rats on the 21.0% NaCl ration were dead at nine months. After fifteen months the per cent survival of the rats on the low salt diet (0.01% NaCl) was markedly lower than the controls (Fig. 19).

Mean adrenal weight (Fig. 20), heart weight (Fig. 21), and kidney weight (Fig. 22) in mg./kg. of rat were increased with added dietary salt. The mean length at death, measured from the nose tip to base of tail at autopsy, followed the maximum weight findings, i. e., significantly greater length in the control rats than in the low NaCl (0.01%) and stepwise decreases as dietary salt increment increased (Fig. 22).

The experiment has continued, although all original animals have died. Others have been started on similar experiments, with particular reference to the relationship of potassium and sodium.[21, 25] Females fed

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high salt diets produced fewer and smaller litters but did not develop symptoms of eclampsia. Pair feeding at the 5.6% NaCl of the purified diet and a commercial ration produced remarkable similar mean systolic blood pressures (Fig. 24).

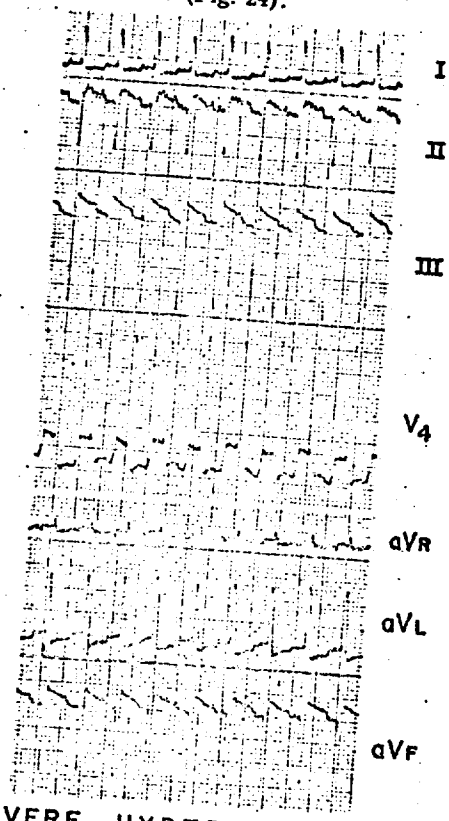

Fig. 14. Electrocardiogram of rat after 19 months on 8.4% NaCl.

It is clear that these findings must be taken only for what they are, that increases of salt in the diet of male rats produced, under the conditions of the experiment, pathologic lesions and increased blood pressure proportional to the concentration of salt. The effect seemed to be greatest on the growing animals. Also it is to be noted that some 1/3rd of the rat's life had passed before the more significant changes appeared. However, they seemed significant enough to extend the study to other species. Therefore, the study has been extended to dogs.

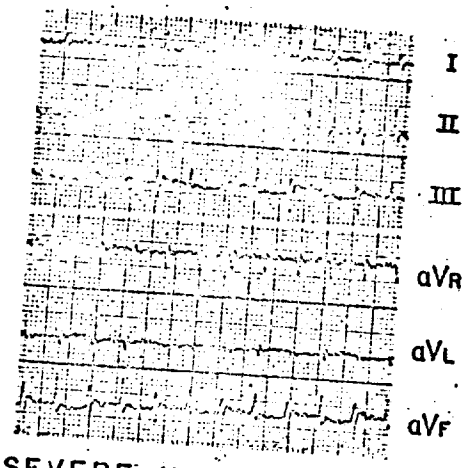

Fig. 15. Electrocardiogram of rat after 19 months on 9.8% NaCl.

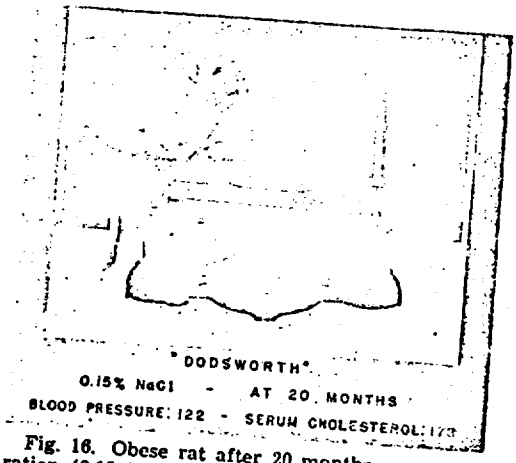

Fig. 16. Obese rat after 20 months on control ration (0.15% NaCl).

Twenty-four litter mate pedigreed male beagles were divided into four matched groups and placed on specially prepared diets* containing 0.2% (low), 1.4% (control), 6.0% and, initially, 12.0% NaCl. After four weeks of feeding, the group on the 12.0% NaCl showed such weight loss and

*Analysis of the dog chow as given by the Ralston Purina Company; moisture 8-10%, protein 20%, fat 6%, carbohydrate 49%, calcium 1.5%, phosphorus 0.8%, vitamin A-5 I. U. per gm., vitamin D-1. U. per gm., thiamine-3 U. per gm., riboflavin-5 U. per gm., panthenic acid-15 U. per gm., NaCl-0.2%.

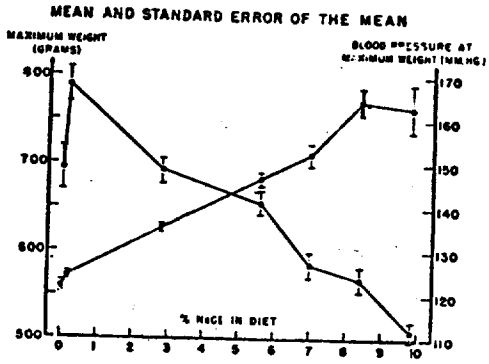

Fig. 17. Maximum weight attained and blood pressure at maximum weight of rats eating 7 levels of NaCl.

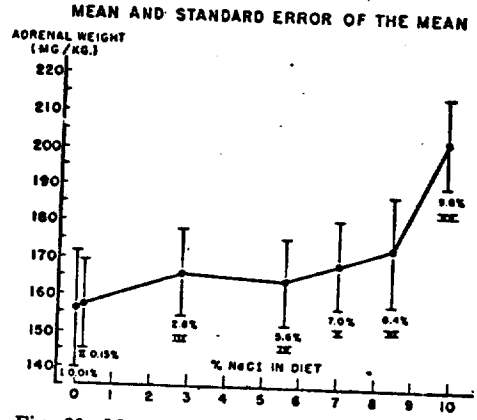

Fig. 20. Mean adrenal weights of rats eating 7 levels of NaCl.

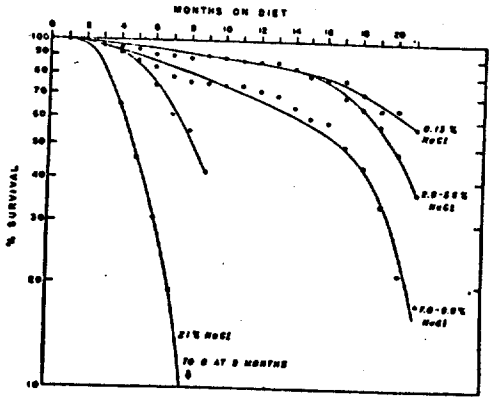

Fig. 18. Longevity % survival of rats eating 0.15 (control), 2.8, 5.6, 7.0, 8.4, 9.8, 14.0 and 21.0% NaCl. The slope of the line indicates mortality rate.

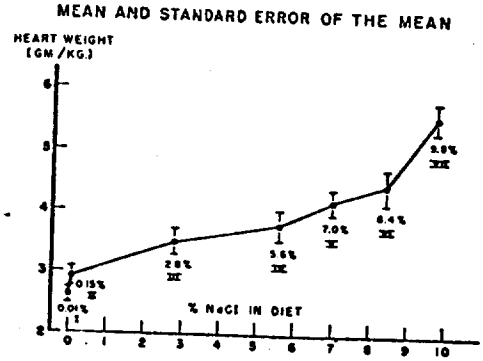

Fig. 21. Mean heart weights of rats eating 7 levels of NaCl.

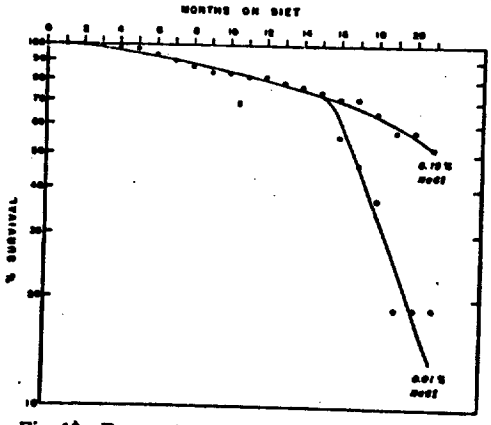

Fig. 19. Per cent survival of low NaCl (0.01%) and control (0.15% NaCl) rats.

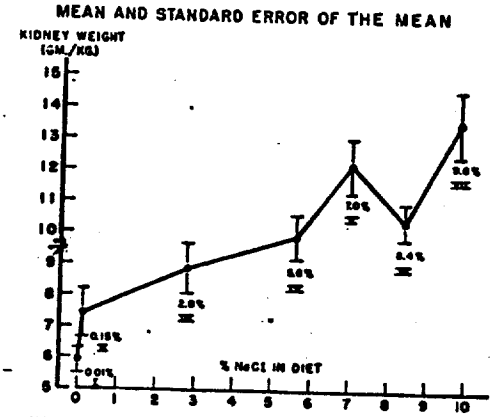

Fig. 22. Mean kidney weights of rats eating 7 levels of NaCl.

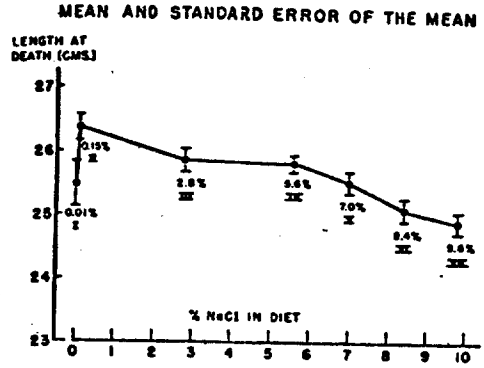

Fig. 23. Length at death measured from tip of nose to base of tail in relation to per cent of NaCl in ration.

HYPERTENSIVE ACTION OF DIETARY NaCl

♀ rats after 10 months

Ration	% NaCl	Systolic Blood Pressure Mean	±	S. D.
Purified Control	0.15	124.2	±	2.1
Purified	5.6	139.3	±	0.4
Purified	9.8	149.8	±	1.3
Commercial	5.6	140.1	±	1.2

Fig. 24. Comparison of hypertensive action of purified and commercial rations containing 5.6% NaCl fed female rats.

anorexia that the dietary level of salt was changed to 9.0%.

The dogs were weighed and examined at weekly intervals and the following measurements made on each animal every three weeks: mean blood pressure by femoral artery puncture, serum cholesterol, hemoglobin determination, hematocrit and complete hematologic study, including erythrocyte, leucocyte and differential counts. The data obtained after 26 weeks are summarized below.

I. *Growth*: There was no difference in the rate of growth of dogs on the low NaCl (0.2%) and the control group receiving 1.4% NaCl. The dogs on the high levels of NaCl grew more slowly than the animals on moderate or low levels of sodium. During the 26-week period the average weight gain for the individual dogs was 7.46 pounds + 1.13 for the group eating 0.2% NaCl, 6.92 pounds + 2.31 for the control group, 3.58 pounds + 2.64 for the dogs at the 6% level,

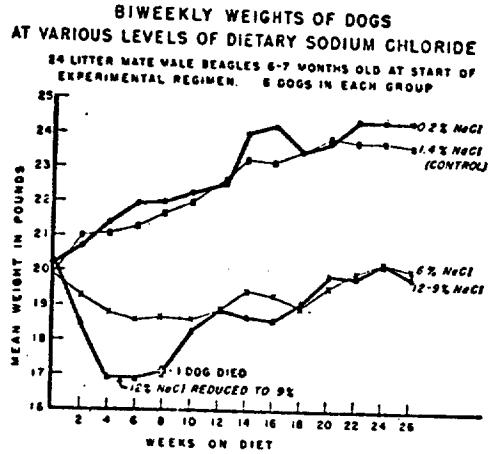

Fig. 25. Weight curves of dogs eating various levels of sodium chloride.

and 2.95 pounds + 1.13 for the group eating the highest level (12% and, later, 9%) of NaCl. These data are shown graphically in Fig. 25.

II. *Blood Pressure*: Several different methods for measuring blood pressure were tried during the earliest phase of this study. The direct method by femoral artery puncture proved most satisfactory. The dogs are now well trained and the readings of mean arterial pressures for individual animals are quite reproducible. No differences developed in the mean blood pressures of dogs on various levels of dietary sodium chloride for the first 26 weeks of feeding. This is to be expected at this time in the experiment since the albino rats do not exhibit the phenomena of chronic hypertension until the ninth month of feeding which represents about 1/3rd of the life span of the rat.

Average arterial blood pressure after 26 weeks on diet:

NaCl content of diet	No. of dogs	Average blood pressure (mm. Hg.) x + S. D.
0.2%	6	124+14
1.4%	6	123+15
6.0%	6	128+16
9.0%	5	127+11

III. *Serum Cholesterol*: The serum cholesterol concentrations in all dogs were within the normal range. The experimental findings range from 120 to 188 mg.% and there were no significant differences in the

average values for the groups. There is no apparent association of individual weight gain and serum cholesterol concentration.

Average serum cholesterol values after 26 weeks on diet:

NaCl content of diet	No. of dogs	Serum cholesterol mg % x + S. D.
0.2%	6	153+21
1.4%	6	144+24
6.0%	6	153+21
9.0%	5	148+26

IV. *Hemoglobin Concentration and Packed Cell Volume*: The average values for hemoglobin concentration and packed cell volume of animals were essentially the same in dogs on the low NaCl diet, the control ration and the 6% NaCl ration but both the hemoglobin and packed cell volume were considerably reduced in the group receiving 9% NaCl (Fig. 26).

HEMOGLOBIN AND PACKED CELL VOLUME AFTER 26 WEEKS ON DIET

% of NaCl in the diet	No. of dogs	Average hemoglobin concentration (gms. per 100ml. blood)	Average packed cell volume
0.2%	6	15.6	46.3
1.4%	6	14.9	44.0
6.0%	6	15.0	44.8
9.0%	5	13.4	41.2

Fig. 26.

V. *Erythrocyte and Leucocyte Counts*: The numbers of erythrocytes and leucocytes per unit volume were reduced in the group on the highest level of NaCl. The blood picture of the other three groups, receiving 0.2, 1.4 and 6.0% NaCl, remained unchanged (Fig. 27).

ERYTHROCYTES AND LEUCOCYTE COUNTS AFTER 26 WEEKS ON DIET

NaCl content of diet	No. of dogs	RBC per cmm. blood x 10^6	WBC per cmm. blood
0.2%	6	6.00	13,200
1.4%	6	5.57	13,600
6.0%	6	5.50	13,400
9.0%	5	5.18	12,600

Fig. 27.

The earliest signs of NaCl toxicity observed in those litter-mate male beagles were slight inhibition of growth and reduction of hemoglobin. Base line values for blood pressure, serum cholesterol, hemoglobin level and blood cell distribution have been firmly established. Serial electrocardiograms were within normal limits. The studies have been continued in order to detect other early changes, particularly in blood pressure and serum cholesterol, which may result from the experimental regimen.

As the animals on high levels of salt begin to show further evidence of deterioration, additional biochemical and physiologic measurements will be made. If hypertension is manifested, these studies will include the measurement of sodium space and electrolytic metabolism, renal function studies, and investigation of lipid metabolism. As the experiment progresses, those lines of investigation which show promise will be pursued and many procedures can be followed which are technically too difficult on a small animal such as the rat.

DISCUSSION

These findings must be interpreted with caution. It is clear that in rats the long continued intakes of large amounts of salt produce morphologic and functional changes even when unlimited water is permitted. These changes increase in severity as the amount of salt ingested daily increases. At certain stages in the course of a high salt diet some animals develop a disease picture closely resembling that of nephrosis in humans. These constitute a smaller proportion of the total number and the incidence is highly individual. The bulk of the animals live and grow (though not normally) until they develop in increasing numbers a condition closely resembling chronic glomerular nephritis, with lesions in arterioles which sometimes resemble those seen in malignant hypertension in man. Despite that, some animals live to an old age, for one animal an age corresponding to almost 90 years for a man.

Two aspects deserve special attention: first, the changes have been produced in young growing animals and, second, the lesions do not appear to any significant degree until approximately one-third of the life span of the animals has been passed. The latter is significant in relation to the result so far observed with the dogs.

The meaning of these findings with respect to the effects of salt intake in humans depends to a great extent on the human requirements of salt. As I have said, these are unknown. It is clear that customary intakes of salt, expressed as aver-

ages, do not by any means necessarily represent requirements or even what may be called normal intakes. There is enough evidence to indicate that such intakes are merely customary intakes, the result of education or artificially developed tastes. Variation in these customary intakes in various populations supports this conclusion. On the other hand there is no doubt of the necessity for salt. This is demonstrated by animal experiments, including the results with rats reported here, by historical evidence and by general zoologic data. Aside from such evidence as the occurrence of salt deficiency disease in hot environments, evidence such as that of Allen and Sherrill,[2] who encountered symptoms of salt depletion in patients with hypertension treated by a low salt diet, gives a rough quantitative measure of minimal needs. It is important to point out, however, that these amounts varied rather widely, individually were affected by the existing disease, and that variations apparently occur in healthy persons. Nevertheless, with symptoms from too little and symptoms from too much we are again on the horns of a dilemma[26] with respect to a prophylactic or therapeutic procedure.

Just what levels of intake of salt are capable of toxic effects, or the factors which may affect one way or another such effects, are generally unknown. However, some rough idea may be gained. It is worth noting that the salt content of experimental diet III used with the rats (2.8% of NaCl) compares roughly on a weight for nutrient basis with the human intake of about 12-15

COMPARISON WITH HUMAN DIET

Protein	100 Gm.	400 Cal.
Fat	100 Gm.	900 Cal.
Carbohydrate	300 Gm.	1200 Cal.
TOTAL	500 Gm.	2500 Cal.

500 Gm. x 0.028=14 Gm. NaCl/day

Fig. 28.

gm. of salt a day, a common estimate of the salt in the diet in this country (Fig. 28). Also, it may be significant that salt intakes very much lower than the customary intakes are effective in a significant number of persons with hypertension, which suggests that the customary level, under some as yet unknown conditions, may possess such a potential.

26. Youmans, J. B.: Editorial: The Horns of a Dilemma, Am. J. Med. 11, 133, 1951.

More General Practitioners Can Now Treat Alcoholism—It is now possible for more and more general practitioners to provide the first necessity in treatment of the chronic alcoholic—which is simply to make him feel better through medical care.

Dr. Marvin L. Block, Buffalo, N. Y., chairman of the American Medical Association's committee on alcoholism, said that patience and understanding, psychotherapy, and sometimes the help of specialists is needed for long-range treatment of the alcoholic.

However, the first steps are now within the reach of more doctors than ever before through improved medical techniques and new drugs, he said in the December 29 Journal of the A. M. A.

The necessary psychotherapy can be administered by the average physician if he will take the time to understand and help the sick alcoholic. However, the medical treatment of alcoholism is rapidly becoming more important in accomplishing recovery, he said. Because of the fear and threat that so many alcoholics feel about any treatment, a medical approach seems much more acceptable. As the patient's physical condition improves, he feels better and is more willing to cooperate and accept psychiatric treatment.

"In the chronic alcoholic we are dealing not only with a sick individual but with that individual in relationship to his environment," Dr. Block said. Therefore, his social situation, religious background, employment or industrial situation, and his relationships with family, friends and fellow employees are pertinent factors.

While it is not always necessary to treat the patient in a hospital, "there is no doubt that this is the best setting for such treatment," he said. Necessary equipment is readily available. In addition, it is frequently advantageous to get the patient away from his family, since "all too frequently the family is in an unfavorable emotional state during the patient's intoxication."

General hospitals should be willing to accept alcoholic patients, he said. Records have shown that alcoholic patients offer no more difficulty for hospitals than any other sick persons. In fact, the hospital's attitude that the acutely ill alcoholic patient is sick and is welcome for treatment makes these patients more cooperative than many others, he said.

Dr. Block then discussed at length the specific medical treatment of alcoholism. He said his discussion was arranged to fit within the framework of the practicing physician's experience and knowledge. His report is the first of a series on the treatment and the physiological, psychiatric and sociological aspects of alcoholism, according to Dr. Richard J. Plunkett, secretary of the A. M. A.'s council on mental health.

Dr. Plunkett said the council and the members of its committee on alcoholism believe that much of the recently developed information on the most effective methods of treating alcoholism in private practice and in the hospital has not had wide circulation. As a result many patients are not receiving the necessary attention and treatment that could and should be given them.

Salt-induced hypertension in the dog

4099

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VOGEL, JAMES A. *Salt-induced hypertension in the dog.* Am. J. Physiol. 210(1): 186-190. 1966.—Arterial hypertension was successfully induced in two groups of dogs by increasing the dietary intake of NaCl to 2 g/kg body wt per day and providing 0.9% saline instead of tap water to drink. In the first group the arterial pressure increased approximately 55% over a period of 26 days. Restriction of dietary potassium intake had no effect on this response. In the second group, catheters were chronically implanted in the right atrium and aorta, prior to salt feeding. Control animals were similarly catheterized. In these conscious, unrestrained animals, salt hypertension was found to be associated with a marked increase in blood volume and cardiac output. The latter was attributable to an increase in stroke volume rather than heart rate. Calculated peripheral resistance was not altered by salt feeding nor was the concentration of plasma sodium. Total exchangeable sodium, however, was approximately doubled.

salt hypertension; essential hypertension; blood pressure; cardiac output; stroke volume; blood volume

HYPERTENSION HAS BEEN INDUCED in several animal species (4, 9, 13), as well as in man (10), with a high dietary intake of sodium chloride. Rats, for instance, may be rendered hypertensive by substituting either 2% saline for their drinking water or by adding as little as 1% sodium chloride to their diet (4, 13). Similar procedures have also been effectively used in chickens (9). In dogs, however, salt hypertension is difficult to induce (14, 16), apparently due to the marked capacity of the canine kidney to excrete exogenous salt (8). On the basis of studies in the rat, Koletsky (7) suggested that salt hypertension and human essential hypertension are similar with respect to their evolution and associated pathological lesions. Whether or not this is true remains debatable since the hemodynamic change associated with the development of salt hypertension has not been studied; hence, little is known about the basic mechanisms which are responsible for the rise in blood pressure. To describe these mechanisms, even grossly, a detailed analysis of central cardiovascular function is required. For obvious reasons, such an analysis is difficult to achieve in the laboratory rat in which the hypertension is so readily induced. Therefore, in the studies presented here a new attempt was made to produce body salt retention and, consequently, hypertension in the dog. On achieving this, the hemodynamics of the phenomenon were studied in detail.

Received for publication 28 June 1965.

MATERIALS AND METHODS

To determine the feasibility of inducing salt hypertension in dogs, four adult mongrels were placed on a normal canine diet of Ken-L-Biskit (Quaker Oats Co.) for 10 days, during which time their base line, i.e., control, blood pressures were established. Two of the animals were then fed a synthetic, low-potassium diet composed of corn starch, corn oil, necessary vitamins, K-free salt mixture, and sufficient NaCl to provide an average intake of 2 g NaCl/kg body wt per day. In addition, 0.9% saline was substituted for tap water as a drinking source. The other two dogs were continued on the Ken-L-Biskit, but at this point, sufficient NaCl was mixed with the diet to provide a daily intake of 2 g/kg body wt. Again, 0.9% saline was substituted for tap water as a drinking source. In all four of these dogs the period of high-salt consumption was maintained for 26 days. The animals were then returned to their control diet for 30 days after which time the high-salt diets were reinstituted for a second period of 26 days. Systolic blood pressure was determined every 3 days by sphygmomanometry, using an Infraton (Spinco Division, Beckman Instruments, Inc.) condensor-microphone pulse pick-up placed at the base of the tail, after the method of Pratt and Winburg (12). This technique was found to be reliable in trained animals when precautions were taken to select the proper cuff size for each dog, to carefully reset the cuff between each reading, and to calculate average pressure on the basis of three to five readings. In addition to blood pressure, daily records of body weight, saline intake, urine volume, and food consumption were maintained. All the dogs were sacrificed and autopsied following the final period of salt feeding.

Following this feasibility experiment, a more detailed analysis of cardiovascular function was made on a group of seven NaCl-treated and four control dogs. In this experiment, as opposed to the first, the period of NaCl feeding was initiated at a level of 1 g/day per kg body wt. The dietary level was then progressively increased

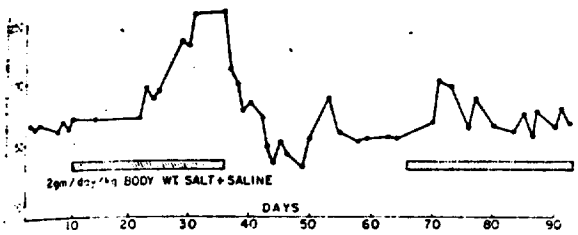

FIG. 1. Effect of dietary sodium chloride on the systolic blood pressure of four dogs. Pressures were determined every 2-3 days by sphygmomanometry. Salt and saline were administered during as covered by hatched bar.

For the next 12 days until the daily intake was 2 g/kg body wt. The animals were maintained at this latter level for a total of 14 days at which time the animals were sacrificed and autopsied. During the entire period of salt feeding, 0.9% NaCl again served as a drinking source. Control animals were handled identically except for the absence of salt in their diet and the retention of tap water rather than saline for drinking. Prior to an initial control period, all 11 dogs were anesthetized (40 mg sodium pentobarbital/kg) and chronically catheterized with polyvinyl chloride tubing (0.042 inch i.d. × 0.074 inch o.d., S-1 formulation, Surprenant Mfg. Co., Clinton, Mass.) via the jugular and common carotid vessels. The exact placement of the catheter tips in the aortic arch and right atrium was ascertained by fluoroscope and X-ray examination during and following the surgical procedure. The external connections of the catheters consisted of Tygon tubing shaped into loops and metal female syringe connectors anchored with two Teflon plates, one above, one below the skin. This connection was located on the neck just above the original incision over the vessels. Patency was maintained during the period of the study by twice-weekly flushing with heparinized (20 mg/liter) saline.

This preparation permitted measurements of cardiovascular function in the conscious, unrestrained animal. The development of salt hypertension was monitored by twice-weekly measurements of arterial blood pressure. This was accomplished with a Statham P 23 Da transducer. All other measurements were made biweekly, as follows: The cardiac output was estimated by dye dilution, using indocyanine dye injected into the right atrium with subsequent blood withdrawals (20 ml/min) being made from the aortic arch for the measurement of the diluted dye concentration in a Gilford densitometer. The densitometer output, as well as that of the pressure transducer, was monitored on a multichannel Electronics for Medicine oscillographic recorder. Total peripheral resistance (TPR) was calculated as: (mean arterial BP × 1,332)/(cardiac output, ml/sec) (expressed as dynes-sec/cm^5). Blood volume was estimated by the chromium 51 label method of Karlson and Sears (6) with hematocrits being determined by high-speed centrifugation in capillary tubes. Total-body exchangeable sodium (TEBS) was estimated with sodium 22. Accordingly, a known amount (about 5 μc) of

TABLE 1. *Salt and water/saline intake and urine output of salt-induced hypertensive dogs*

	Body Wt, kg	Water Saline Intake, ml/day	Total Added NaCl Intake, g/day	Urine Output, ml/day
Control (10 days)				
	12.4	1,079	00	478
Salt feeding				
1st Week	12.1	1,666	20*	1,034
2nd Week	11.8	1,896	36	1,005
3rd Week	11.9	2,281	40	1,347
4th Week	11.9	2,507	42	1,402

Values are means of four dogs. *2 g/day per kg minus that in feed not consumed plus 0.9% saline ad lib.

$Na^{22}Cl$, mixed with 0.9% saline, was injected into the animal. Twenty-four hours later, a 0.2 ml-blood sample was withdrawn and counted in a TriCarb (Packard) scintillation counter along with a blank and a standard. The amount of Na^{22} excreted in the urine during this 24-hr period was also determined. The plasma and urine levels of nonradioactive sodium were also determined in a Baird Atomic flame photometer. The total-body exchangeable sodium was then calculated by the following formula:

$$\text{TEBS (mEq)} = \frac{(\text{total } NA^{22} \text{ counts/min injected}) - (\text{total } NA^{22} \text{ counts/min in 24-hr urine})}{\dfrac{NA^{22} \text{ counts/min per ml plasma})(1{,}000)}{\text{mEq Na per liter plasma}}}$$

RESULTS

The initial experiment demonstrated the feasibility of inducing hypertension in dogs with a massive dietary sodium chloride load. Since level of dietary potassium did not affect this response, the measurements made on two animals receiving a low-K diet were combined with those of animals receiving a normal-K diet. As shown in Fig. 1, the average systolic pressure began to rise after 12 days of salt administration, and during the subsequent 14 days it reached a maximum value of 255 mm Hg or 55% above control level. On cessation of the treatment, pressure fell quickly to a level (130 mm Hg) significantly below base line. From there it gradually returned to control level over the next 2 weeks. When NaCl was again administered after a lapse of 30 days, no sustained increase in arterial pressure was elicited; rather, the arterial pressure was quite erratic during this period.

Two of the dogs showed no sustained weight changes during the course of the study while the other two exhibited a small decline (Table 1). Fluid intake and urine output increased two- to threefold when the animals were subjected to the high-salt intake. The average total intake of NaCl by the 4th week reached 42 g/day (Table 1). On a body-weight basis this represented an intake of 3.53 g/day per kg. The animals did not appear edematous and all remained in a healthy, active state. Autop-

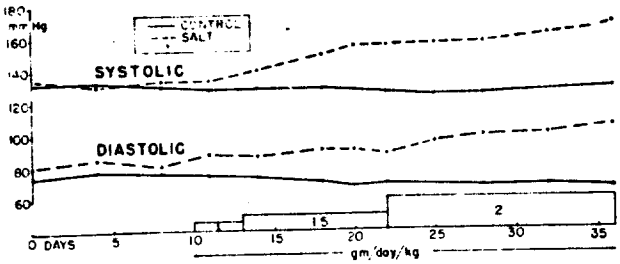

FIG. 2. Arterial blood pressure of seven dogs fed sodium chloride in increasing amounts and four control dogs.

sies at the termination of this experiment revealed no significant cardiovascular or other lesions in any of the dogs.

In the second experiment, where seven dogs were subjected to gradually increased dietary salt loads, the rise in systolic pressure (Fig. 2) was not nearly so marked as that observed in the previous experiment. Accordingly, a significant ($P < .05$) rise in systolic pressure was first observed after 10-12 days of salt feeding, but this rise, even after 14 more days, only reached a maximum average value of 167 mm Hg. The latter figure represents a relative increase in systolic pressure of about 24%. Over the entire period of salt feeding the diastolic pressure also showed a significant increase, but to a lesser extent than the systolic pressure. Consequently, the average pulse pressure during the hypertensive period was elevated by 10 mm Hg. The four control dogs who were fed a normal diet maintained relatively constant systolic and diastolic pressures throughout the entire experiment.

During the period of salt-induced hypertension, a significant ($P < .05$) elevation of the cardiac output (cardiac index) was observed (Table 2). At the end of 24 days of salt feeding the magnitude of this elevation w about 19%. It was accomplished entirely by an increa in stroke volume. Thus, the mean stroke volume creased from a normal-diet value of 27 ml to a value 43 ml after 9 days and 46 ml after 24 days of s feed At the same time this was occurring, the he rate tually showed a significant ($P < .05$) decrea from average control rate of 111 beats/min to rate 92 beats/min after 9-24 days of salt feeding, it is to be noted, furthermore, that the increa in str volume (74%) during salt hypertension i relati greater than the increase in cardiac output

Since both blood pressure and flow rose ately the same, total peripheral resistance did not cha during salt feeding. This was true whether the salt dogs were compared to themselves prior to salt feed or to their counterparts who were maintained on a no mal diet throughout the experiment.

During the period of salt-induced hypertension, blo volume rose from an initial value of 73 ml kg to ml/kg after 9 days and 89 ml/kg after 24 days (a 21 increase) of salt feeding. Both of the values were nificantly higher ($P < .05$) than the initial control va This change was primarily the result of a plasma vol increase, though cell volume was slightly higher (P .05) at the end of the treatment period. There were significant changes in either blood, plasma, or cell ume in the control animals at 9 or 24 days.

During the course of this experiment, the salt sumption of the seven hypertensive dogs reached average value of 3.9 g/day per kg while the uri output of NaCl rose from 2 mEq to 41.3 mEq/day kg. In both instances the increase was approxim 20-fold. This period of high-salt intake was associ with an increase in the body sodium pool (TEBS) 50.9 to 108.3 mEq/kg. The concentration of pla

TABLE 2. *Central cardiovascular and blood volume responses to salt-induced hypertension*

Treatment	Normal Diet		High-Salt Diet			
			9 Days		24 Days	
	Control (4)	Salt (7)	Control (4)	Salt (7)	Control (4)	Salt (7)
NaCl intake, g/day per kg	.2	.2	.2	3.1	.2	3.9
Body wt, kg	17.2±1.6	18.9±2.0	17.3±1.5	18.0±1.9	18.2±1.5	18.3±1.8
Mean BP, mm Hg	115±5	100±6	109±5	121±5*	111±4	130±4*
Heart rate, beats/min	109±2	111±2	103±2	85±3*	106±3	92±3*
Cardiac index, liters/min per m²	4.05±.30	4.14±.20	3.75±.25	4.44±23*	4.45±.28	4.96±.3
Stroke vol, ml	26±1	27±1	28±1	43±4*	32±2	46±7*
Total periph. res., dynes-sec/cm⁵	3,193±125	2,697±119	3,067±94	2,934±148	2,625±99	2,739±17
Blood vol, ml/kg	64±2	73±3	63±2	80±5*	66±3	89±11*
Cell vol, ml/kg	25±1	26±1	24±1	27±2	26±1	30±4*
Plasma vol, ml/kg	39±3	47±2	39±3	52±3*	40±2	59±7*
Hct, %	43.9±1.2	41.1±1.6	44.4±1.6	38.3±20	45.5±1.6	38.0±2.5
Urinary Na, mEq/day per kg	2.8±.7	2.0±.4	1.9±.4	33.1±.9*	2.1±.5	41.3±1.1
TEBS,† mEq/kg	46.8±1.3	50.9±2.4	53.9±1.8	78.8±7.5*	59.0±1.7	108.3±12.
Plasma Na, mEq/liter	157.3±3.6	154.0±3.9	159.0±3.4	154.8±2.8	158.6±3.8	160.7±4.

Values are mean ± SE. No. of dogs given in parentheses. * Difference between control and salt group significant at P level, or better. † Total exchangeable body sodium.

ium, on the other hand, was not altered by the high-t diet, although the absolute amount of sodium in e plasma obviously did increase in proportion to the rease in plasma volume noted above.

Electrocardiograms taken periodically throughout the dy gave no indications of sinus arrhythmias or axis viations. No significant cardiovascular or renal lesions re revealed at autopsy.

SCUSSION

Waldman and Wilhelmj (14) were unable to produce sustained hypertension in dogs by feeding 2.5 g salt/kg r day and then subsequently adding saline to this. rther work by Wilhelmj's group (15, 16) indicated a finite adaptation to such high-salt load; nevertheless, ey were able to raise blood pressure consistently by perimposing an additional stress to the salt-fed dog. ahl (3), on the other hand, was never able to elicit pertension in dogs even though they were fed 1-6% aCl for as long as 4 years. The difficulty encountered these investigators appears to be explained by Ladd nd Raisz's (8) experiments which document the dog's arked ability, many times that of man, to excrete xogenous salt.

The successful induction of hypertension in the studies ported here would appear to be attributable to the ct that the dogs were started immediately on a large ad of salt, both in the diet and drinking water. The se of saline along with a dietary salt may be a key fac-or since it limits the water intake, thus restricting the ability to excrete the imposed load. In this respect Sapir-tein (13) has suggested that the ratio of NaCl to water atake is more important than the actual amount of salt onsumed. In contrast to the present experiments, thers (3, 14–16) have permitted their dogs to adapt radually to the salt load or did not include saline ini-ally in the treatment. It would appear then that the ad given in the studies presented here was above that hich could be excreted completely and, therefore, aCl retention and hypertension resulted. Further sup-port for this interpretation is found when the data from he first and second experiments are compared. Thus, he hypertension observed in the second experiment, here the salt load was given gradually, was not nearly o marked as in the first experiment where the entire oad was given initially. The results from the first ex-periment also indicate that an initial salt load caused some sort of an adaptation, probably similar to that reported by Wilhelmj et al. (15, 16), which rendered the dogs better able to cope with subsequent salt loads.

The manner whereby high levels of dietary salt produce hypertension is not understood. It is also not known whether this type of elevated pressure is similar to essential hypertension as found in man. The data presented here strongly suggest that hypertension induced in dogs by salt loading is primarily a volume phenomenon rather than an effect on arterial smooth muscle tone. As NaCl is retained in the body, as evidenced by a greater total exchangeable body sodium, extracellular water increases, including plasma volume, thus maintaining a normal blood sodium concentration. The expanded blood volume apparently results in a larger venous return and stroke volume and thus a higher cardiac output. The induced stroke volume increase appears to elicit a compensating decline in heart rate as the hypervolemia develops. Since the arterial pressure increased in proportion to this augmented blood flow, there is no indication that peripheral resistance was significantly altered.

The possible importance of an expanded blood volume in essential hypertension has been pointed out by Page (11) and Borst (1) and more strongly supported by the renal hypertension studies of Douglas et al. (5). The latter investigators suggested a possible relationship between blood volume and arterial pressure in dogs rendered hypertensive by 70% removal of renal tissue and an increased dietary salt intake.

It can be concluded from this work that the hemodynamic mechanisms underlying short-term salt and essential hypertension are probably not similar. Thus, the hypertension induced by feeding only a high level of NaCl, at least in the dog, is characterized by an expanded volume of blood and an increase in blood flow without any change in peripheral resistance. Essential hypertension in man, by way of contrast, is characterized by an increased peripheral resistance with a normal cardiac output. Therefore, it may be misleading to extrapolate findings of salt-induced hypertension in animals to essential hypertension in man since the central hemodynamic characteristics of the two conditions are quite dissimilar.

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