MONOGRAPH ON CELLULOSE AND DERIVATIVES VOLUME I

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INFORMATICS INC.
6000 Executive Boulevard
Rockville, Maryland 20852

CELLULOSE AND DERIVATIVES

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Summary

Cellulose is the most abundant carbohydrate in nature, comprising more than 50% of the total organic carbon. It is a regular component of the normal human diet functioning to provide bulk and to promote intestinal peristalis. The derivatives of cellulose used as food additives - methyl cellulose, the propylene glycol ether of methyl cellulose, ethyl cellulose, carboxymethyl cellulose, and sodium carboxymethyl cellulose (see *footnote) - are synthetic products manufactured from cellulose and are not naturally occuring compounds.

In the tests reported to date, cellulose and its derivatives have been found to pass through the digestive tracts of rats and man unchanged. By tracing the distribution of orally administered ^{14}C-labelled SCMC in rats, Frawley et al. (137) resolved that SCMC remains totally unabsorbed while passing through the gastrointestinal tract of the rat. Baker (21) reported that fecal recoveries, from 4 rats fed 14C-labelled microcrystalline cellulose at 10 and 20% of their diets, ranged from 96 to 104%, no evidence of degredation or digestion was observed. Shelanski and Clark (399) recovered from the feces of 5 rats, 90% of the 5 g of SCMC which had been fed to them. Ziegelmayer et al. (483) reported that SCMC is almost quantitatively recovered from the feces of rats and man whereas rabbits apparently digest approximately 50% of the ingested amount. It was suggested that this apparent digestion was the result of bacterial action present only in herbivorous animals (483). After feeding a total of 110 g of SCMC to each of two female dogs over a period of 6 days, Massatsch and Steudel (294) found that all of the substance was excreted unchanged in the feces. In a series of experiments by Machle et al. (285) in which human subjects were given single oral doses of 5-10 g of MC, analysis of the feces indicated that essentially all of the MC had passed through the digestive tract unchanged. They found that the formation and absorption of methanol, and the consequent formation of formic acid, following the ingestion of MC is negligible (285). After feeding PGMC to 25 human adults in 3 graduated doses of 0.6-8.9 g one week apart, Knight et al (244) found that essentially all (97%) of the ingested dose was eliminated in the feces within 96 hours. Baker (21) reported quantitative recovery of radioactivity following administration to one human subject of 150 g of ^{14}C-labelled microcrystalline cellulose in 2 portions in one day and 150 g unlabelled microcrystalline cellulose daily for the subsequent 10 days.

Upon finding that SCMC can support the rapid growth of hematopoietic cells in an agitated culture even though it is not metabolized by the cells, Mizrahi and Moore (317) suggested that SCMC probably protects the cells against physical stress in suspended cultures.

*Throughout this monograph the following abbreviations will be used: MC-methyl cellulose; PGMC-propylene glycol ether of methyl cellulose; EC-ethyl cellulose; CMC-carboxymethyl cellulose; SCMC-sodium carboxymethyl cellulose.

Ziegelmayer et al. (483) found that ingestion of 20-30 g of SCMC daily by 3 human subjects resulted in a depression in the digestion of protein, while a slight increase occurred in the digestion of fats. This effect was also noted in rats and rabbits (483).

According to Schneider et al. (391), the addition of CMC to penicillin preparations may result in accumulation of penicilloyl-CMC conjugates, which could act as elicitors of penicilloyl-specific anaphylaxis.

That nitrofurantoin is excreted more slowly when suspended in a MC solution than when suspended in water was reported by Seager (394).

On the basis of clinical and electrocardiographic studies, Feinblatt et al. (124) reported that the simultaneous administration of CMC minimizes the acute toxicity of digitoxin.

Six articles dealing with the carcinogenicity of cellulose derivatives have been published. In the first of these, Lusky and Nelson (279) reported that weekly subcutaneous injections of 1 ml of 2% CMC solution to 30 rats for 73 weeks resulted in the development of tumors (fibrosarcomas) at the site of injection in 13 of the rats. Hueper (203) injected 25 rats subcutaneously with 500 mg of powdered MC and another 25 rats with the same level of EC. After 2 years, histological examination of the animals revealed that the increase of tumor development was within the control range for the MC-injected rats but that 3 (12%) of the EC-injected rats had developed tumors; 9% of the 200 untreated controls had developed tumors (203). Lazar and Lazar (262) administered MC to rats intraperitoneally in conjunction with subcutaneous transplantation of Murphy-Sturm lymphosarcoma. The percentage of regressions of the tumors was found to be greatly increased in the MC-treated rats (262). Histopathological examination of MC-treated rats sacrificed after complete regression of the tumors showed a striking hepatosplenomegaly as well as abdominal lymphadenopathy (262). The MC-treated rats also exhibited numerous vacuolated cells in glomeruli, proteinaceous material in tubules of the kidney, and foamy macrophages in the liver, spleen, thymus, and abdominal lymph nodes (262). In experiments similar to those of Lazar and Lazar (262), Hruban et al. (197) also found that MC treatment of rats bearing Murphy-Sturm lymphosarcoma produces a significant increase in the percentage of complete regressions. Lazar and Lazar (264) reported that Murphy-Sturm lymphosarcoma could be successfully reintroduced in rats in which MC-induced regression of the tumors had previously occurred. However, a third innoculation failed to produce any detectable growth of the tumors (264). The intraperitoneal administration of 2 ml of 2.5% aqueous suspension of MC, 3 times weekly, to rats receiving subcutaneous implantation of Walker tumor 256 was found by Lazar and Lazar (265) to have no beneficial effect on the size of the tumor or the longevity of the rats.

The feeding of cellulose to rats at a dietary level of 30% for 3 generations revealed an adverse effect on reproduction, manifested in relatively low fertility, live birth, and lactation indices and unsatisfactory physical condition of the offspring (354). These effects were attributed to nutritional deficiencies of the basal diet rather than to any toxic effects of the cellulose (354). However, Shelanski and Clark (399) found that when rats were given a dietary level of SCMC as high as 1.0 g/kg for 3 generations no untoward effects were

evident. In fact, the treated rats exhibited a slight increase in weight gain over the controls (399). Bauer (29) reported that three generations of rats maintained on a basal diet supplemented with 5% MC showed a definitely greater average increase in body weight than did the controls. No effects on reproductive functions were observed (29). In a similar study by Bauer and Lehman (31), in which 3 generations of rats were fed 1.7 or 5% MC, reproduction was again found to be unaffected.

Attempts to produce local irritation by application of cellulose derivatives have so far been unsuccessful. Khadzhy and Shaposhnikova (238) reported that application of a MC solution to the eyes of rabbits failed to produce irritation of the conjunctiva. Rowe (378) found that neither CMC nor SCMC induce irritation when applied to the shaven bellies of rabbits 5 times per week for 4 weeks. And through both patch tests and application to the vaginal area of human subjects, Shelanski and Clark (399) found no evidence of irritation or other untoward effects produced by SCMC.

Franciosi (136) reported that cases of immediate hypersensitivity to CMC have been observed in cattle which have been immunized against foot-and-mouth disease with vaccine containing CMC.

Obolentseva (338) found that SCMC given orally to mice (0.12-0.5 g/kg), rats (0.8-1.1 g/kg), and dogs (0.06-0.13 g/kg) exerted a marked laxative effect, dependent upon dosage. The mechanism of SCMC's laxative effect was found to be related to its colloidal swelling, which results in a mechanical stimulation of motor activity in the intestines (338).

A table of acute toxicity is presented on page 22.

Due to the large number of feeding studies reported in this monograph, a tabulated summary of those experiments disclosing no adverse effects will be presented at the end of this section. Those experiments in which adverse effects were manifested will be discussed more fully.

In a feeding study using 20 day-old Arbor Acres chicks, Kratzer et al. (252) found that a dietary level of 2% CMC, given for a period of 3 weeks, produced a significant growth depression, reduction in feed efficiency, and a significant reduction in fat absorption.

Avicel (microcrystalline cellulose) in dry commercial form and in gel form was fed to groups of 50 male and 50 female albino rats at a dietary level of 30% for 72 weeks (Groups No. 2 and No. 3), while a control group was maintained on 30% 'Alphacel (alpha cellulose, a fibrous cellulose) (Group No. 1) (353). In Group No. 3, body weight gains for the males at 52 weeks and for the females throughout the first year were significantly higher than the controls (353). The terminal body weights and heart weights for the females and the liver weights for the males in Group No. 2 were significantly lower at 26 weeks than those for the controls (353). At 72 weeks, significant increases were noted in the terminal body weights and heart, liver, and kidney weights for the males and females of Group No. 3 as well as increases in the liver and kidney ratios for the males of this group (353). Reductions in the thyroid, liver, and kidney ratios for

the females of Group No. 3, compared to the controls, were found at 72 weeks (353). Gross changes were found in the kidneys of the females, particularly in groups No. 1 and No. 2 while the kidneys of the males appeared to be normal (353). Severe dystrophic calcification of the terminal proximal tubule was disclosed by microscopic examination of kidney sections from the female rats (353). Involutional changes in the ovaries were observed in the majority of females from Group No. 2 sacrificed after 52 weeks and in several females from Groups No. 1 and No. 3 (353).

Following single intraperitoneal doses of Cellan 300 to groups of five male albino rats at dosage levels of 10.0, 31.6, 100, 316, 1000, and 3160 mg/kg, gross autopsies performed on the animals at the 1000 and 3160 mg/kg levels revealed adhesions between the liver and the diaphragm and peritoneal wall, and conjestion of the kidneys (349).

A 14% reduction of growth rate, compared to control values, was reported by Tainter (428) for female rats given MC at a dietary level of 10% for a period of 95 days.

Methocel 70HG was fed to groups of 10 male and 10 female white albino rats, 45 days of age, for a period of 90 days at dietary levels of 0, 0.3, 1.0, 3.0, 10.0, and 20.0% (92). At the 10% level the male rats showed slight but significant growth retardation and at the 20% level moderate growth retardation was observed in both sexes (92). Hodge et al. (193) observed growth retardation in rats given Methocel 65HG at dietary levels of 20 and 25% for 1 year. Groups of 20 rats were fed Methocel 65HG for 30 days at dietary levels of 0, 2, 10, and 25% (193). Hodge et al. (193) reported that the rats fed the highest dose exhibited loss in weight, early deaths and severe diarrhae. When Methocel 90HG was fed to rats for 3 months at dietary levels of 0, 0.3, 1, 3, 10, and 20%, a marked growth retardation was noted in the male rats receiving 20% PGMC and a slight growth retardation in the male rats at the 10% level (494). Of rats fed diets containing 0, 1, 3, 10 and 30% Methocel 2602 for 121 days, those given the 30% diet became rough and unkempt and exhibited a marked growth retardation, 10% of the rats failed to survive the full 121 days, while at the 10% level there was a slight retardation of growth for the male rats (297). In an experiment by Hodge et al. (193) in which rats were fed 0, 1, 5, and 20% Methocel 65HG in their diet for 2 years, the male rats given the 20% diet showed a retardation in growth rate.

Kiriyama (241) found that a 5% dietary supplement of CMC counteracted the rise in plasma cholesterol and the increase in accumulation of liver cholesterol effected in rats by a hypercholesterolemic diet.

Rowe et al. (378) noted a very slight growth-curve depression of questionable significance when 10 female rats were given a 20% supplement of SCMC with their diet for 63 days. Retardation of growth occurred at the higher SCMC level when McElligott and Hurst (299) fed SCMC to rats at levels of 0, 1, and 10% for 104 weeks.

Hodge et al. (193) fed PGMC to two dogs for 30 days at levels of 25 and 50 g/day. The dog fed the higher level had diarrhea, lost weight and was anemic (193).

The following table summarizes those feeding studies in which no adverse effects were manifested. In each case the maximum administered dose is listed along with the number of animals receiving that dosage.

Animal	Substance	No. & Sex	Maximum dosage	Duration	Ref.
Mice	CMC	10	5% of diet	Weaning till death	335
Mice	SCMC	50 M + 50 F	10% of diet	100 weeks	299
Chicks	MC	--	2% of diet	20 days	453
Chicks	MC	20	2% of diet	3 weeks	252
Chicks	SCMC	--	2% of diet	20 days	453
Rats	SCMC	3	14% of diet	5 weeks	483
Rats	Cellulose	5 M	3160 mg/kg	single dose	349
Rats	PGMC	11	4000 mg/kg	single dose	193
Rats	CMC	--	5 g/kg	single dose	378
Rats	SCMC	--	3 g/kg	3 divided doses over a 6-hr period	378
Rats	MC	--	0.5 g/kg	4 weeks	238
Rats	MC	3 M + 6 F	50% of diet	90 days	31
Rats	MC	5	5% of diet	184 days	30
Rats	MC	80	0.8% of diet plus 1.0% of drinking water	8 months	103
Rats	MC	40	200 mg/kg/day	28 days	117
Rats	EC	80	1.2% of diet	8 months	103
Rats	CMC	10	5% of diet	1 year	335
Rats	CMC	10 F	20% of diet	63 days	378
Rats	CMC	10 M + 15 F	5% of diet	8 months	378
Rats	SCMC	10 F	20% of diet	63 days	378
Rats	SCMC	100	0.5 g/kg/day	6 months	399
Rats	SCMC	10	0.5 g/day	2 months	461
Rats	SCMC	10 M + 10 F	5% of diet	8 months	378
Rats	MC	20 M + 20 F	5% of diet	2 years	92
Guinea Pigs	CMC	--	5 g/kg	single dose	378
Guinea Pigs	SCMC	--	3 g/kg	3 divided doses over a 6-hr period	378
Guinea Pigs	SCMC	100	1 g/kg/day	6 months	399
Guinea Pigs	SCMC	20	1 g/kg/day	1 year	399
Rabbits	CMC	--	5 g/kg	single dose	378
Rabbits	SCMC	--	3 g/kg	3 divided doses over a 6-hr period	378
Rabbits	PGMC	6	25% of diet	30 days	193
Dogs	MC	4	3 g/kg/day	1 month	238
Dogs	PGMC	2	3 g/kg/day	1 year	193
Dogs	SCMC	10	1 g/kg/day	6 months	399
Dogs	SCMC	5	3 mg/kg/day	4 months	203
Man	Cellulose	8 M + 8 F	30 g/day	6 weeks	441
Man	Cellulose	8 M	30 g/day	2 weeks	11
Man*	MC	--	10 g/day	--	428
Man*	MC	37	6 g/day	240 days	393
Man*	MC	30	200 mg/kg/day	--	25
Man*	MC	25	0.6-8.9 g	single dose	244
Man*	SCMC	250	133 mg/kg/day	--	392
Man*	SCMC	22	166.6 mg/kg/day	6 months	69
Man*	SCMC	34 M + 94 F	133 mg/kg/day	--	130

*In these experiments the compound under consideration showed laxative and/or constipating effects.

Chemical Information

Carboxy Methyl Cellulose and Sodium Carboxy Methyl Cellulose

I. Nomenclature

A. Common Names

1. Sodium cellulose glycolate
2. Cellulose gum
3. Carboxy methyl-cellulose ethers

B. Chemical Names

1. Cellulose, carboxymethyl ether
2. Carboxymethyl cellulose
3. Cellulose carboxymethylate
4. Cellulose ether of glycolic acid

(Carboxymethyl cellulose is used as the sodium salt in most commercial applications).

C. Trade Names

1. Na-CMC
2. CMC-CT (CMC)
3. Cel-O-Brandt
4. Cethylose
5. Glykocellon
6. Carbose D
7. Thylose
8. Xylo-Mucine
9. Tylose MGA
10. Cellolax
11. Polycell
12. CM-Cellulose

D. Chemical Abstracts Registry No.

CMC PM9000117
SCMC 977000002

II. Empirical Formula

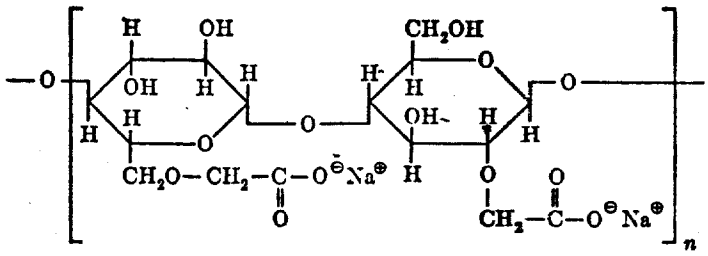
$$(C_6H_7O_2(OH)_x(OCH_2COONa)_y)_n$$

where $x = 2.00$ to 2.40
$y = 0.60$ to 1.00
$x+y = 3.00$

III. Structural Formula

Sodium carboxymethyl cellulose is a synthetic ether of cellulose, a polysaccharide made up of beta-anhydroglucopyranose units.

On each anhydro glucopyranose unit there are three possible sites of substitution for a carboxymethyl group, suggesting a maximum degree of substitution (D.S.) of 3.0.



However, materials with a lower D.S. (0.95) have better solubility and other physical properties.

IV. Molecular Weight

Unsubstituted structural unit	162.14
Monosubstituted structural unit	242.16

The average degree of polymerization (D.P.) is 500 to 2000 giving a molecular weight range of 85,000 to 480,000.

V. Specifications

Food Chemicals Codex

Assay. Not less than 99.5% of sodium carboxymethyl cellulose, on the dried basis.

Degree of substitution. Not more than 0.95 carboxymethyl groups ($-CH_2COOH$) per anhydroglucose unit after drying.

Viscosity of a 2%, weight in weight, solution. Not less than 25 cps.

Limits of impurities.

Arsenic (as As)	Not more than 3 ppm.
Heavy metals (as Pb)	Not more than 40 ppm.
Lead	Not more than 10 ppm.
Loss on drying	Not more than 10%.

VI. Description

A. General Characteristics

Sodium carboxy methyl cellulose is a white or slightly yellowish, odorless and tasteless powder.

B. Physical Properties (SMCM)

Hygroscopic
Soluble in hot and cold water
Insoluble in ethanol, ether, benzene, carbon tetrachloride and hexane
Viscosity of 2% solution ranges from 10,000 to 50,000 cps.
Viscosity decreases on heating-thermoplastic
Viscosity decreases below pH 5 - stable at pH 5-11.

C. Stability

Sodium carboxymethyl cellulose solutions can be stored for indefinite periods with no loss of viscosity, provided they remain free of mold and bacterial growths. If the solution is to be stored for a long time, a preservative should be added.

VII. Analytical Methods

Carboxymethyl cellulose (CMC) occurs in many commercial products.

Graham isolated carboxymethyl cellulose (CMC) from milk, chocolate milk, chocolate, and vanilla ice cream as the papain digest of the food sample. He precipitated out other gum contaminants such as alginate, pectic acid, and carrageenan. Then CMC was precipitated by cetyl-pyridinium chloride, collected on a Hyflo Super Cel column, washed, and finally dispersed by sulfuric acid. The eluate contained CMC (166).

When the gum is isolated, it can be determined qualitatively by a systematic analytical scheme such as one devised by Ewart and Chapman (121). This method is based on precipitation reactions with calcium chloride, sodium hydroxide, barium hydroxide, and lead acetate. This scheme distinguishes between pectin, alginate, gelatin, starch, carboxymethyl cellulose, methylcellulose, and several other gums, including carrageenan, tragacanth, agar, locust, karaya, and ghatti.

Croessman et al. contrived a series of colorimetric and precipitation reactions to differentiate between sodium carboxymethylcellulose and methylcellulose. It is based on foaming power, heat coagulation, and the reagents iodine/potassium iodide, tannin, Zephirol R, chromotropic acid, copper sulfate, and lead acetate (94).

McNulty has identified carboxymethyl cellulose from frozen desserts using infra red spectrometry. Carboxymethyl cellulose can be distinguished from agar, locust bean, Irish moss, pectin, and gum arabic by their spectra (302).

Padmoyo and Miserez have used microelectrophoresis to separate and identify the gums: carboxymethyl cellulose, methyl cellulose, agar, arabic, tragacanth, carrageenan, carob and guar, as well as, gelatin, pectin, starch, dextrin, and sodium alginate. The electrophoresis is carried out on cellulose acetate strips. Substances with similar motility are differentiated by staining (348).

Hildago used moving boundary electrophoresis to separate and identify carboxymethyl cellulose from guar gum, gum arabic and carrageenan (190).

CMC can be reacted with chromatropic acid (1,8 dihydroxynapthanene-3,6-disulonic acid) to form a purple color with a maximum absorbance at 570 nm. By preparing a standard curve, a quantitative determination can be made (166).

Anthrone reacts with CMC in a 60% sulfuric acid solution to form a green colored complex with a maximum absorbance at 625 nm. CMC in 50% sulfuric acid reacts with 2,7-dihydroxynapthalene to form a purple color with a maximum absorbance at 540 nm. By preparing a standard curve, a quantitative determination can be made for both methods (28).

VIII. Occurrence

Synthetic

Cellulose (wood pulp or cotton linter) is treated with NaOH. The alkali cellulose is reacted with sodium monochloroacetate or monochloroacetic acid.

$$R\text{-}OH + NaOH \rightarrow R\text{-}ONa + H_2O$$

$$R\text{-}ONa + Cl\text{-}CH_2COONa \rightarrow R\text{-}O\text{-}CH_2COONa + NaCl$$

Food grade CMC is washed with an alcohol-water mixture to remove the salt.

The reaction is controlled to give the desired degree of substitution (D.S.) degree of polymerization (D.P.) and uniformity of substitution, which in turn determine the properties of the finished product.

By varying the properties of D.S., D.P., and uniformity of substitution, materials with a wide variety of solution properties are produced. In addition, materials with various particle sizes, shapes, and densities are produced.

Methyl Cellulose

I. Nomenclature

A. Common Names

1. Methyl Cellulose
2. MC
3. Cellulose methyl ester

B. Chemical Names

Cellulose, methyl ether
Cellulose methylate

C. Trade Names

1. Methocel
2. Cellothyl
3. Syncelose
4. Bagolax
5. Cethylose
6. Cethytin
7. Cologel
8. Cellomuth
9. Hydrolose
10. Tylose
11. Culminal
12. Celacol
13. Celofas
14. Edifas

D. Chemical Abstracts Registry No.

PM9004675

II. Empirical Formula

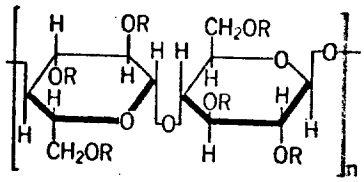
$$(C_6H_7O_2(OH)_x(OCH_3)_y)_n$$

where $x = 0.80$ to 1.40
$y = 1.60$ to 2.20
$x+y = 3.00$

III. Structural Formula

Methyl cellulose is a synthetic ether of cellulose, a polysaccharide made up of beta-anhydroglucopyranose units (490).

On each anhydroglucose unit there are three possible sites of substitution for a methyl group suggesting a degree of substitution (D.S.) of three.



However, materials with a lower D.S. have the better solubility and other physical properties.

IV. Molecular Weight

Unsubstituted structural unit		162.14
Monosubstituted structural unit		176.17
Disubstituted structural unit		190.20
Trisubstituted structural unit		204.3
Low molecular weight polymers (n = ± 200)	in range of	40,000
High molecular weight polymers (n = ± 900)	in range of	180,000

V. Specifications

Food Chemicals Codex

Assay. Between 27.5 - 31.5% methoxyl (-OCH3) after drying.
Viscosity. The apparent viscosity of a solution containing 2 g in each 100 ml is between 80-120% of that stated on the label for viscosity types of 100 centipoises or less; and between 75-140% of that stated on the label for viscosity types higher than 100 centipoises.

Limits of Impurities

Arsenic (as As)	Not more than 3 ppm.
Heavy metals (as Pb)	Not more than 10 ppm.
Loss on drying	Not more than 5%
Residue on ignition	Not more than 1.5%

VI. Description

A. General characteristics

Methyl cellulose is a white, fibrous powder or granules; it is odorless and tasteless.

B. Physical properties

Soluble in cold water and glacial acetic acid.
Insoluble in ether, ethanol, and chloroform.
Solutions are neutral and relatively stable over a pH range of 3-11.

Aqueous solutions are surface active, form films upon drying, and undergo reversible transformations from solution to gel upon heating and cooling, respectively.
Solution viscosity depends on the degree of polymerization and ranges from 10-8,000 cps. for a 2% solution.
Apparent density, g/cc
for 4,000 cps is 0.35
for 400 cps is 0.41
for 15 cps is 0.46
Charring temperature is 300-305 deg. C.
Freezing point is 0.0 deg. C.
Refractive index (2%) is 1.3355.
Specific gravity
1% = 1.0012
5% = 1.0017
10% = 1.0245

C. Stability

Methyl cellulose is stable under alkaline conditions but will undergo intrinsic viscosity reduction when oxygen is present (28).

VII. Analytical Methods

Methyl cellulose is used in many commercial products. It can be separated from starches and gums by extraction with alcohol. Water soluble impurities may be separated by hot water extraction (386).

When the gum is isolated, it can be determined qualitatively by a systematic analytical scheme such as one devised by Ewart and Chapman (121). This method is based on precipitation reactions with calcium chloride, sodium hydroxide, barium hydroxide, and lead acetate. This scheme distinguishes between pectin, alginate, gelatin, starch, carboxymethyl cellulose, methyl cellulose, and several other gums including carrageenan, tragacanth, locust, karaya, and ghatti.

Croessmann et al. devised a series of color and precipitation reactions to differentiate between sodium carboxymethyl cellulose and methyl cellulose based on foaming power, heat coagulation, and the reagents iodine/potassium iodide, tannin, Zephirol R, chromotropic acid, copper sulfate, and lead acetate (94).

Padmoyo and Miserez have used microelectrophoresis to separate and identify the gums: methyl cellulose, agar, arabic, tragacanth, carrageenan, carob, and guar, as well as gelatin, pectin, starch, dextrin, sodium alginate, and carboxymethyl cellulose. The electrophoresis is carried out on cellulose acetate strips. Substances with similar motility are differentiated by staining (348).

Becker suggests a scheme for identification of methyl cellulose in foodstuffs using paper chromatography of the gum after hydrolytic cleavage. Hydrolysis is made with sulfuric acid and boiling for three hours. After removal of sulfuric acid by barium hydroxide and concentration of the filtrate under vacuum, the sample is chromatographed.

Methyl cellulose is identified by the presence of glucose and non-hydrolyzed methylated glucoses. Pectin, alginate, carob-seed flour, carboxymethyl cellulose, gum arabic, and tragacanth can be identified as well. When several thickners are present identification on the basis of the chromatogram can become difficult. It then seems preferable to make identification less through the spots that do show but rather through those absent (36).

A quantitative determination of methyl cellulose can be made using the anthrone reagent. A green color is formed with a maximum absorbance at 625 nm (386).

Croessmann et al. suggest the determination of methoxy content as a quantitative measure of methyl cellulose (94).

VIII. Occurrence

Synthetic

Methylcellulose is prepared by reacting cellulose fibers (either cotton linters or wood pulp) with caustic soda to produce alkali cellulose. Alkali cellulose is reacted with methyl chloride to produce the methyl ether of cellulose.

$$R\text{-}OH + NaOH \rightarrow R\text{-}ONa + H_2O$$

$$R\text{-}ONa + CH_3Cl \rightarrow R\text{-}OCH_3 + NaCl$$

Propylene Glycol Ether of Methyl Cellulose

I. Nomenclature

A. Common Name

Propylene Glycol ether of methyl cellulose

B. Chemical Names

Cellulose, hydroxypropyl methyl ether
Hydroxy propyl methyl cellulose

C. Trade Name

Methocel HG

D. Chemical Abstracts Registry No.

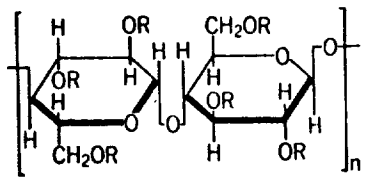
000000000

II. Empirical Formula

$$(C_6H_7O_2(OH)_x(COCH_3)_y(COCH_2OHCH_3)_z)_n$$

where $x = 0.88 - 1.85$
$y = 1.08 - 1.82$
$x+y+z = 3.0$

III. Structural Formula



R is H, CH_3, or CH_3CH_2OHCO

This anhydroglucose unit contains one primary hydroxyl group in the 6- position and two secondary hydroxyl groups in the 2- and 3- positions. Thus the propylene glycol ether of methycellulose is fundamentally a carbohydrate derivative containing a basic structure of condensed glucose rings.

The average degree of substitution for "Methocel" is 1.83. The propylene glycol degree of substitution is a minor portion of the total substitution and is present in a range of only 0.07 to 0.3. The methoxy degree of substitution is 1.08 to 1.82 (494).

IV. Molecular Weight

Equivalent weight varies from 162.4 to 235.2 dependent upon degree of substitution.

V. Specifications

Not available

VI. Description

A white, fibrous powder or granule. Viscosity of 2% solution ranges from 10 to 8,000 cps dependant upon degree of substitution (D.S.) and degree of polymerization (D.P.)

Thermo-gel point 50 to 55 deg. C. is raised as high as 85 deg. with increase in number of hydroxypropyl groups.

VII. Analytical Methods

See methyl cellulose

VIII. Occurrence

Synthetic

The basic reactions required for the manufacture of methyl cellulose are:

R—OH	+	NaOH	=	R—ONa	+	H_2O
Cellulose		Sodium hydroxide		Alkali cellulose		Water

R—ONa	+	CH_3Cl	=	R—OCH_3	+	NaCl
Alkali cellulose		Alkyl halide		Methyl cellulose		Sodium chloride

R—ONa	+	CH_2—CH_3 (O)	=	$ROCH_2CH_2OH$
Alkali cellulose		Propylene oxide		Hydroxypropyl cellulose

If in addition to methyl chloride the alkali cellulose is reacted with propylene oxide, the mixed ether hydroxypropyl methyl cellulose is produced.

Since the viscosity of the cellulose ether is related to the polymer molecular weight, control of the air oxidation of alkali cellulose (depolymerization) offers a means of producing cellulose ethers in a wide range of viscosities.

Cellulose Acetate

I. Nomenclature

A. Common Name

Acetate
Cellulose acetate

B. Chemical Name

Acetyl cellulose

C. Trade Names

1. Drawinella
2. Estron
3. Acetate cotton
4. Cellulose ester CA

D. Chemical Abstracts Registry No.

000000000

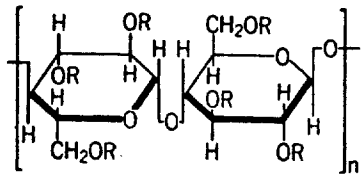
II. Empirical Formula

$(C_6H_7O_2(OH)_x(CH_3CO)_y)_n$

III. Structural Formula

Cellulose acetate is a synthetic ester of cellulose, a polysaccharide made up of beta-anhydroglucopyranose units.

On each anhydroglucose unit there are three possible sites for esterification of an acetyl group. These are the primary 6 positions, and the secondary 2- and 3- positions (371).



R is H or CH_3CO

IV. Molecular Weight

Equivalent weight varies from 162.14 to 288.6.

V. Specifications

Not available

VI. Description

A. General Characteristics

Cellulose acetates are amorphous, colorless to yellowish masses.

B. Physical Properties

Non-flammable,
Insoluble in water, alcohol, ether, but soluble in glacial acetic acid.

VII. Analytical Methods

Cellulose acetate occurs in many commercial products such as non-flammable photographic film, rayon, varnish, lacquer, phonograph records, and in waterproofing fabrics.

No information is available on separating or identifying cellulose acetate from food products.

However, Hekeler has paper chromatographed cellulose acetate and separated it from cellulose formate, propionate and butyrate (184).

VIII. Occurrence

Cellulose acetate is obtained by treating cellulose with acetic anhydride at various temperatures and for different lengths of time, depending on the degree of acetylation desired.

Ethyl Cellulose

I. Nomenclature

A. Common Name

Ethyl cellulose

B. Chemical Names

Cellulose, ethyl ether
Cellulose ethylate

C. Trade Names

Cellulose ether - CE
Ethocel

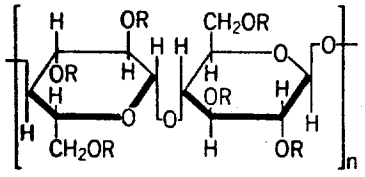
D. Chemical Abstracts Registry No.

PM9004573

II. Empirical Formula

$(C_6H_7O_2(OH)_x(CH_3CH_2O)_y)_n$

III. Structural Formula



R is H or CH_3CH_2

VI. Molecular Weight

Equivalent weight varies from 162.14 to 237.54

V. Specifications

Assay. Not less than 44.0% and not more than 50.0% of ethoxyl groups ($—OC_2H_5$), after drying (equivalent to not more than 2.6 ethoxyl groups per anhydroglucose unit).

Viscosity. Not less than 90% and not more than 110 % of that stated on the label, in centipoises, except that the 4 cps viscosity grade may vary between 3 and 5.5 cps, and the 7 cps viscosity grade may vary between 6 and 8 cps.

Limits of Impurities

Arsenic (as As)	Not more than 3 ppm (0.0003%).
Heavy metals (as Pb)	Not more than 40 ppm (0.004%)
Lead	Not more than 10 ppm (0.001%).
Loss on drying	Not more than 3%.
Residue on ignition	Not more than 0.4%.

VI. Description

A. General Characteristics

A free-flowing white to light tan powder.

B. Physical Properties

It is practically insoluble in water, in glycerin, and in propylene glycol, but is soluble in varying proportions in certain organic solvents, depending upon the ethoxyl content. Ethyl cellulose containing less than 46-48 percent of ethoxyl groups is freely soluble in tetrahydrofuran, in methyl acetate, in chloroform, and in aromatic hydrocarbon-alcohol mixtures. Ethyl cellulose containing 46-48% or more of ethoxyl groups is freely soluble in alcohol, in methanol, in toluene, in chloroform, and in ethyl acetate. A 1 in 20 aqueous suspension of the sample is neutral to litmus.

C. Stability

Stable if stored in tightly closed containers.

VII. Analytical Methods

See methyl cellulose.

VIII. Occurrence

Synthetic

Prepared from wood pulp or chemical cotton by treatment with alkali and ethylation of the alkali cellulose with ethyl chloride.

Cellulose, Microcrystalline

I. Nomenclature

A. Common Name

Cellulose

B. Chemical Name

Cellulose

C. Trade Names

Cellulose, microcrystalline
Cellulose gel
Avicel

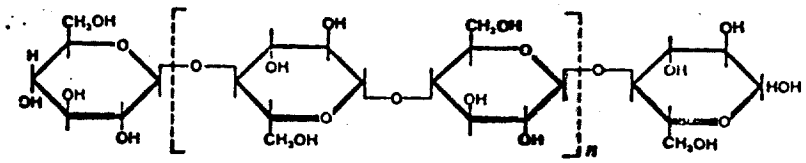
D. Chemical Abstracts Registry No.

977006657

II. Empirical Formula

$(C_6H_{10}O_5)_n$

III. Structural Formula



CELLULOSE

IV. Molecular Weight

In the range of 50,000

V. Specifications

Loss on drying	Not more than 5%
pH	Between 5.5 and 7.0
Limits of Impurities	
Arsenic (as As)	Not more than 3 ppm (0.0003%)
Heavy metals (as Pb)	Not more than 10 ppm (0.001%)
Residue on ignition	Not more than 0.05%
Water-soluble substances.	Not more than 0.16%

VI. Description

A. General Characteristics

Microcrystalline cellulose is purified, partially depolymerized cellulose. It occurs as a fine, white, odorless, crystalline powder. It consists of free-flowing, non-fibrous particles.

B. Physical Properties

Insoluble in water, dilute acids and most organic solvents.
Slightly soluble in sodium hydroxide.
Refractive Index: 1.55.
Bulk density: 18-19 lb/cu.ft.

C. Stability

Stable if stored in tightly closed containers.

VII. Analytical Methods

Cellulose has often been determined by oxidation with dichromate to avoid lengthy oven methods. Standard methods in use at present call for dissolving the cellulose in 12M sulfuric acid prior to the addition of dichromate. The heat-of-dilution dichromate method dispenses with these time-consuming techniques. Dilution of concentrated sulfuric acid provides instantaneous, reproducible, and more favorably applied heat, and prior dilution of the cellulose is thus eliminated. The method is very simple and rapid (5 min. per weighed specimen). The results are highly reproducible and more accurate than usual because carbon monoxide formation, causing 1-1.5% error with external heating, is essentially negligible in the heat-of-dilution method. The method has been applied to the determination of moisture in fruits and vegetables and in beet sugar pulp (93).

VIII. Occurrence

Synthetic

Microcrystalline cellulose is prepared by treating alpha cellulose, obtained in pulp form from fibrous plant material, with mineral acids.

Biological Data

I. Acute toxicity

Substance	Animal	No.	Route	Dosage (mg/kg body wt)	Measurement	Ref.
Methocel HG	Mice	138	i.p.	5000	LD_{50}	193
SCMC	Rats		p.o.	20000	ED_0	399
SCMC	Rats		p.o.	27000	LD_{50}	399
SCMC	Rats		p.o.	40000	LD_{100}	399
SCMC	Guinea Pigs		p.o.	10000	ED_0	399
SCMC	Guinea Pigs		p.o.	16000	LD_{50}	399
MC(5%)	Mice	180	i.p.	147.0*	LD_0	475
MC(5%)	Mice	180	i.p.	1.0*	ED_0	475
MC	Rabbits	5	i.v.	140	MLD	97
MC	Dogs	5	i.v.	200	MLD	97

*ml/kg

A. Cellulose

Rats

Single oral doses of Cellan 300 (a refined form of alpha cellulose) were administered to groups of five male albino rats (Sprague-Dawley strain, weight 94-125 g), by stomach tube, at dosage levels of 10.0, 31.6, 100, 316, 1000, and 3160 mg/kg. Observation of the animals for a period of 7 days followed by gross autopsies performed on all animals revealed no pathological effects (349).

Single intraperitoneal doses of Cellan 300 (a refined form of alpha cellulose) were administered to groups of five male albino rats (Sprague-Dawley strain, weight 103-124 g) at dosage levels of 10.0, 31.6, 100, 316, 1000, and 3160 mg/kg. The animals were closely observed for 7 days, after which gross autopsies were performed on all animals. Within 10 minutes after administration, the rats at the two highest levels exhibited depression characterized by inactivity, labored respiration, and ataxia. Ptosis and sprawling of the limbs were also seen in the rats given 3160 mg/kg. At the end of 24 hours, these animals appeared to be fully recovered and showed no further abnormalities during the 7 days. Gross autopsies performed on the animals at the 1000 and 3160 mg/kg levels revealed adhesions between the liver and the diaphragm and peritoneal wall, and congestion of the kidneys. Numerous discrete white masses appearing to be unabsorbed cellulose were found in the animals given the 3 highest doses, adhering to the organs contained within the abdominal cavity (349).

B. Methyl cellulose

Mice

In a study using 18 groups of 10 male, Swiss albino, Caesarean-derived mice, 20-30 g in weight, Worthley and Schott determined the LD_0 and ED_0 of a 5% solution of methyl cellulose to be 147.0 and 1.0

ml/kg, respectively, when injected intraperitoneally (475).

Rabbits

Wiedersheim, et al. reported that intravenous injections of 10 mg of methyl cellulose/kg body weight caused leucopenia in rabbits. However, injections of 10-100 mg/kg of a 1% solution had no effect on the blood pressure or respiration (466).

Man

Knight et al. administered single oral doses, 0.6-8.9 g, of Methocel HG (a high-gel-point methyl cellulose) to 25 adult human subjects. Mild laxative effects were noted in 11 cases and mild constipating effects were noted in 16 cases. Minor side effects (cramping, flatus, headache, and tenesmus) were reported in some cases but these apparently were not related to the ingestion of Methocel HG (244).

C. Propylene glycol ester of methyl cellulose.

Mice

Hodge, et al., administered Methocel HG (a water soluble cellulose ether) i.p. to 138 adult albino mice; the LD_{50} was found to be on the order of 5000 mg/kg (193).

Rats

Hodge, et al., administered Methocel HG orally by stomach tube to eleven fasted adult albino rats. A level of 4000 mg/kg produced no sign of injury (193).

D. Ethyl Cellulose

None

E. Carboxymethyl cellulose

Rats

Rowe et al. administered single oral doses of 5 g CMC/kg to rats (unspecified number) in suspensions of olive oil and aqueous gum arabic by means of a stomach tube. Observation of the animals for two weeks indicated no toxic effects. Gross and microscopic examination of tissues from the rats taken at daily intervals after the forced feeding revealed no pathological lesions except those which might be expected from the administration of large volumes of material (378).

Guinea pigs

Rowe et al. administered single oral doses of 5 g CMC/kg to guinea pigs (unspecified number) in suspensions of olive oil and aqueous gum arabic by means of a stomach tube. Observation of the animals for two weeks indicated no toxic effects. Gross and microscopic examination of tissues from the animals taken at daily intervals after the forced feeding revealed to pathological lesions except those which might be expected from the administration of large volumes of material (378).

Rabbits

Rowe et al. administered single oral doses of 5 g CMC/kg to rabbits (unspecified number) in suspensions of olive oil and aqueous gum arabic by means of a stomach tube. Observation of the animals for two weeks indicated no toxic effects (378).

F. Sodium carboxymethyl cellulose

Rats

Intubation was used by Shelanski and Clark to determine the acute toxicity of SCMC in white rats (unspecified number). They found the LD_{100} to be 40 g/kg, the LD_{50} to be 27 g/kg, and a no-effect level of 20 g/kg. Autopsies of the animals showed no evidence of gross pathology (399).

Forty-eight hours after intraperitoneal injection of SCMC (1 ml of a 1.6% solution) into 6 rats, Jasmin and Bois noted the presence of CMC particles in lung reticulo-endothelial cells. A few phagocytes were found in the liver and speen (218).

SCMC was administered to rats (unspecified number) at a level of 3 g/kg in 3% aqueous solution in 3 divided doses over a 6-hour period. Observation of the animals for two weeks indicated no toxic effects. Gross and microscopic examination of tissues from the rats taken at daily intervals after the forced feeding revealed no pathological lesions except those which might be expected from the administration of large volumes of material (378).

Guinea pigs

SCMC was administered to guinea pigs (unspecified number) at a level of 3 g/kg in 3% aqueous solution in 3 divided doses over a 6-hour period. Observation of the animals for two weeks indicated no toxic effects. Gross and microscopic examination of tissues from the guinea pigs taken at daily intervals after the forced feeding revealed no pathological lesions except those which might be expected from the administration of large volumes of material (378).

To determine acute toxicity, Shelanski and Clark administered SCMC to guinea pigs (unspecified number) by stomach tube. Ten g/kg was reported as the LD_0 while the LD_{50} was listed as 16 g/kg. Post mortem examination of the test animals revealed no evidence of gross pathology (399).

Rabbits

SCMC was administered to rabbits (unspecified number) at a level of 3 g/kg in 3% aqueous solution in 3 divided doses over a 6-hour period. Observation of the animals for two weeks indicated no toxic effects (378).

II. Short Term Studies

A. Cellulose

Man

The free choice diets of 16 human volunteers (8 male and 8 female) were supplemented with 30 g of Avicel (microcrystalline cellulose) daily for a period of six weeks. Gross observations of the subjects and subject interviews and examinations, as well as the results of hematological and biochemical examinations of blood and urine for a period starting two weeks before Avicel administration and lasting 10 weeks, revealed no changes or pathological effects attributable to Avicel injestion (441).

Various examinations were performed on 8 healthy human males, age 18 to 34, before, during, and after a two week period during which they each ingested daily 30 g of Avicel (microcrystalline cellulose). Observations on digestion, assimilation, chemical composition of blood and urine, blood tests, influence upon intestinal bacterial flora, and influence upon vitamin B_1 assimilation revealed no unfavorable effects attributable to Avicel consumption (11).

B. Methyl cellulose

Chicks

Vohra and Kratzer found no effect of methyl cellulose on the growth rate of day-old Arbor-Acres chicks (unspecified number) when fed ad lib at a level of 2% of the diet for a period of 20 days (453).

Kratzer, et al., fed twenty day-old Arbor Acres chicks a 2% methyl cellulose (MC) diet ad libitum for 3 weeks. MC had no growth depressing properties for chicks (252).

Rats

No appreciable discrepancies in the behavior, appetite, or bodily weight gain curve of the animals were noted when rats (unspecified number) weighing 60-70 g were given MC at a daily dosage (method unspecified) of 0.5 g/kg for a period of 4 weeks (238).

Supplementation of Tioga meal with 10% MC (Methocel (Dow) 4000 cps) was implemented by Tianter in an investigation of the possible toxic effects of MC. This mixture was fed to 5 male and 5 female rats for 95 days while a like group of animals was maintained on Tioga meal as a control. Food consumption was such that an average of 11.4 g/kg/day of MC was ingested by the test rats. Growth rate of the male rats was

unaffected, but a 14% reduction in growth rate was manifested in the females, apparently as a result of decreased food intake. There was demonstrable change in weights of the heart, liver, spleen and kidneys, however the stomachs of the methyl cellulose animals were 15% heavier than the controls. Microscopic examination revealed no significant pathological changes in the test animals (428).

Bauer and Lehman studied the effect of 50% methyl cellulose on 9 Sprague-Dawley rats (3 male; 6 female) for 90 days.

Rat	Diet	Schedule	Growth	feces	urine
1	500,000 ppm MC	ad lib	low	larger	smaller
2	500,000 ppm cellulose flour	ad lib	low	smaller	larger
3	standard	paired to Rat 1	low		
controls*	standard	ad lib	high		

*controls are from a previous experiment

This system was used to determine the effect of massive dosage and the "bulk" producing effect. For 40 days all rats were given standard diet ad lib; growth appeared normal for all. All rats reproduced as normal. MC females produced half as much formic acid in their urine as female controls. Male cellulose flour rats produced 4 times as much formic acid as male controls. It is concluded that the growth depression of bulk containing diets is the result of nutritional want, not toxicity (31).

Bauer et al. investigated the chronic toxicity of methyl cellulose in rats. Four groups of 5 white rats were employed, 3 groups of females and one of males. Steenbach's whole artificial diet was supplied ad lib to one group of females which served as the control. The remaining 3 groups were placed on this basic diet in which 1.66, 1.66, and 5.0% MC, respectively, had replaced carbohydrate. Feeding of these diets continued for 184 days. The MC-fed rats gained weight more rapidly, attained greater average weight at the conclusion of the experiment, and consumed more food per day than did the controls. A mineral oil type of stool was excreted by the test rats. Pathologic examination of one rat from each group revealed no gross or microscopic effects (30).

Deichmann and Witherup supplied 80 young albino rats with MC at a level of 0.8% in their diet and 1.0% in their drinking water for a period of 8 months. This provided a total daily intake of 436 mg of MC per animal. Food and water intake was normal for the test animals and no effect on growth rate was observed. Post mortem investigation of the animals revealed no significant gross or microscopic abnormalities in the tissues (list of tissues examined was not given). It was concluded that the feeding of MC at this level is harmless to rats (103).

Ellingson and Musengale studied the effect of ingested methyl cellulose on growth of normal and vitamin-depleted rats to minimal dosages of thiamine and vitamin A.

Group	No. of Rats	Vit. fed	MC	Growth effect
1 normal	10	vit. A	200 mg/kg	none
2 depleted vit. A for 18 days	10	vit. A	200 mg/kg	none
3 normal	10	thiamine	200 mg/kg	none
4 depleted thiamine for 18 days	10	thiamine	200 mg/kg	none

(vit. A = 3 units, thiamine = 6 micrograms thiamine hydrochloride)

The vitamin was dissolved in a solution of MC and administered daily by stomach tube. No decrease in growth was noted. The authors conclude that daily ingestion of large amounts of MC does not decrease absorption of water-soluble or oil-soluble vitamins (117).

Dogs

Khadzhay and Shaposhnikova administered MC (method unspecified) to 4 dogs at levels of 0.1, 1.0, 2.0, and 3.0 g/kg for a period of one month. Except for a general weakening 2.5-4 hours after administration no undesirable or toxic effects were observed (238).

Two dogs which were fed 2 to 100 g of MC daily for one month showed no significant increase in the weight of the stools for the different levels of intake. The slight increases observed approximately equalled the dry weight of the MC administered (29).

Man

In studying the use of methyl cellulose as a laxative, Bargen administered, orally, 33.3 mg MC/kg 4 times daily to 6 human adults while another 30 patients received 50 mg/kg 4 times daily. MC proved to be an effective laxative and no toxic effects were found (25).

For the majority of 37 patients, 1-6 g of MC daily proved effective for the relief of chronic or acute constipation. Prolonged use at this level (maximum 6 g daily for 240 days) produced no evidence of systemic changes or toxicity (393).

Bauer found that doses of 2.5 to 5.25 g of MC taken orally as gels in 250 ml of water had a tendency to be mildly constipating in man (29).

Tainter reported that daily doses of 10 g of MC approximately doubled the volume of the stools and increased their frequency in normal human subjects. Each gram of MC injected increased the stools approximately 10 g (428).

C. Propylene glycol ester of methyl cellulose

Rats

Methocel 70HG, a mixed methyl and hydroxyl propyl ether of cellulose containing 25-27% methoxyl groups and 7-10% hydroxy propyl groups, was fed to groups of 10 male and 10 female white albino rats, 45 days of age, for a period of 90 days at dietary levels of 0, 0.3, 1.0, 3.0, 10.0 and 20.0%. Gross appearance and behavior of the animals was observed throughout the experiment and records of body weights and food consumption were kept. At conclusion of the feeding period, the animals were sacrificed and autopsied. Gross pathological findings were recorded and the lungs, heart, liver, kidneys, spleen, and testes were removed and weighed. Sections of the adrenals, pancreas, and the aforementioned organs were examined microscopically and blood hematocrit values were determined. The criteria examined revealed no evidence of adverse effects in the rats fed 0.3, 1.0, and 3.0% Methocel 70HG. At the 10% level, the male rats, but not the females, showed slight but significant growth retardation. These animals had distended abdomens and their feces were bulky and light colored. Of the group receiving 20% Methocel 70HG, 3 males and 3 females died during the course of the experiment from indeterminant causes. Moderate growth retardation was observed in both sexes at this level accompanied by a decrease in feed efficiency. On a percent of body weight basis, there was an apparent increase in the weight of the testes of the male rats on the 20% level. However, on an absolute weight basis there was no effect on the testes. No histopathological effects due to the Methocel 70HG were found in the rats of either sex receiving the 10 or 20% dietary levels (494).

For a period of 121 days, McCollister and Oyen fed groups of rats (10 males and 10 females per group, starting age 2-3 months) basal diets containing 0, 1, 3, 10, and 30% Methocel 2602 (a hydroxypropyl methyl cellulose preparation in which methoxyl substitution amounts to 29-30% and hydroxypropoxyl substitution is 6-7%). The rats were caged in groups of five and allowed food and water ad lib. Records were kept of body weights, general appearance, mortality, and average daily food consumption. Hematological studies and histological examinations of the organs were performed at the end of the experiment. At the 30% level, the rats became rough and unkempt in appearance and there was marked retardation of growth. Four male rats and six female rats given the 30% diet failed to survive the full 121-day period. Autopsies of the animals which survived the full 121 days at this level revealed no change in organ weights or pathological effects. In the group fed 10% Methocel 2602, there was a slight retardation of growth for the male rats but no other effects were found. No evidence of any adverse effects were found in the 1% or 3% Methocel 2602-fed rats. McCollister and Oyen suggested that under-nutrition, due to non-nutritive bulk in the diet, was responsible for the effects on growth (297).

Methocel 90HG was fed to rats (20/group) for 3 months at the following concentrations: 0, 0.3, 1, 3, 10 and 20% of the diet. There was marked growth retardation in the male rats receiving the 20% dose and a slight growth retardation in the male rats on the 10% dose. No ill effects were observed in the other groups. Organ weights were normal and histopathology of the tissues was negative in all groups (494).

Rats (20/group) were fed Methocel 65HG for 30 days at the following levels: 0, 2, 10 and 25%. At the highest dose there was a loss in weight, early deaths and severe diarrhea. The results of urine and hematological examinations were normal except for a decreased red count in the 25% group. Organ weights were normal as compared to the controls, and there was no evidence of histological injury (193).

Hodge et al. in a preliminary experiment fed Methocel 65HG to rats (20/group) for one year at the following levels: 0, 20 and 25%. Growth retardation occurred at both test levels; however, there were no after effects as judged by urine and blood analyses, organ weights and histopathology (list of organs examined was not given) (193).

Rabbits

Six rabbits per group were fed Methocel 65HG for 30 days at the following levels: 0, 10 and 25%. Those on the 25% dose maintained body weight. Urinalysis and organ weights were normal and histopathology was negative (193).

Dogs

Hodge et al. fed two dogs per group for one year at the following doses of Methocel 65HG: 0, 0.1, 0.3, 1 and 3 g/kg/day. All dogs maintained their body weights, and no toxicity was seen as judged by urinalyses, periodic hematological examinations, gross and histological examinations of the heart, lung, spleen, stomach, intestines, pancreas, liver, gall bladder, adrenal, kidney, bladder, gonads, thyroid, bone marrow, and brain tissues (193).

Two dogs were fed Methocel 65HG for 30 days at a level of 25 g and 50 g/day. The dog fed the 50 g dose had diarrhea, lost weight and was anemic. Urinalysis and organ weights, however, were normal in both. Histopathology (of unnamed organs) was negative (193).

D. Ethyl cellulose

Rats

In a feeding study by Deichman and Witherup, 80 young albino rats were supplied with diets containing 1.2% ethyl cellulose for a period of 8 months. The average daily intake of ethyl cellulose by the rats was 182 mg. Food and water intake was normal for the test animals and no effect on growth rate was observed. Post mortem investigation of the animals revealed no significant gross or microscopic abnormalities in the tissues (unnamed). It was concluded that the feeding of ethyl cellulose at this level is harmless to rats (103).

E. Carboxymethyl cellulose

Chicks

Kratzer, et al., fed a low fat diet, a high fat diet, and a high protein diet to twenty day-old Arbor Acres chicks each for 3 weeks. Every diet contained a 2% level of carboxymethyl cellulose; every diet

was slightly deficient in vitamin D_3. The chicks in all three diets developed ricketts, and had reduced growth and fat absorption. In the high fat diet, the pancreatic weight was significantly increased (252).

Later Kratzer, et al., repeated the above experiment but increased the vitamin D_3 concentration to six times the minimal level. Growth was depressed on the high fat diet, and the pancreas was enlarged on both fat diets (252).

Rats

The hypocholesterolemic effect of CMC was investigated by Kiriyama et al. through feeding tests using Wistar rats maintained on a hypercholesterolemic diet. The animals were individually housed and fed a basal diet for 5-8 days, after which they were given the control diet (hypercholesterolemic) with or without a 5% supplement of CMC for either 8 days or 2 weeks. A rise in the plasma cholesterol level was observed with the control diet but was depressed by the addition of CMC to the diet. The CMC-fed rats accumulated less cholesterol in the liver than did the controls, the liver cholesterol level apparently paralleling the plasma cholesterol level. Absorption of ingested cholesterol, however, was somewhat higher in the test animals than in the controls (241).

Groups of 10 rats, both male and female, were given a basal diet with or without 5% CMC for a period of 1 year. Records of body weight and food and water intake were nearly the same for both groups. Gross and microscopic examination of the sacrificed rats disclosed no evidence of pathological effects (335).

Groups of 10 practically mature female rats were given a basal diet with or without a 20% supplement of CMC for a period of 63 days. Growth curves, organ weights, gross observations and histopathological examination revealed no discernible ill effects produced by the supplement (378).

Ten male and 15 female rats (average age 25 days) were given a basal diet supplemented with 5% CMC for 8 months and a like group was maintained on the basal diet as a control. Growth curves, organ weights, gross observations, and histopathological examination revealed no discernible ill effects produced by the supplement (378).

F. Sodium carboxymethyl cellulose

Chicks

Vohra and Kratzer found no effect of sodium carboxymethyl cellulose on the growth rate of day-old Arbor-Acres chicks (unspecified number) when fed ad lib at a level of 2% of the diet for a period of 20 days (453).

Rats

Groups of 10 practically mature female rats were given a basal diet with or without a 20% supplement of SCMC for a period of 63 days. A very slight growth-curve depression of questionable significance was noted. This was explained by the decrease in nutrient food intake resulting from the bulkiness of the diet. The general appearance of

the animals was normal except that their feces were somewhat soft throughout the experiment. No histopathological changes or effects on organ weight were observed (378).

By supplementation of the diet, 100 white rats received 0.5 g SCMC/kg daily for 6 months, 100 received 1.0 g/kg and 40 were kept as controls. Normal weight gain was observed except in some cases in which the test animals showed greater weight gain than the controls. No effect on fertility was observed. Examination of the urine, blood, and tissue sections (liver, kidney, spleen, stomach, intestines, and heart) exposed no evidence of gross or microscopic pathology (399).

Ten male and 15 female rats (average age 25 days) were given a basal diet supplemented with 5% SCMC for 8 months and a like group was maintained on the basal diet as a control. Growth curves, organ weights, gross observations, and histopathological examination revealed no discernible ill effects produced by the supplement (378).

In a feeding test in which Ziegelmayer et al. fed 3 rats, 7-week-old, a diet containing 14% Cellugel (SCMC) for a period of 5 weeks, no growth inhibition or damage to the health of the rats was observed (483).

For a period of approximately 2 months, Werle fed 10 grown rats diets containing SCMC such that consumption of SCMC was 0.3 or 0.5 g per day. Observation of general appearance and body weight disclosed no untoward effects resultant from ingestion of SCMC (461).

Guinea pigs

By supplementation of the diet 100 guinea pigs received 0.5 g SCMC/kg daily for 6 months, 100 received 1.0 g/kg and 40 were kept as controls. Normal weight gain was observed except in some cases in which the test animals showed greater weight gain than the controls. No effect on fertility was observed. Examination of the urine, blood, and tissue sections (liver, kidney, spleen, stomach, intestine, and heart) exposed no evidence of gross or microscopic pathology of liver, kidney, spleen, stomach, intestine and heart (399).

Schelanski and Clark fed 20 guinea pigs 0.5 g/kg and 20 others 1.0 g/kg of SCMC daily in their diet for 1 year. Weight gain of the animals was normal and post mortem examination of sections of liver, kidney, spleen, stomach, intestine, and heart revealed no pathological or histological effects (399).

Dogs

Shelanski and Clark fed 3 groups of 10 dogs each 0, 0.5, and 1.0 g SCMC/kg daily for 6 months. Weight curves revealed no effect of SCMC on growth rate. Histological examination of the stomach, intestines, spleen, liver, kidneys, heart, lungs and pancreas revealed no deviations from the normal control animals (399).

Hueper gave 5 dogs varying amounts of sodium carboxymethyl cellulose for 3-4 months. He started with 12.50 mg/kg daily and increased to 31 mg/kg daily, and then to 47 mg/kg daily. No gross pathological changes occurred. Histopathological studies revealed uptake in the reticulo-endothelial cells in the aorta (203).

Man

Fittipoldi and Davis used sodium carboxymethyl cellulose as a laxative on 128 human adults (94 female, 34 male). The dose was 66.6 mg/kg given orally (tablet) twice daily. Nineteen subjects complained of cramps, belching, fullness, gas, constipation, griping, or a combination thereof. No serious side effects were noted (130).

Brick studied the effect of sodium carboxymethyl cellulose on blood cells when used as a laxative. He gave 166.6 mg/kg orally (tablet) daily for six months to 22 adult humans. Seven patients required reduced dosage due to diarrhea, abdominal discomfort, or a feeling of urgency to have a bowel movement (4 patients dropped out of study). Red blood cell count, white blood cell count, and hematocrit showed no significant change from control period after 6 months. Differential smear, hemaglobin, and buffy coat determinations were essentially normal as well. No toxic effects were noted (69).

Schultz treated 250 adult humans for constipation using sodium carboxymethyl cellulose as a colloid laxative over a period of three years. At first, 33.3 - 66.6 mg/kg sodium carboxymethyl cellulose were administered orally (tablet) twice a day. The dosage was then cut by 50% and still worked effectively. No toxic effects were found at either level (392).

III. Long Term Studies

A. Cellulose

Rats

Two groups (Groups No. 2 and No. 3, respectively) of young albino rats (Charles River Caesarean-derived strain), 50 males and 50 females per group, received Avicel (microcrystalline cellulose) in dry commercial form or in gel form in a synthetic diet at a level of 30% for 72 weeks. A control group (Group No. 1) was maintained on the same synthetic diet containing 30% Alphacel (fibrous cellulose). Observations on physical appearance and behavior of the rats revealed no discrepancies between the test animals and the controls. In Group No. 3, body weight gains for the males at 52 weeks and for the females throughout the first year were significantly higher than the controls. No differences were seen in food consumption or survival of the animals. Hematological values for the test groups were generally within normal limits. The terminal body weights and heart weights for the females and the liver weights for the males in Group No. 2 were significantly lower at 26 weeks than those for the controls. At 72 weeks, significant increases were noted in the terminal body weights and heart, liver, and kidney weights for the males and females of Group No. 3 as well as increases in the liver and kidney ratios for the males of this group. Reduction in the thyroid, liver, and kidney ratios for the females of Group No. 3, compared to the controls, were found at 72 weeks. Gross changes were found in the kidneys of the females, particularly in groups No. 1 and No. 2 while the kidneys of the males appeared to be normal. Severe dystrophic calcification of the terminal proximal tubule was disclosed by microscopic examination of kidney sections from the female rats. Involutional changes in the ovaries were observed in the majority of females from Group No. 2

sacrificed after 52 weeks and in several females from Groups No. 1 and No. 3. Histological examination of the other organs revealed no further abnormalities (353).

B. Methyl cellulose

Rats

Groups of 20 male and 20 female rats, 50 days old, were placed on diets, ad lib, containing 0, 1.0, or 5% of 15 cps., 400 cps., or 4000 cps. of Methocel MC (Dow methyl cellulose) for a period of 2 years. Additional groups of 10 males and 10 females for each dietary level were set up and designated to be sacrificed for examination after 12 and 18 months of feeding. Records of general appearance and behavior, growth, mortality, incidence of tumorous growths, food consumption, hematological studies, serum urea nitrogen and alkaline phosphatase determinations, final average body and organ weights, and gross and microscopic examination of tissues (lungs, heart, liver, kidneys, spleen, testes, adrenal, and pancreas) gave no evidence of any toxic effects produced by the feeding of methyl cellulose to rats at the indicated levels (92).

C. Propylene glycol ether of methyl cellulose

Rats

Hodge et al. performed a long term study on the toxicity of Methocel 65HG in rats. Four groups of 50 male and 50 female rats each were fed 0, 1, 5, and 20% levels ad libitum for two years. During the first year, the 20% male rats grew less than the controls by 30 g, all other levels of males and all of the females grew equally as well as the controls for the first year. This trend continued throughout the second year until near the end, when disease caused many fluctuations in the growth curve. Blood analyses, urine analyses, and mortalities were normal for the ageing groups. No tissue injuries were found in the stomach, small bowel, large bowel, liver, kidneys, lungs, heart, brain, bladder, spleen, testes, or bone marrow and tumor incidence was found to be normal in the treated rats (193).

D. Ethyl cellulose

None

E. Carboxymethyl cellulose

Mice

Nilson and Bender fed both male and female mice a basal diet with (10 mice) or without (5 mice) a supplement of 5% CMC from weaning until death. Records of body weight and food and water intake revealed no differences between the control and the test groups. No gross symptoms characteristic of any toxic condition were found during post-mortem examination of the animals (335).

F. Sodium carboxymethyl cellulose

Mice

McElligott and Hurst fed sodium CMC to mice at levels of 0, 1, and 10% (50 males and 50 females each) for a period of 100 weeks.

Body weight (g) at week	0	25	50	75	100
Males					
0	37	42	46	49	45
1%	37	41	42	44	38
10%	38	42	43	43	39
Females					
0	29	35	42	42	37
1%	29	34	38	40	34
10%	30	34	37	38	36

Mortalities occurring in the first 1.5 years resulted from pulmonary infections; later deaths were due to neoplasia common to ageing animals. No indication of CMC absorption or storage was found. Tumor frequency was normal (299).

Rats

McElligott and Hurst fed sodium CMC to Alderly Park rats at levels of 0, 1, and 10% (50 males and 50 females each) for 104 weeks.

	Initial		Terminal		
	No. of rats	Body wt (g)	No. of rats	Body wt (g)	Avg. wt. gain (g)
Males					
0	50	310	25	511	201
1%	50	287	30	498	211
10%	50	299	30	451	152
Females					
0	50	207	27	344	137
1%	50	208	23	337	129
10%	50	200	24	287	87

Mortalities occurred occasionally in the first 1.5 years of the test from pulmonary infections; in the late part of the test, death was due primarily to neoplasia common to ageing rats. Retarded growth occurred in both males and females on the higher SCMC diet although these rats had a higher feed intake. Haematology and tumor frequency were normal. No indication of CMC absorption or storage was found (299).

IV. Special Studies

A. Cellulose

Reproduction

Sixteen female and eight male rats each were selected from Groups No. 1, No. 2, and No. 3 (30% Alphcel, 30% dry Avicel, and 30% Avicel gel, respectively) of a nutritional balance study (see long term studies-cellulose-rats; ref. 354). These rats were maintained on their respective diets and were mated. Their offspring were treated similarly. Second and third generation litters revealed an adverse effect of the cellulose on reproduction. Relatively low fertility, live birth, and lactation indices were observed. Unsatisfactory physical condition of the offspring was noted in all three generations, but no deformities were found in any of the pups produced. The workers concluded that the adverse effects noted were related to nutritional deficiences of the basal diet and not to any toxic effects of the cellulose (354).

B. Methyl cellulose

Carcinogenicity

Intraperitoneal injections of 2 ml of a 2.5% aqueous solution of methyl cellulose were administered to Sprague-Dawley rats 3 times per week starting at times varying from 10 days before to 8 days after subcutaneous transplantation of Murphy-Sturm lymphosarcoma. The injections had no effect on establishment or growth of the tumor for the first 10 days after transplantation. However, between the 17th and 21st days after transplantation, complete regression of the tumor was observed in 38 of the 40 rats begun on injections 10 days before transplantation, 15 of 18 rats begun on injections 1 day after transplantation, and 12 of 18 rats begun on injections 8 days after transplantation. In 6 of 26 untreated control animals regression of the tumor occurred spontaneously. Biopsy of the tumors, performed on half the animals in each group 11 days after transplanting, revealed some degree of necrosis in all of the animals, with an average of 10-20% for all of the groups. No correlation of degree of necrosis and subsequent growth or regression could be made. Histopathological examination of MC-treated rats sacrificed at the end of the experiment (after complete regression) showed a striking hepatosplenomegaly as well as abdominal, but not peripheral, lymphadenopathy. The MC-treated rats also exhibited: numerous vacuolated cells in glomeruli and proteinaceous material in tubules of the kidney; foamy macrophages in the liver, spleen, thymus, and abdominal lymph nodes with the extent of involvement paralleling the amount of MC injected. Three of these animals also had a marked medial thickening of the pulmonary arterioles (262).

In experiments similar to those of Lazar and Lazar (ref. 262; see previous paragraph), Hruban et al. also found that MC treatment of rats bearing Murphy-Sturm lymphosarcoma produces a significant increase in the percentage of complete regressions. Electrophoretic studies of the sera of the MC-treated animals indicated, at the time of regression, high levels of gramma-globulin, elevated beta-globulins, and pretreatment levels of total serum proteins (197).

Complete, spontaneous regression of the Murphy-Strum lymphosarcoma in rats results in life-long resistance of the animal to the successful re-introduction of this tumor. If re-introduced, the tumor will not grow. However, Lazar and Lazar reported re-introduction and growth of the tumors in 9 of 12 rats in which MC-induced regression of the tumors had previously occurred (caused by i.p. injection of 2 ml of a 2.5% aqueous solution of MC three times weekly starting 10 days before implantation of the tumor). Complete regression occurred in all nine rats, but a third inoculation of these animals failed to produce any detectable growth of the tumors (264).

Forty adult female Sprague-Dawley rats were subjected to i.p. injections of 2 ml of 2.5% aqueous suspension of MC three times weekly starting 10 days before subcutaneous implantation of Walker tumor 256 and continuing until the death of the rats (20 rats) or for only two injections after implantation (20 rats). Observation of the size of the tumor and the longevity of the rats indicated no beneficial effect attributable to the methyl cellulose (265).

Hueper injected 25 Bethesda black rats subcutaneously with 500 mg of powered methyl cellulose. After 2 years, histological examination of the animals revealed that the incidence of tumor development was within the control range (203).

Reproduction

Bauer reported that three generations of rats maintained on a basal diet supplemented with 5% MC showed a definitely greater average increase in body weight than did the controls. No effect on reproductive functions were observed (29).

Bauer and Lehman studied the effect of methyl cellulose on growth, food intake, focal output, urinary formic acid production, and reproduction. Albino rats of the Sprague-Dawley strain (35-40 days old; 55-84 g) were divided into 3 groups of 10 (5 males, 4 females). The diets were 50,000 ppm MC (high), 1700 ppm MC (low) and a standard diet (control). The average daily intake (ad lib) of MC was 690 mg/kg (males) and 777 mg/kg (females) from the high MC diet, and 61 mg/kg (males and 57 mg/kg (females) on the low MC diet. After 8 months they were mated. The young of the f1 and f2 generations were placed on the 50,000 ppm MC diet and later mated. Representative animals from each group (1st generation) were sacrificed after 8 months. Both experimental groups ate more than controls. All generations produced healthy offspring which grew at the same rate as the 1st generation (normally). Macroscopic and microscopic pathologic examination of the first generation showed no damage to the tissues (31).

Local irritant

Khadzhay and Shaposhnikova reported that 0.1-1.0% solutions of MC do not change the contraction amplitude or the tone of smooth muscle of isolated sections of rabbit intestine. Application of the compound, in a 1-2% solution, to the eye of a rabbit failed to produce irritation of the conjunctiva (238).

C. Propylene glycol ether of methyl cellulose

None

D. Ethyl cellulose

Carcinogenicity

Hueper injected 25 Bethesda black rats subcutaneously with 500 mg of powdered ethyl cellulose. After 2 years, histological examination of the animals revealed that three of the rats (12%) had developed tumors. Of the 200 untreated controls, 9% had developed tumors (203).

E. Carboxymethyl cellulose

Carcinogenicity

Lusky and Nelson administered weekly subcutaneous injections of 1 ml of 2% CMC solution to 30 rats (10 male and 10 female Osborne-Mendel and 10 Bethesda Black males) for 73 weeks. At this point 13 of the rats (43%) had developed tumors at the site of injection. It was noted that injection of CMC left persisting deposits of it at the injection site. Microscopically, the injection site tumors were fibrosarcomas of moderate histological malignancy (279).

Local Irritant

Application of CMC to the shaven bellies of rabbits 5 times per week for 4 weeks indicated that CMC is not irritating to the skin of rabbits (378).

Allergenic

Franciosi reported that clinical phenomena of allergic type may be induced in cattle by preparations with a basic combination of corticosteroids, but that these phenomena are not connected with the administration of active constituents but rather to a particular suspension element, CMC. He pointed out that cases of immediate hypersensitiveness to CMC were observed only in regions in which the cattle had been immunized against foot-and-mouth disease with vaccine containing CMC (136).

F. Sodium carboxymethyl cellulose

Reproduction

White rats in groups of 25 each received in their diet 0, 0.1, 0.5, and 1.0 g SCMC/kg, respectively, for a period of 25 months. Litters produced by these animals were kept in their respective groups and placed on the same diet as their parents. Records of fertility, which include third generation animals, revealed no effects attributable to SCMC. Weight curves for the test rats showed a slight increase in weight gain for the SCMC-fed animals. Examination of the blood, urine, and tissue sections (heart, liver, kidney, stomach, intestines, spleen,

and adrenals) revealed no gross or microscopic pathology associated with the feeding of SCMC (399).

Local Irritant

Application of SCMC to the shaven bellies of rabbits 5 times per week for 4 weeks indicated that SCMC is not irritating to the skin of rabbits (378).

Application of 5 g of SCMC powder per subject in the treatment of vaginal infections in 134 cases revealed no evidence of irritation or untoward reactions to the vaginal mucosa or external genitalia (399).

Patch tests on two hundred unselected human subjects indicate that SCMC is not a primary irritant nor does it appear to be a sensitizer (399).

Laxative

Obolentseva et al. investigated the laxative effect of SCMC using white mice, rats and dogs. They found SCMC to be an effective laxative; doses of 0.5 g/kg were effective in 63% of the white mice, 1.1 g/kg effective in 83% of the rats, and 0.27 g/kg effective for 50% of the dogs. Prolonged administration of the compound decreased the dosage required for effective treatment. The mechanism of SCMC's laxative effect was found to be related to its colloidal swelling, which results in a mechanical stimulation of motor activity in the intestines. The experiments also disclosed that SCMC decreases the volume and activity of gastric juice in dogs (338).

I. Breakdown

CMC is readily hydrolyzed by microorganisms, but MC is usually resistant to microbial attack. The degree of substitution has an effect on enzymatic hydrolysis as measured by the production of reducing sugars. When 4 samples of CMC were hydrolyzed, the amount of reducing sugar obtained was an inverse function of the degree of substitution. The data indicated that the presence of one substituent on every anhydroglucose molecule renders the chain refractory to attack (368).

II. Absorption-Distribution

Gastrointestinal absorption of SCMC by the rat was investigated by Wiebe et al. An aqueous solution of SCMC labelled with ^{14}C in the carboxymethyl moiety was administered to 10 male albino rats (250-275 g) in doses of 0.40 g by means of a stomach tube. Each animal was restrained on a wire rack to prevent any movement of the posterior portion of the body and urine was collected for 48 hours in 12-hour increments. After 48 hours the animals were sacrificed and their liver and kidneys removed and tested for ^{14}C. No radioactivity could be detected in the pooled liver and kidney samples from all the rats, indicating no absorption of the SCMC. Radioactivity equivalent to 0.17% and 0.10% of the administered SCMC was found in the total 48 hours urine collections of the two groups. However, the SCMC used contained up to 0.34% ^{14}C-tagged sodium glycolate and further tests indicated that the radioactivity measured in the urine could be attributed to this impurity. Therefore, it was concluded that SCMC remains totally unabsorbed while passing through the gastrointestinal tract of the rat (137).

III. Metabolism and Excretion

In vitro experiments by Letzig indicate that while saliva, pepsin, and pancreatin do not produce enzymatic changes in methyl cellulose, Diastase puriss (an enzyme preparation from the Merck Co.) and Filtragol (an enzyme preparation obtained from certain mildew fungi produce an enzymatic cleavage of the compound, as evidenced by reduction of viscosity (271).

In a digestibility study, Ziegelmayer et al. found that when Cellugel (SCMC) is fed to rats at a dietary level of 14.29%, 98.8% of the Cellugel is excreted unchanged in the feces; at a dietary level of 10%, 96.3% is excreted in the feces; and at a level of 5%, 100% is excreted. When rabbits were given Cellugel at dietary levels of 9.09 and 4.79%, they were found to excrete 47.85 and 57.4% of the compound in their feces. In human subjects receiving dietary levels of 2.46-5.91% Cellugel, approximately 90% of the ingested SCMC was recovered from the feces unchanged. It was suggested that the digestion of the Cellugel by the rabbits was the result of bacterial action and that rats and man lack the bacteria needed for this process (483).

After 5 rats were fed 5 g of SCMC in one dose, collectively, collection and examination of the feces revealed that 90% of the SCMC had been passed through unchanged (399).

Fecal recoveries of radioactivity from 4 rats fed ^{14}C-labelled microcystalline cellulose at 10 and 20% of the diet ranged from 96 to 104%. No radioactivity appeared in the urine. No evidence of degradation of digestion was noted (21).

After feeding a total of 110 g of SCMC to each of two female dogs over a period of 6 days, Massatsch and Steudel found that all of the substance was excreted unchanged in the feces (294).

Man

Knight et al. fed Methocel 65HG to 25 normal (23 male and 2 female) adults in three graduated doses of 0.6 to 8.9 grams one week apart. After each dose, stool samples were collected and combined at intervals of 24 hours up to 96 hours and the amount of Methocel was determined in each. Essentially all (97%) of the ingested dose was eliminated in the feces within 96 hours. The major portion of the dose was eliminated between zero and 72 hours. The side effects were minor and mild laxative or constipating effects were noted in several cases (244).

In 10 experiments, by Machle et al., in which 2 men and one 10-year-old girl were given single oral doses of 5-10 g of methyl cellulose, analysis of the feces indicated that essentially all of the MC passed through the digestive tract unaltered. They found that the formation of formic acid, following the ingestion of MC is negligible (285).

Following administration to one human subject of 150 g of ^{14}C-labelled microcrystalline cellulose in two portions on one day and 150 g unlabelled microcrystalline cellulose daily for the subsequent 10 days, no radioactivity appeared in the urine or in the expired CO_2. All administered radioactivity was recovered from the feces within 2 days (22).

Upon examination of the stools of 1 male and 1 female patient given 30 g microcrystalline cellulose as dry flour or gel for 5.5 weeks, Tusing et al. confirmed the presence of undegraded material of the same birefringence as the original microcrystalline cellulose administered. No significant effects on the human gastrointestinal tract were noted during the administration (442).

IV. Effects on Enzymes and Other Biochemical Parameters

In vitro experiments by Mizrah and Moore indicate that SCMC can support rapid growth of hematopoietic cells in an agitated culture. SCMC was not metabolized by the cells. Lower rates of glucose utilization and lactic acid production were observed in the presence of SCMC and the uptake of glucose by the cells decreased as the SCMC concentration in the medium was increased. On the basis of these results, it was suggested that SCMC probably protects the cells against physical stress in suspended cultures (317).

Ziegelmayer et al. found that ingestion of 20-30 g of SCMC daily by 3 human subjects resulted in a depression in the digestion of protein, while a slight increase occurred in the digestion of fat. This effect was also noted in rats and rabbits (483).

V. Drug Interaction

CMC is frequently added to clinically used penicillin preparations. It has been found that this may result in accumulation of penicilloyl-CMC conjugates in these preparations. According to Schneider et al., these conjugates are potentially dangerous to allergic patients since they could act as elicitors of penicilloyl-specific anaphylaxis (391).

Seager found that nitrofurantoin, an insoluble urinary antiseptic, is excreted more slowly when suspended in a methyl cellulose solution then when suspended in water. After an overnight fast, nine men were given orally 0.5% nitrofurantoin (1.5 mg/kg) in a water suspention and nine men received 0.5% nitrofurantoin (1.5 mg/kg) in a 5.0% methyl cellulose (15 mg/kg) solution. Urine samples showed that the concentration of the nitrofurantoin rose less rapidly and the peak was delayed by 1 hour for the methyl cellulose suspention (394).

On the basis of clinical and electrocardiographic studies, Feinblatt et al. (124) reported that the simultaneous administration of carboxymethyl cellulose minimizes the acute toxicity of digitoxin.

VI. Consumer Exposure Information

The cellulose derivatives are used as substitutes for natural gums, since the desired degree of viscosity can be controlled. They find use as stabilizers and emulsifiers for various dairy and imitation dairy products, desserts, beverages, and salad dressings, and as a film-forming coating for meat, fish and other products. Cellulose and derivatives are also used as bulking agents in baked goods and diet foods.

The following tables were compiled from data submitted by user firms. Food consumption values for each food category were derived from the Market Research Corporation of America (MRCA) data on frequency of eating and from the USDA data on mean portion size of foods in each food category. The food consumption values thus derived were coupled with the usage level data obtained in the surveys to calculate the daily intake of each substance.

Table 2 reports the usage of alginates in regular foods. There was no report of their use in infant formulas or baby foods. Table 11 reports the annual poundage data for the various alginates. Table 13 reports the possible daily intake per food category and total dietary based on food consumption by total sample. Tables 14 and 15 report potential daily intakes in mg of NAS Appendix A substances (Groups I and II) (table 14) and additional NAS and FEMA substances (Group IV) (table 15) per food category reported, based on food consumption by eaters only.

TABLE 2 -- USAGE LEVELS REPORTED FOR NAS APPENDIX A SUBSTANCES (GROUP I) USED IN REGULAR FOODS(R)

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO.	NAME	# FIRMS REPORTING	*** USUAL USE *** WTD MEAN, %	*** MAXIMUM USE *** WTD MEAN, %
CARBOXYMETHYL CELLULOSE NAS 0263 FEMA 2239	01	BAKED GOODS(R)	*	.11985	.30150
	03	OTHER GRAIN(R)	*	.00800	.00830
	05	MILK PRODS(R)	*	.02000	.03500
	06	CHEESE(R)	*	.40000	.40000
	07	FROZN DAIRY(R)	8	.01497	.03746
	08	PROCSD FRUT(R)	*	.07929	.07938
	10	MEAT PRODS(R)	*	.05600	.12659
	11	POULTRY(R)	*	.36000	********
	14	PROCSD VEGS(R)	*	.07240	.49000
	17	CONF FROST(R)	*	.25000	.25000
	19	SWEET SAUCE(R)	*	.07000	********
	20	GELATIN PUD(R)	*	.20000	1.00000
	23	BEV TYPE 1(R)	8	.01237	.02158
	27	GRAVIES(R)	*	.22312	.45719
	49	MISC UNCLAS(R)	*	.80000	.80000
CELLULOSE NAS 0265	01	BAKED GOODS(R)	*	4.83354	29.00125
	04	FATS OILS(R)	*	3.00000	4.50000
	05	MILK PRODS(R)	*	.25000	.38609
	06	CHEESE(R)	*	1.00000	1.00000
	07	FROZN DAIRY(R)	*	.40000	.85000
	10	MEAT PRODS(R)	*	.94312	1.40704
	11	POULTRY(R)	*	2.00000	3.00000
	13	FISH PRODS(R)	*	2.50000	3.00000
	14	PROCSD VEGS(R)	*	.00062	********
	17	CONF FROST(R)	*	.40000	.67500
CELLULOSE NAS 0265	19	SWEET SAUCE(R)	*	.90000	1.03024
	20	GELATIN PUD(R)	*	.75000	1.00000
	21	SOUPS(R)	*	.35000	.75000
	25	NUT PRODS(R)	*	.54000	.54000
	27	GRAVIES(R)	*	.75000	1.00000
	28	IMIT DAIRY(R)	*	.52395	1.25000

TABLE 2 -- USAGE LEVELS REPORTED FOR NAS APPENDIX A SUBSTANCES (GROUP I) USED IN REGULAR FOODS(R)

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO.	NAME	# FIRMS REPORTING	*** USUAL USE *** WTD MEAN, %	*** MAXIMUM USE *** WTD MEAN, %
METHYLCELLULOSE NAS 0127 FEMA 2696	01	BAKED GOODS(R)	4	.14831	.35766
	03	OTHER GRAIN(R)	*	.75000	1.00000
	04	FATS OILS(R)	*	.06860	.12020
	07	FROZN DAIRY(R)	4	.41189	.46206
	10	MEAT PRODS(R)	*	.04536	.07482
	11	POULTRY(R)	*	.01250	.02500
	14	PROCSD VEGS(R)	*	.20000	.66510
	16	SOFT CANDY(R)	*	.00111	.00261
METHYLCELLULOSE (CONT.) NAS 0127 FEMA 2696	19	SWEET SAUCE(R)	*	.00045	.00110
	23	BEV TYPE I(R)	*	.00480	.01050
	25	NUT PRODS(R)	*	1.00000	1.00000
	27	GRAVIES(R)	*	.00100	.00250
	28	IMIT DAIRY(R)	*	.15000	.20000
	31	CHEWING GUM(R)	*	2.20280	2.20280
SODIUM CHC NAS 0186	01	BAKED GOODS(R)	10	.21416	.30919
	03	OTHER GRAIN(R)	*	.07345	.07655
	04	FATS OILS(R)	*	.59656	.78063
	05	MILK PRODS(R)	5	.05275	.36072
	06	CHEESE(R)	*	.07720	.08324
	07	FROZN DAIRY(R)	7	.09205	.17479
	08	PROCSD FRUT(R)	6	.05742	.07591
	09	FRUIT ICES(R)	4	.06669	.11538
	10	MEAT PRODS(R)	*	.00800	.00850
	11	POULTRY(R)	*	.20000	.35000
	12	EGG PRODS(R)	*	.06250	.06250
	14	PROCSD VEGS(R)	*	.00561	.02825
	16	SOFT CANDY(R)	*	.10300	.10300
	17	CONF FROST(R)	*	.13650	.15675
	18	JAM JELLY(R)	*	.49699	.49699
	19	SWEET SAUCE(R)	*	.50645	1.00051
	20	GELATIN PUD(R)	4	.05999	.06003
	21	SOUPS(R)	*	.01000	.02000
	23	BEV TYPE I(R)	6	.07090	.08746
	27	GRAVIES(R)	8	.52571	.52813
	28	IMIT DAIRY(R)	4	.22894	.46800

TABLE II, PART A — ANNUAL POUNDAGE DATA FOR NAS APPENDIX A SUBSTANCES (GROUPS I & II)

SUBSTANCE NAME (SURVEY NO.)	# REPORTS TO NAS 1960/1970	POUNDAGE REPORTED TO NAS (MATCHING REPORTS FOR BOTH YEARS) 1960	1970	TOTAL 1970 POUNDAGE REPORTED TO NAS	# REPORTS TO FEMA	POUNDAGE REPORTED TO FEMA-- 1970 ONLY	TOTAL 1970 POUNDAGE NAS + FEMA
CARBOXYMETHYL CELLULOSE NAS 0263 FEMA 2239	4/ 12	40,000	538,452	590,237	22	69,307	659,544
CELLULOSE NAS 0265	7/ 10	33	468,814	578,854			578,854
METHYLCELLULOSE NAS 0127 FEMA 2696	9/ 11	46,050	99,215	104,565	7	13,824	118,389
SODIUM CMC NAS 0186	34/ 44	3,084,300	2,442,619.	3,727,594			3,727,594

TABLE 14

Food Category	Substance	# of Firms	Age	Average	High A	High B	Very High
Baked Goods	Cellulose	*	0-5 mo	749.199	2479.606	4495.194	14877.641
			6-11 mo	1387.226	2634.279	8323.359	15805.681
			12-23 mo	2653.613	4340.519	15921.686	26043.123
			2-65 yr	6636.450	9855.588	39818.716	59133.549
Fats, Oils	Cellulose	*	0-5 mo	147.000	417.000	220.500	625.500
			6-11 mo	123.000	249.000	184.500	373.500
			12-23 mo	195.000	363.000	292.500	544.500
			2-65 yr	534.000	954.000	801.000	1431.000
Milk Products	Cellulose	*	0-5 mo	130.500	283.250	201.539	437.440
			6-11 mo	328.250	999.000	506.936	1542.816
			12-23 mo	211.000	768.750	325.860	1187.227
			2-65 yr	142.000	388.500	219.299	599.984
Cheese	Cellulose	*	0-5 mo	7.000	12.000	7.000	12.000
			6-11 mo	87.000	163.000	87.000	163.000
			12-23 mo	115.000	249.000	115.000	249.000
			2-65 yr	130.000	271.000	130.000	271.000
Frozen Dairy	Cellulose	*	0-5 mo	28.000	64.800	59.500	137.700
			6-11 mo	61.200	123.200	130.050	261.800
			12-23 mo	73.600	146.800	156.400	311.950
			2-65 yr	134.800	274.400	286.450	583.100
Meat Products	Cellulose	*	0-5 mo	102.800	257.472	153.367	384.122
			6-11 mo	283.879	577.189	423.519	861.108
			12-23 mo	298.969	495.138	446.032	738.696
			2-65 yr	746.951	1229.828	1114.376	1834.780
Poultry	Cellulose	*	0-5 mo	200.000	500.000	300.000	750.000
			6-11 mo	218.000	412.000	327.000	618.000
			12-23 mo	256.000	436.000	384.000	654.000
			2-65 yr	408.000	784.000	612.000	1176.000
Fish Products	Cellulose	*	0-5 mo	80.000	147.500	96.000	177.000
			6-11 mo	102.500	192.500	123.000	231.000
			12-23 mo	220.000	392.500	264.000	471.000
			2-65 yr	472.500	897.500	567.000	1077.000

TABLE 14

Food Category	Substance	# of Firms	Age	Average	High A	High B	Very High
Procsd Vegs	Cellulose	*	0-5 mo	.051	.097	---	---
			6-11 mo	.187	.364	---	---
			12-23 mo	.250	.407	---	---
			2-65 yr	.530	.889	---	---
Conf Frost	Cellulose	*	0-5 mo	.800	.800	1.350	1.350
			6-11 mo	3.200	8.800	5.400	14.850
			12-23 mo	10.400	22.400	17.550	37.800
			2-65 yr	13.600	24.800	22.950	41.850
Sweet Sauce	Cellulose	*	0-5 mo	73.800	189.000	84.480	216.350
			6-11 mo	22.500	44.100	25.756	50.482
			12-23 mo	43.200	77.400	49.451	88.601
			2-65 yr	110.700	219.600	126.719	251.378
Soups	Cellulose	*	0-5 mo	14.700	24.500	31.500	52.500
			6-11 mo	169.050	392.000	362.250	840.000
			12-23 mo	173.600	377.650	372.000	809.250
			2-65 yr	177.100	373.450	379.500	800.250
Gravies	Cellulose	*	0-5 mo	8.250	15.750	11.000	21.000
			6-11 mo	23.250	44.250	31.000	59.000
			12-23 mo	52.500	105.000	70.000	140.000
			2-65 yr	99.75	198.000	133.000	264.000
Imit. Dairy	Cellulose	*	0-5 mo	.000	.000	.000	.000
			6-11 mo	86.976	109.506	207.500	261.250
			12-23 mo	43.488	78.593	103.750	187.500
			2-65 yr	30.389	89.072	72.500	212.500
Gellatin Pud	Cellulose	*	0-5 mo	176.250	541.500	235.000	722.000
			6-11 mo	171.750	358.500	229.000	478.000
			12-23 mo	150.750	311.250	201.000	415.000
			2-65 yr	231.750	469.500	309.000	626.000
Nut Prods	Cellulose	*	0-5 mo	2.160	2.700	2.160	2.700
			6-11 mo	59.940	129.900	59.940	129.900
			12-23 mo	31.320	72.900	31.320	72.900
			2-65 yr	58.860	127.980	58.860	127.980

TABLE 14

Food Category	Substance	# of Firms	Age	Average	High A	High B	Very High
Baked Goods	Carboxymethyl Cellulose	*	0-5 mo	18.577	61.483	46.733	154.670
			6-11 mo	34.397	65.318	86.531	164.318
			12-23 mo	65.798	107.625	165.524	270.747
			2-65 yr	164.554	244.374	413.960	614.759
Other Grain	Carboxymethyl Cellulose	*	0-5 mo	.712	1.488	.739	1.544
			6-11 mo	1.448	3.320	1.502	3.445
			12-23 mo	1.592	3.176	1.652	3.295
			2-65 yr	2.736	5.384	2.839	5.586
Milk Prods	Carboxymethyl Cellulose	*	0-5 mo	10.440	22.660	18.270	39.655
			6-11 mo	26.260	79.920	45.955	139.860
			12-23 mo	16.880	61.500	29.540	107.625
			2-65 yr	11.360	31.080	19.880	54.390
Cheese	Carboxymethyl Cellulose	*	0-5 mo	2.800	4.800	2.800	4.800
			6-11 mo	34.800	65.200	34.800	65.200
			12-23 mo	46.000	99.600	46.000	99.600
			2-65 yr	52.000	108.400	52.000	108.400
Frozen Dairy	Carboxymethyl Cellulose	8	0-5 mo	1.048	2.425	2.622	6.069
			6-11 mo	2.290	4.611	5.731	11.538
			12-23 mo	2.754	5.494	6.893	13.748
			2-65 yr	5.045	10.269	12.624	25.698
Procsd Fruit	Carboxymethyl Cellulose	*	0-5 mo	20.060	51.380	20.083	51.438
			6-11 mo	53.124	117.508	53.185	117.641
			12-23 mo	93.086	169.046	93.192	169.238
			2-65 yr	102.126	203.696	102.241	203.927
Meat Prods	Carboxymethyl Cellulose	*	0-5 mo	6.104	15.288	13.798	34.559
			6-11 mo	16.856	34.272	38.104	77.473
			12-23 mo	17.752	29.400	40.129	66.460
			2-65 yr	44.352	73.024	100.259	165.073
Poultry	Carboxymethyl Cellulose	*	0-5 mo	36.000	90.000	---	---
			6-11 mo	39.240	74.160	---	---
			12-23 mo	46.080	78.480	---	---
			2-65 yr	73.440	141.120	---	---

TABLE 14

Food Category	Substance	# of Firms	Age	Average	High A	High B	Very High
Procsd Vegs	Carboxymethyl Cellulose	*	0-5 mo	5.937	11.367	40.180	76.930
			6-11 mo	21.865	42.499	147.980	287.630
			12-23 mo	29.250	47.567	197.960	321.930
Conf Frost	Carboxymethyl Cellulose	*	0-5 mo	.500	.500	.500	.500
			6-11 mo	2.000	5.500	2.000	5.500
			12-23 mo	6.500	14.000	6.500	14.000
			2-65 yr	8.500	15.500	8.500	15.500
Sweet Sauce	Carboxymethyl Cellulose	*	0-5 mo	5.740	14.700	---	---
			6-11 mo	1.750	3.430	---	---
			12-23 mo	3.360	6.020	---	---
			2-65 yr	8.610	17.080	---	---
Bev Type I	Carboxymethyl Cellulose	*	0-5 mo	4.033	16.749	7.035	29.219
			6-11 mo	5.826	13.038	10.164	22.745
			12-23 mo	10.044	23.095	17.523	40.290
			2-65 yr	17.603	39.448	30.708	68.819
Gelatin Pud	Carboxymethyl Cellulose	*	0-5 mo	47.000	144.400	235.000	722.000
			6-11 mo	45.800	95.600	229.000	478.000
			12-23 mo	40.200	83.000	201.000	415.000
			2-65 yr	61.800	125.200	309.000	626.000
Gravies	Carboxymethyl Cellulose	*	0-5 mo	2.454	4.686	4.029	9.601
			6-11 mo	6.917	13.164	14.173	26.974
			12-23 mo	15.618	31.237	32.003	64.007
			2-65 yr	29.675	58.904	60.806	120.698
Baked Goods	Sodium CMC	10	0-5 mo	33.195	109.864	47.924	158.614
			6-11 mo	61.464	116.717	88.738	168.509
			12-23 mo	117.574	192.316	169.745	277.653
			2-65 yr	294.042	436.672	424.518	630.438
Other Grain	Sodium CMC	*	0-5 mo	6.537	13.662	6.813	14.238
			6-11 mo	13.294	30.482	13.856	31.768
			12-23 mo	14.617	29.160	15.233	30.390
			2-65 yr	25.120	49.432	26.180	51.518

TABLE 14

Food Category	Substance	# of Firms	Age	Average	High A	High B	Very High
Fats, Oils	Sodium CMC	*	0-5 mo	29.231	82.922	38.251	108.508
			6-11 mo	24.459	49.514	32.006	64.792
			12-23 mo	38.776	72.184	50.741	94.456
			2-65 yr	106.188	189.706	138.952	248.240
Milk Prods	Sodium CMC	5	0-5 mo	27.536	59.766	188.296	408.696
			6-11 mo	69.261	210.289	473.625	1441.437
			12-23 mo	44.521	162.206	304.448	1109.214
			2-65 yr	29.962	81.974	204.889	560.559
Cheese	Sodium CMC	*	0-5 mo	.540	.926	.583	.999
			6-11 mo	6.716	12.584	7.242	13.568
			12-23 mo	8.878	19.223	9.573	20.727
			2-65 yr	10.036	20.921	10.821	22.558
Frozen Dairy	Sodium CMC	7	0-5 mo	6.444	14.912	12.235	28.316
			6-11 mo	14.084	28.351	26.743	53.835
			12-23 mo	16.937	33.782	32.161	64.148
			2-65 yr	31.021	63.146	58.904	119.906
Procsd Fruit	Sodium CMC	*	0-5 mo	14.527	37.208	19.205	48.190
			6-11 mo	38.096	85.096	50.860	112.499
			12-23 mo	67.411	122.419	89.118	161.840
			2-65 yr	73.957	147.512	97.772	195.013
Fruit Ices	Sodium CMC	4	0-5 mo	.000	.000	.000	.000
			6-11 mo	3.335	7.203	5.769	12.461
			12-23 mo	4.402	9.203	7.615	15.922
			2-65 yr	7.669	15.939	13.269	27.576
Meat Prods	Sodium CMC	*	0-5 mo	.872	2.184	.927	2.321
			6-11 mo	2.408	4.896	2.559	5.202
			12-23 mo	2.536	4.200	2.695	4.463
			2-65 yr	6.336	10.432	6.732	11.084
Egg Prods	Sodium CMC	*	0-5 mo	1.500	2.063	1.500	2.063
			6-11 mo	2.750	4.625	2.750	4.625
			12-23 mo	3.560	7.438	3.563	7.438
			2-65 yr	5.438	9.938	5.438	9.938

TABLE 14

Food Category	Substance	# of Firms	Age	Average	High A	High B	Very High
Procsd Vegs	Sodium CMC	*	0-5 mo	.460	.881	2.317	4.435
			6-11 mo	1.694	3.293	8.532	16.583
			12-23 mo	2.266	3.686	11.413	18.560
			2-65 yr	4.797	8.045	24.154	40.511
Soft Candy	Sodium CMC	*	0-5 mo	5.768	9.682	5.768	9.682
			6-11 mo	7.725	17.510	7.725	17.510
			12-23 mo	7.622	16.480	7.622	16.480
			2-65 yr	11.742	25.441	11.742	25.441
Conf Frost	Sodium CMC	*	0-5 mo	.273	.273	.317	.317
			6-11 mo	1.092	3.003	1.270	3.493
			12-23 mo	3.549	7.644	4.128	8.890
			2-65 yr	4.641	8.463	5.398	9.843
Jam, Jelly	Sodium CMC	*	0-5 mo	3.479	4.970	3.479	4.970
			6-11 mo	90.949	203.766	90.949	203.766
			12-23 mo	28.825	64.112	28.825	64.112
			2-65 yr	54.669	125.738	54.669	125.738
Gelatin Pud	Sodium CMC	4	0-5 mo	14.098	43.313	14.107	43.312
			6-11 mo	13.738	28.675	13.747	28.694
			12-23 mo	12.058	24.896	12.066	24.912
			2-65 yr	18.537	37.554*	18.549	37.579
Soups	Sodium CMC	*	0-5 mo	.420	.700	.840	1.400
			6-11 mo	4.830	11.200	9.660	22.400
			12-23 mo	4.960	10.790	9.920	21.580
			2-65 yr	5.060	10.670	10.120	21.340
Gravies	Sodium CMC	8	0-5 mo	5.783	11.040	5.809	11.091
			6-11 mo	11.297	31.017	16.372	31.160
			12-23 mo	36.800	73.599	36.969	73.938
			2-65 yr	69.919	138.787	70.241	139.426
Imit. Dairy	Sodium CMC	4	0-5 mo	.000	.000	.000	.000
			6-11 mo	38.004	47.848	77.688	97.812
			12-23 mo	19.002	34.341	38.844	70.200
			2-65 yr	13.279	38.920	27.144	79.560

TABLE 14

Food Category	Substance	# of Firms	Age	Average	High A	High B	Very High
Baked Goods	Methyl Cellulose	4	0-5 mo	22.988	76.083	55.437	183.480
			6-11 mo	42.565	80.829	102.648	194.925
			12-23 mo	81.422	133.182	196.355	321.179
			2-65 yr	203.630	302.404	491.067	729.269
Other Grain	Methyl Cellulose	*	0-5 mo	66.750	139.500	89.000	186.000
			6-11 mo	135.750	311.250	181.000	415.000
			12-23 mo	149.250	297.750	199.000	397.000
			2-65 yr	256.500	504.750	342.000	673.00
Fats, Oils	Methyl Cellulose	*	0-5 mo	3.361	9.535	5.890	16.708
			6-11 mo	2.813	5.694	4.928	9.977
			12-23 mo	4.459	8.301	7.813	14.544
			2-65 yr	12.211	21.815	21.396	38.224
Frozen Dairy	Methyl Cellulose	4	0-5 mo	28.832	66.726	32.344	74.854
			6-11 mo	63.019	126.862	70.695	142.314
			12-23 mo	75.788	151.164	85.019	169.576
			2-65 yr	138.807	282.557	155.714	316.973
Meat Prods	Methyl Cellulose	*	0-5 mo	4.944	12.383	8.155	20.426
			6-11 mo	13.653	27.760	22.521	45.780
			12-23 mo	14.380	23.814	23.718	39.281
			2-65 yr	35.925	59.149	59.257	97.565
Procsd Vegs	Methyl Cellulose	*	0-5 mo	16.400	31.400	54.538	104.420
			6-11 mo	60.400	117.400	200.860	390.414
			12-23 mo	80.800	131.400	268.700	436.971
			2-65 yr	171.000	286.800	568.660	953.753
Soft Candy	Methyl Cellulose	*	0-5 mo	.062	.104	.146	.245
			6-11 mo	.083	.189	.196	.444
			12-23 mo	.082	.178	.193	.418
			2-65 yr	.127	.274	.298	.645
Sweet Sauce	Methyl Cellulose	*	0-5 mo	.037	.094	.090	.231
			6-11 mo	.113	.022	.028	.054
			12-23 mo	.022	.039	.053	.095
			2-65 yr	.055	.110	.135	.268

TABLE 14

Food Category	Substance	# of Firms	Age	Average	High A	High B	Very High
Bev Type I	Methyl Cellulose	*	0-5 mo	1.565	6.499	3.423	14.217
			6-11 mo	2.261	5.059	4.946	11.067
			12-23 mo	3.897	8.912	8.526	19.604
			2-65 yr	6.830	15.307	14.942	33.485
Nut Prods	Methyl Cellulose	*	0-5 mo	4.000	5.000	4.000	5.000
			6-11 mo	111.000	235.000	111.000	235.000
			12-23 mo	58.000	135.000	58.000	135.000
			2-65 yr	109.000	237.000	109.000	237.000
Gravies	Methyl Cellulose	*	0-5 mo	.011	.021	.028	.053
			6-11 mo	.031	.059	.078	.148
			12-23 mo	.070	.140	.175	.350
			2-65 yr	.133	.264	.333	.666
Imit. Dairy	Methyl Cellulose	*	0-5 mo	.000	.000	.000	.000
			6-11 mo	24.900	31.350	33.200	41.800
			12-23 mo	12.450	22.500	16.600	30.000
			2-65 yr	8.700	25.500	11.600	34.000
Chewimg Gum	Methyl Cellulose	*	0-5 mo	---	---	---	---
			6-11 mo	17.622	30.839	17.622	30.839
			12-23 mo	13.217	28.636	13.217	28.636
			2-65 yr	15.420	33.042	15.420	33.042

TABLE 15

Food Category	Substance	# of Firms	Age	Average	High A	High B	Very High
Meat Prods	Cellulose, Micro-Crystalline	*	0-5 mo	10.246	25.662	10.246	25.662
			6-11 mo	28.294	57.528	28.294	57.528
			12-23 mo	29.798	49.350	29.798	49.350
			2-65 yr	74.448	122.576	74.448	122.576
Hard Candy	Ethyl Cellulose	*	0-5 mo	---	---	---	---
			6-11 mo	.001	.001	.001	.002
			12-23 mo	.002	.005	.003	.005
			2-65 yr	.006	.012	.006	.001
Chewing Gum	Ethyl Cellulose	*	0-5 mo	---	---	---	---
			6-11 mo	.204	.356	.299	.524
			12-23 mo	.153	.331	.224	.486
			2-65 yr	.178	.382	.262	.561

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EFFECT OF INGESTION OF AVICEL-CONTAINED FOODS ON LIVING ORGANISMS

(January 1966)

Yoshitoshi Internal Seminar
Medical Department
Tokyo University, Tokyo

and

Biochemical Department
Toranomon Hospital, Tokyo

Asahi Chemical Industry Co., Ltd.

Effect of Ingestion of Avicel-contained Foods on Living Body

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INTRODUCTION

Cellulose has long been known to be useful as the excipient for cakes and tabloid preparations, but its consumption for this purpose has been very small.

Where it is desired to control the calorie of staple food, low-caloric food is made by mixing cellulose in bread, noodle and the like. This practice has been recognized to be effective for the cure of certain diseases: In this case, the quantity of cellulose itself to be ingested becomes considerably large. The authors carried out some extent of observations on the possible effect which foods containing Avicel, a nonfibrous microcrystalline cellulose would have when ingested on the process of digestion and assimilation; the assimilation of vitamin B_1, the chemical composition of urine and blood, the subjects' body weight, and the intestional bacterial flora, in an effort to determine whether or not the oral ingestion of this cellulose will cause troubles to human body.

As the Avicel-contained food, mostly a piece of bread (containing 10 g of Avicel) was given to supplement each meal, three times daily, to effect daily administration of 30 g of Avicel per subject.

On a total of 8 healthy males of age range of 18 to 34, the above-mentioned observations were made several times each before, during the after the oral administration of Avicel.

All the subjects took Avicel-added bread without any particular complaint.

1. Effect of Avicel-contained food products on digestion and assimilation test

Before and during the supplemental dietary intake by 8 healthy males of Avicel-added bread, D-xylose test and ^{131}I-triolein test were carried out repeatedly with a view to studying the effect which Avicel may manifest on the assimilation test on these substances.

Methods

(1) D-xylose test

As the control test, a piece of bread having the same weight as the Avicel-added bread was ingested together with 25 g of D-xylose and 360 ml milk by each subject at an early hour of the day, when his stomach was empty. Blood was collected at 30th, 60th, 90th, 120th, 180th and 240th minutes and all the urine eliminated within 5 hours was gathered, the blood and the urine being subjected to the determination proposed by Roe & Rice et al to find the xylose concentration in the blood and the xylose content in the urine. On the first day of the diet phase of 2 weeks' duration and after the termination of the 2 weeks' diet phase, 25 g of xylose and 360 ml of milk were given simultaneously with a piece of Avicel-added bread (similar in quality and weight to the bread used for the control test excepting its containing Avicel, each piece weighing 80 g, having 160 cal., and containing about 10 g of Avicel), to study the effect of the first intake of Avicel and the effect of continuous intake of Avicel on the assimilation. In one week after the termination of diet phase on Avicel-added bread, the same test was carried out, this time by using Avicel-free bread to study the quantity of xylose eliminated in the urine collected within 5 hours of ingestions.

Results:

1) Quantity of xylose eliminated in urine collected within 5 hours of ingestion

The test results for different diet phases are as follows:

No. 1: Prior to ingestion of Avicel-added bread
7.43 g/5 hours on the average

No. 2: After the first ingestion of Avicel-added bread
4.52 g/5 hours on the average

No. 3: After 2 weeks' ingestion of Avicel-added bread
3.8 g/5 hours on the average

No. 4: After one week's control diet following 2 weeks' ingestion of Avicel-added bread
5.5 g/5 hours on the average

The preceding results suggest presence of significant difference between No. 1 and No. 2 and between No. 1 and No. 3 on 1% level of significance, and between No. 1 and No. 4 and between No. 3 and No. 4 on 5% level of significance.

ii) Concentration of xylose in blood

As shown in the diagram, the xylose concentration in the blood showed, in more cases, generally flat curves as compared with those obtained during the ingestion of control diet. Considering that the concentration in the blood is affected largely by the velocity of discharge from the stomach, however, it is judged difficult to find any definite interpretation from these results.

(2) ^{131}I-triolein test:

As the control diet, each subject received one piece of ordinary bread having the same weight as Avicel-added bread, one ^{131}I-triolein capsule (containing 50 μc of ^{131}I per capsule), 0.5 ml/Kg of peanut oil and 180 ml of milk. From each subject, blood was collected at one hour interval between the 3rd and the 7th hours, and 2.0 ml of blood plasma was obtained from the blood. All the urine discharged during the 72 hours following the completion of the dietary test was gathered, and homogenized. Then 5.0 ml each of the homogenized urine was place in 3 tubes, and measured on a scintillation counter for radioactivity. Based on the values of radioactivity thus measured, the radioactivity of ^{131}I in the plasma and the discharge of ^{131}I into the urine against the total quantity ingested were studied. The same absorption test was carried out after the first ingestion of Avicel-added bread and after the completion of 2 weeks' continuous ingestion of Avicel-added bread.

In the test, the circulated quantity of blood plasma was assumed to be 7.2% of the body weight. Throughout the entire test, no marked change of body weight was recognized in any subject.

Results:

i) Discharge rate of ^{131}I in the urine over 72-hour period:

The following results were obtained for the test listed correspondingly.

No. 1: Before ingestion of Avicel-added bread
2.67%/72 hours on the average
No. 2: After the first ingestion of Avicel-added bread
2.2%/72 hours on the average
No. 3: After 2 weeks' ingestion of Avicel-added bread
2.7%/72 hours on the average

The preceding results suggest absence of significance difference between No. 1 and No. 2 and between No. 1 and No. 3.

ii) Radioactivity within blood plasma

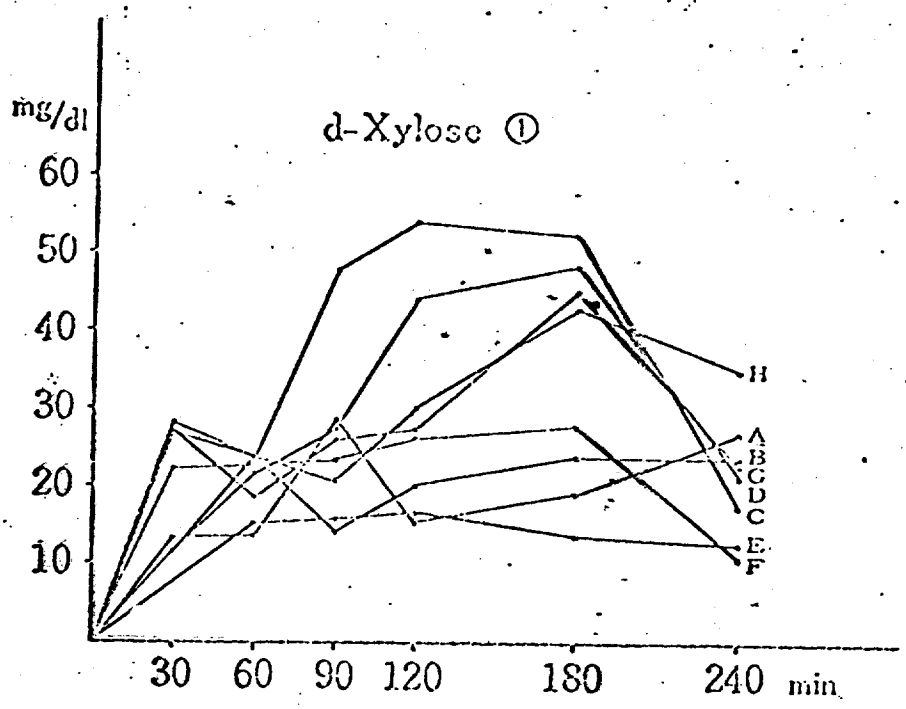
As shown in the diagram, addition of Avicel manifested substantially no effect.

Conclusion

i) As a result of the ingestion of Avicel-added bread, the 5-hour discharge quantity of xylose showed a clear decline. Although no definite conclusion can be made of its cause, adsorption by Avicel can be inferred as one contributive factor. If the 24-hour discharge quantity had been measured, no change would have been detected. That is to say, addition of Avicel might have caused a delay in the absorption. In the present study, however, no further investigation was conducted on this point.

ii) Ingestion of Avicel-added bread had no effect in ^{131}I-triolein test.

Tsuneo Jindate
Yoshitoshi Internal Seminar
Tokyo University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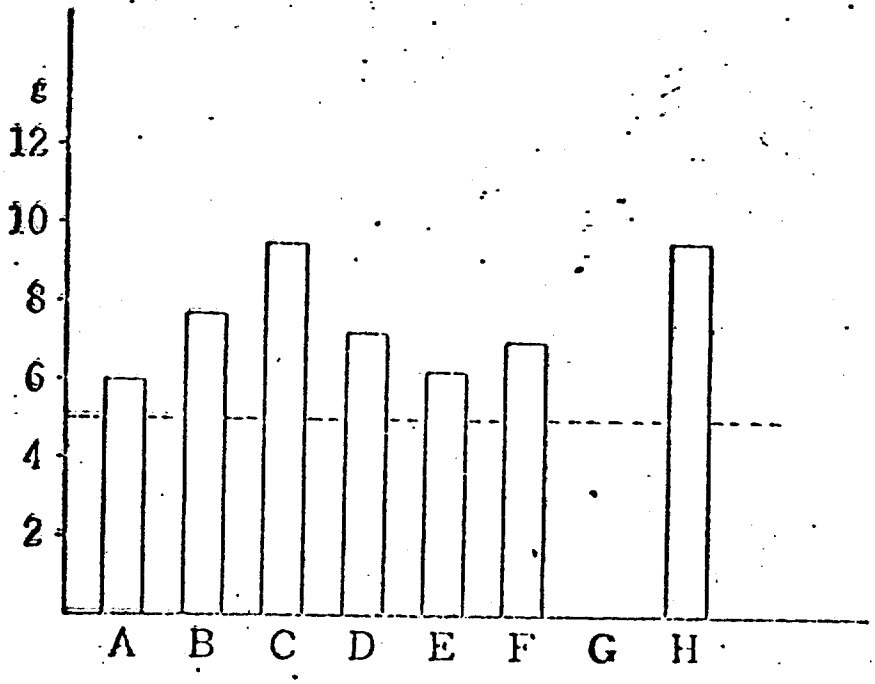
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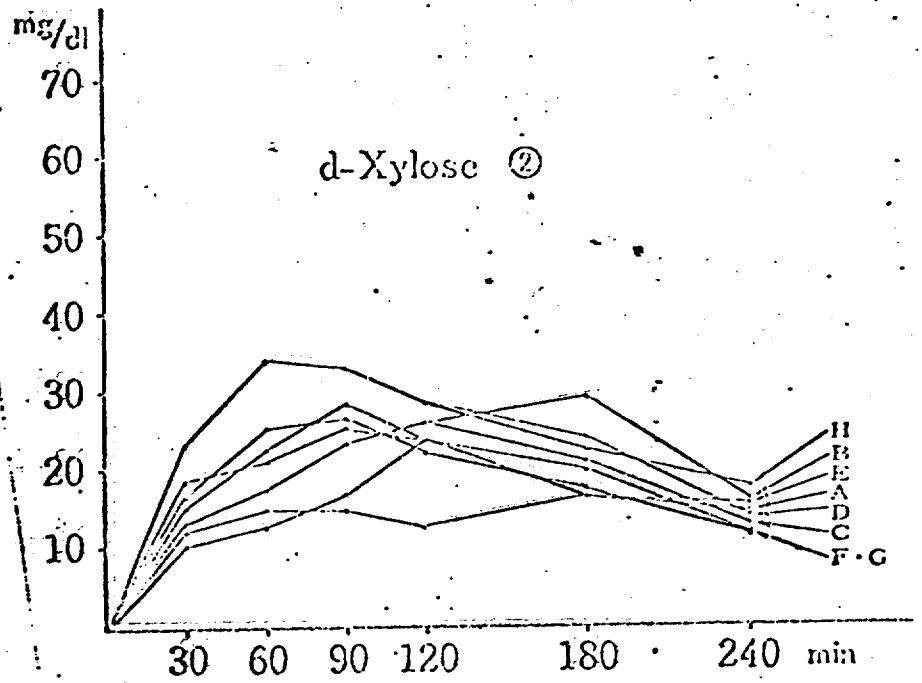
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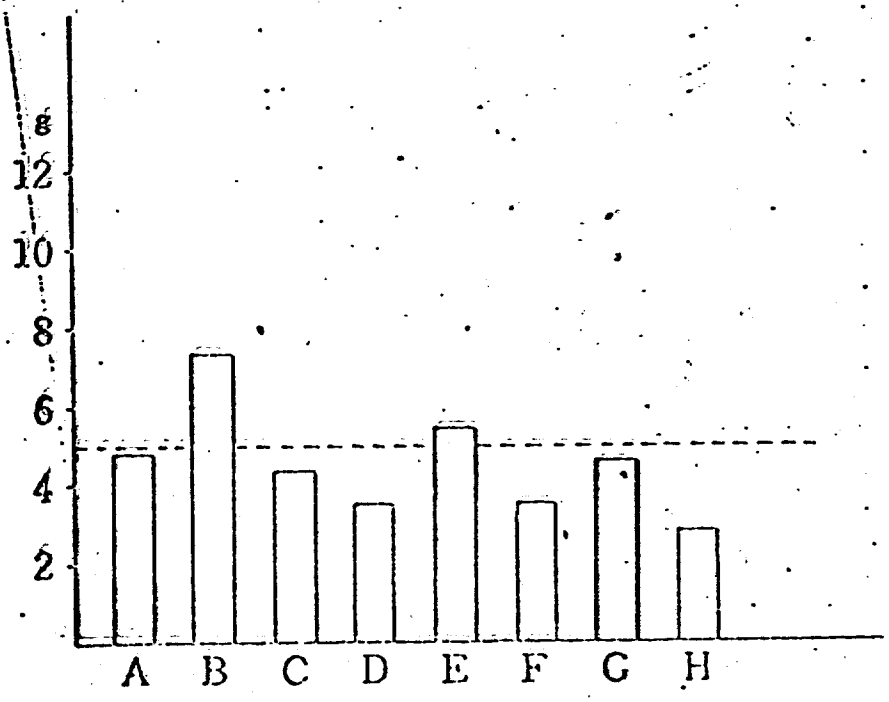
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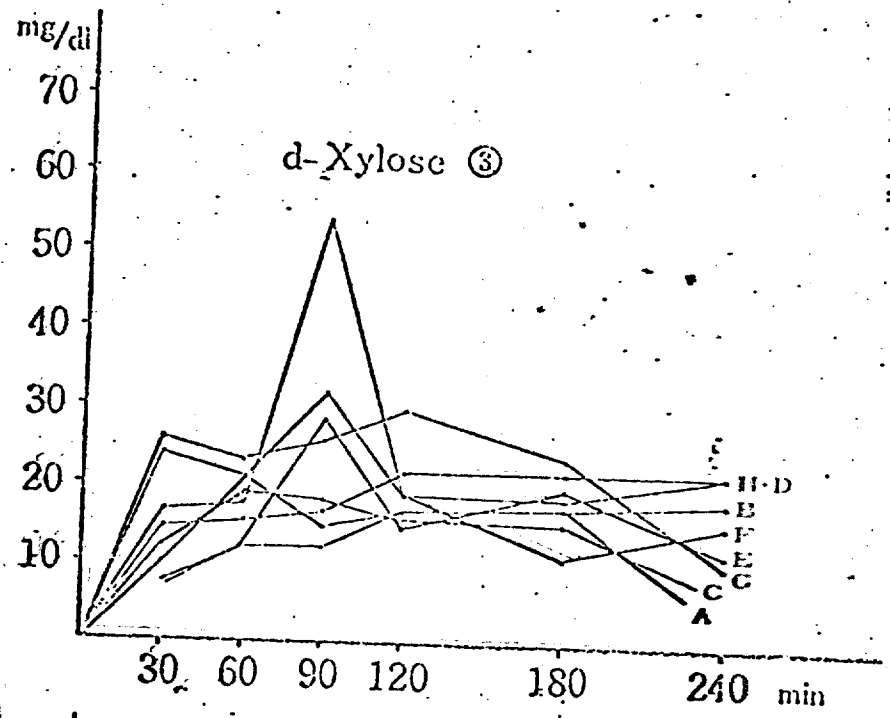
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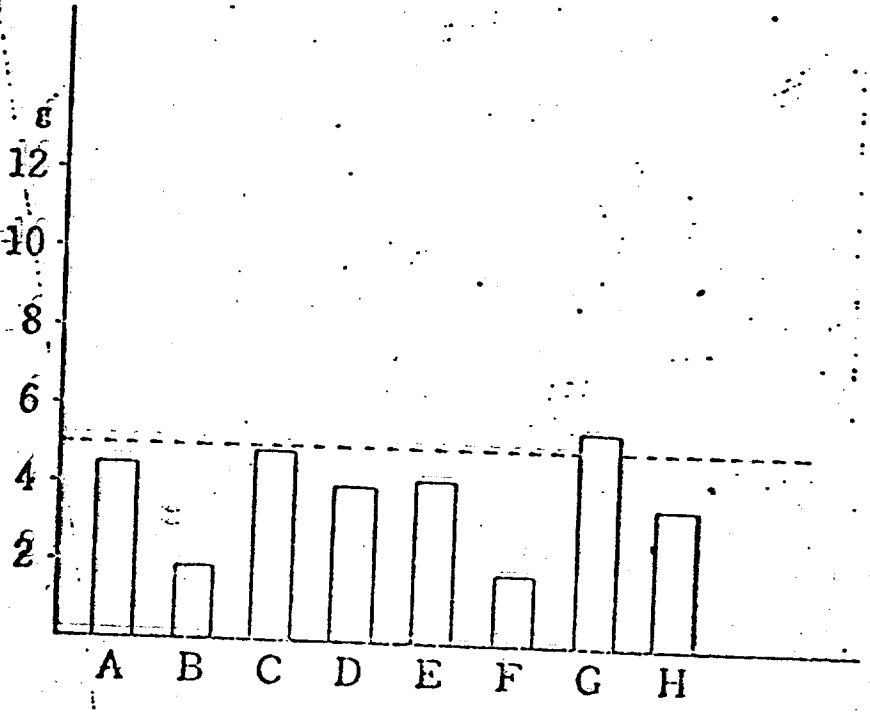
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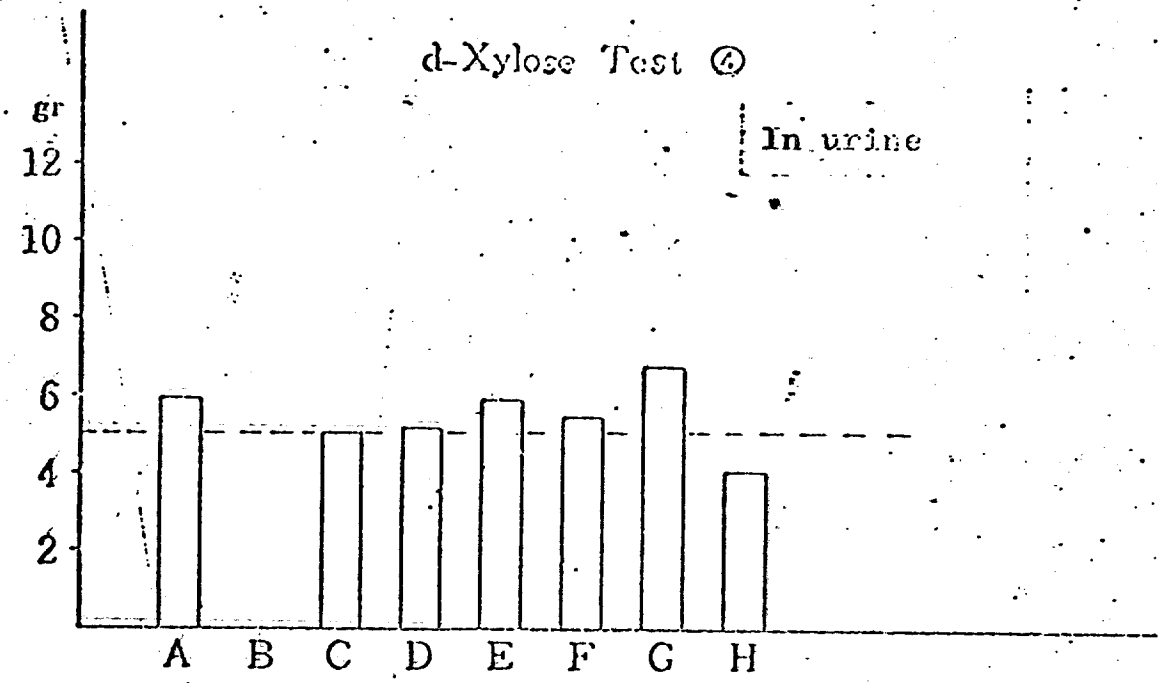

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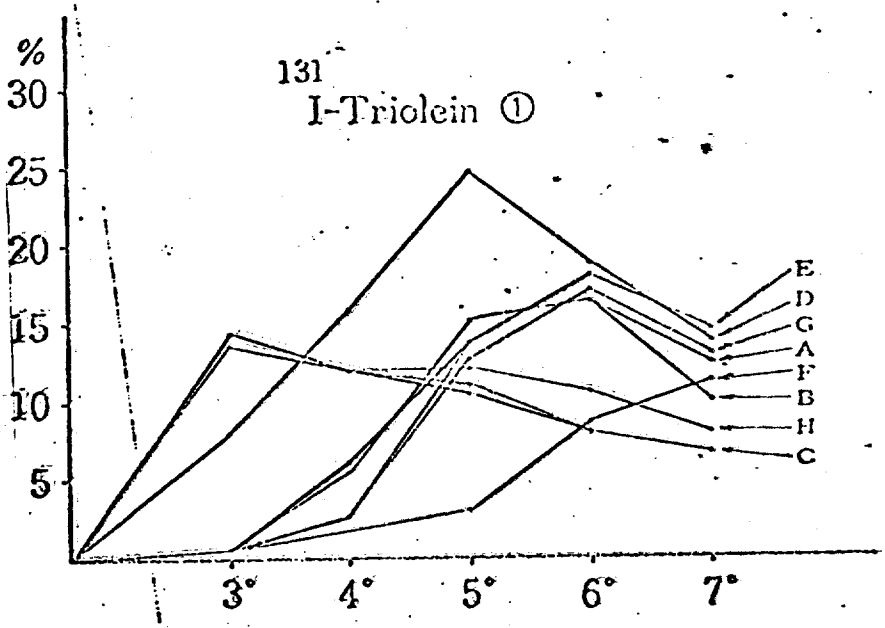
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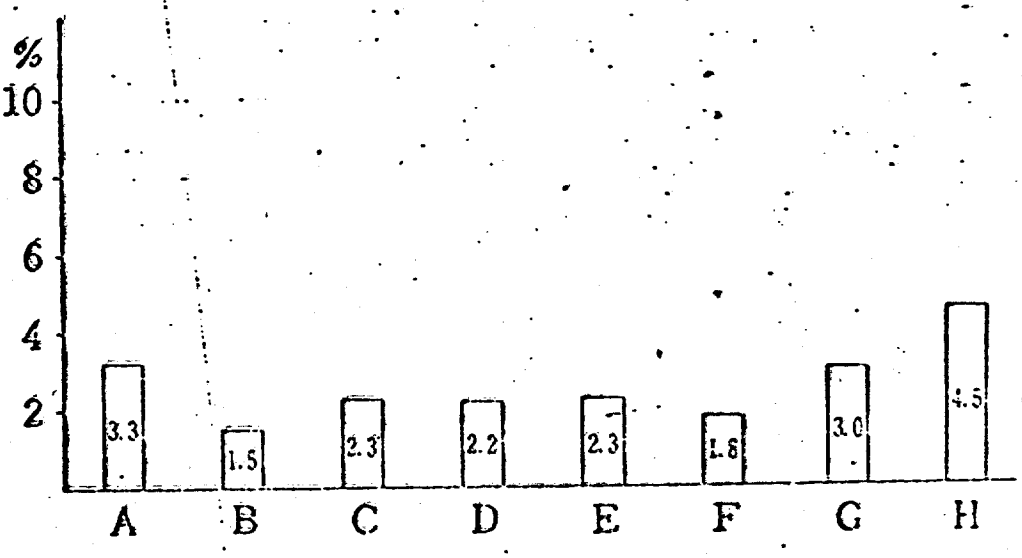
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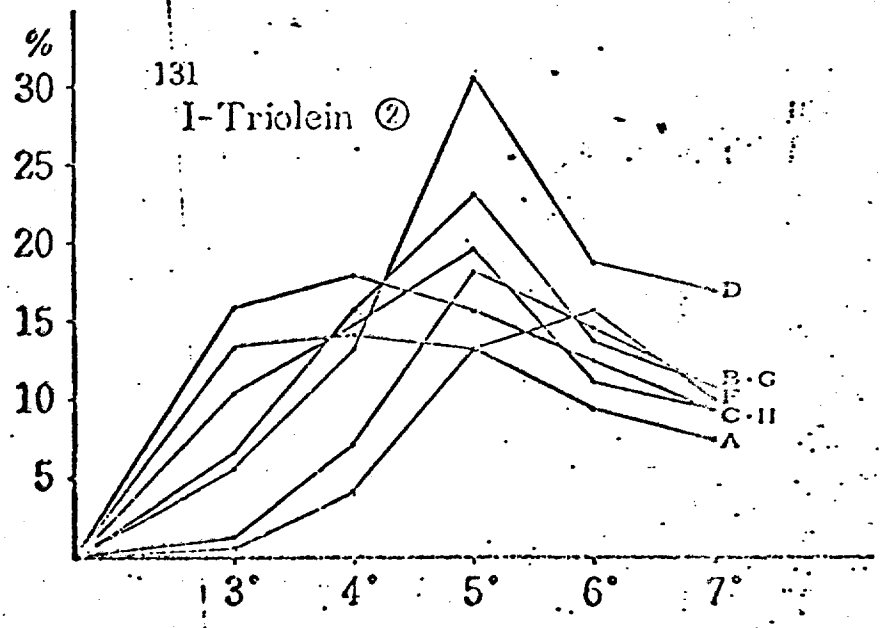
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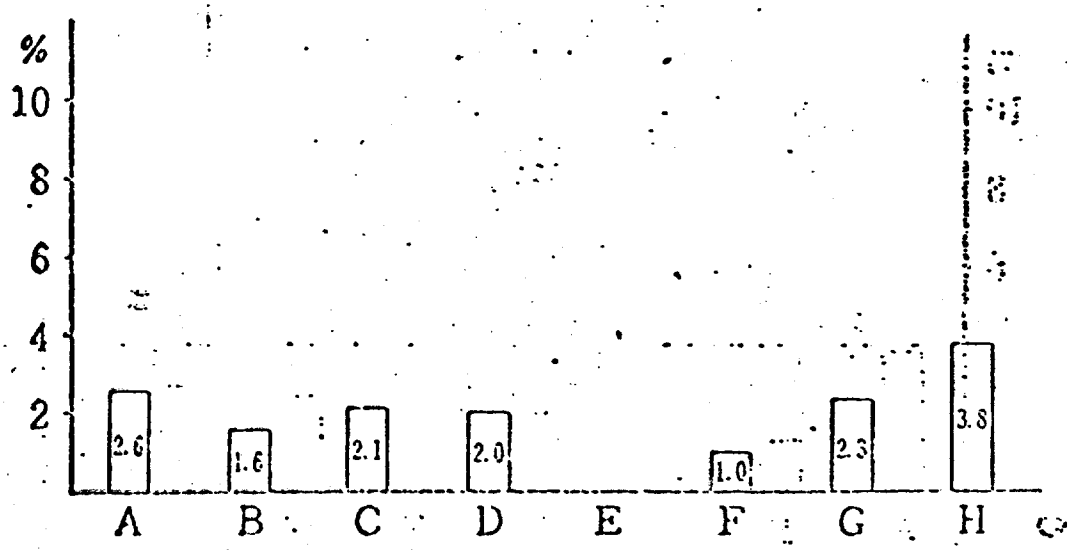
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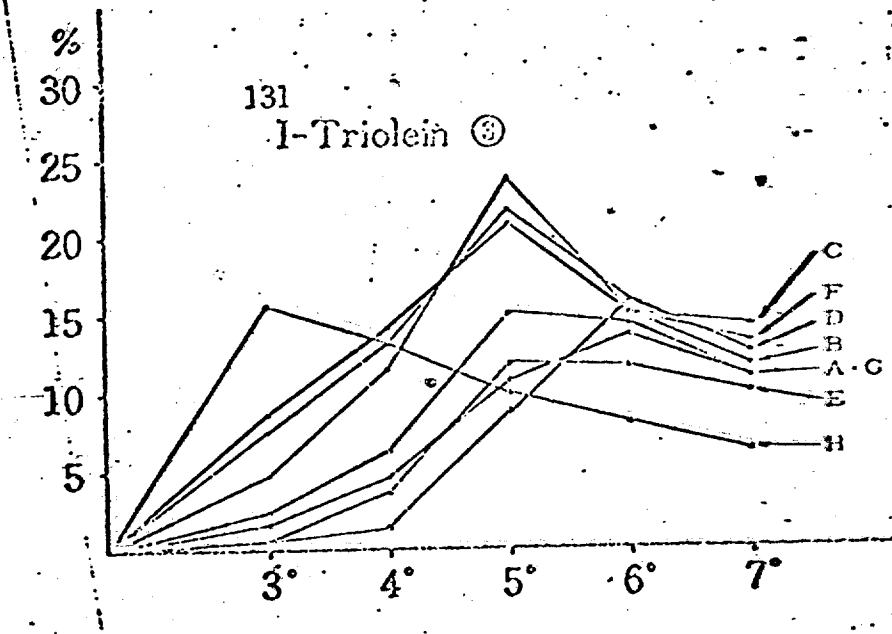
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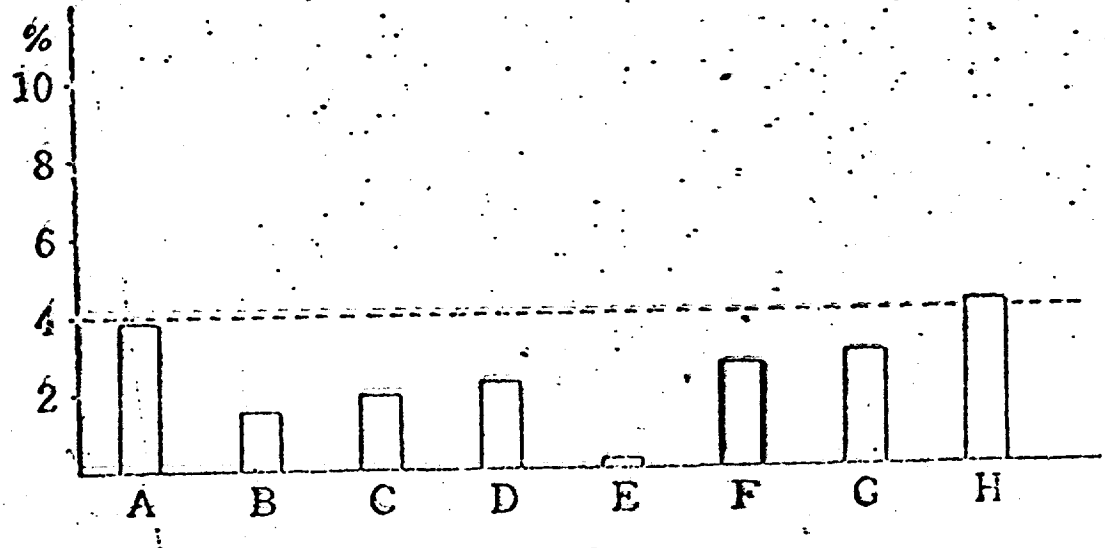
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2. Effect of Avicel-contained food products on vitamin B1 assimilation

Microcrystalline cellulose (Avicel) is composed, to about 80% of its total volume, of minute particles below 200 meshes in size and, therefore, shows rich adsorbability. So, it seems necessary to investigate preliminarily whether the cellulose ingested orally entails the danger of obstructing the assimilation through the intestinal canal of a trace of nutrient, particularly vitamins. Incidentally, an observation has been reported that when albino rats were fed on cellulose-added feed, vitamin B1 content of their excretions increased. As the possible cause for this phenomenon, there has been cited increase of B1 synthesis due to intestinal bacteria. So, the authors fed Avicel-added feed to albino rats and Avicel-added bread to human subjects, and followed them as to the possible influence of Avicel on the intestinal assimilation of B1.

(I) Avicel administration to albino rats:

Experiment

Male albino rats each weighing about 70 g were divided into 3 subgroups (each consisting of 5 heads). To the control subgroup, synthetic feed composed of casein, starch, soybean oil, liver oil, and mixed salts was fed as the basic feed. To the 2nd and the 3rd subgroups, feeds retaining the same ratio of mixed salts only and incorporating Avicel by concentrations of 5% and 10% respectively were fed to be taken freely. As to vitamin B complex, 40 mg of vitamin B1 and sufficient amounts of other vitamin members were administered per head daily. The three subgroups were fed in this way for 8 weeks and were followed as to such developments as quantity of feed ingested, weight curve, and B1 discharge in the urea and feces. In the post-dietary phase, they were further followed as to the peripheral blood picture, total serum protein, protein demarcation picture, total plasma cholesterol, and B1 concentrations in blood and principal organs. The determination of B1 was based on the thiochrome fluorescence method (Fujiwara).

Except in the initial stage of dietary feeding, no clear difference was recognized in the quantity of feed ingested among the control subgroup, 5% Avicel subgroup, and 10% Avicel sub[illegible]

Table 1. Composition of synthetic feed for albino rats

Basic feed		*Salt mixture		Water-soluble vitamin	
Refined casein	20%	NaCl	20.2	B_1	40 g
Starch	68	$MgSO_4 \cdot 7H_2O$	63.5	B_2	40
Soybean oil	5.5	K_2HPO_4	111.5	B_6	50
Liver oil	2.5	CaCl acetate	28.7	Nicotinic acid	200
*Salt mixture	4	$Na_2HPO_4 \cdot 12H_2O$	52.6	Pantothenic acid	200
These ingredients were added with about 4/3 volume of water, heated, and fed in the form of dumpling.		$Ca_2H_2(PO_4)_2 \cdot H_2O$	111.6	Choline chloride	900
		Fe citrate	13.8	Inositol	180
				B_{12}	0.024
				These ingredients were ingested with use of a pipet.	

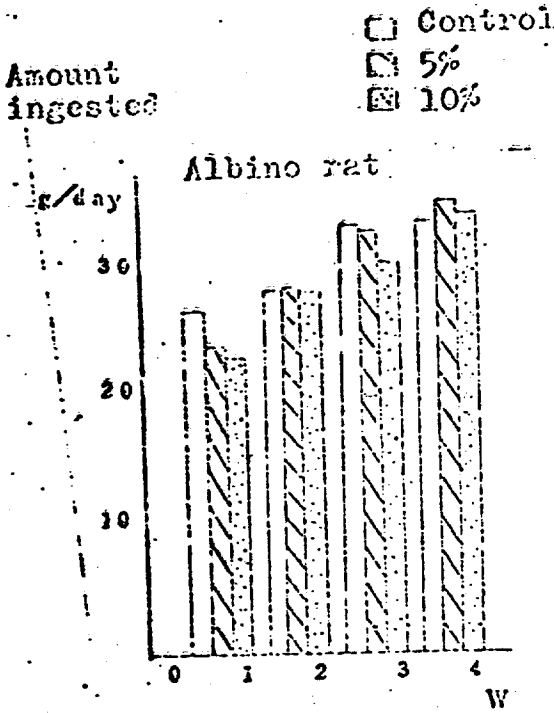


Fig. 1

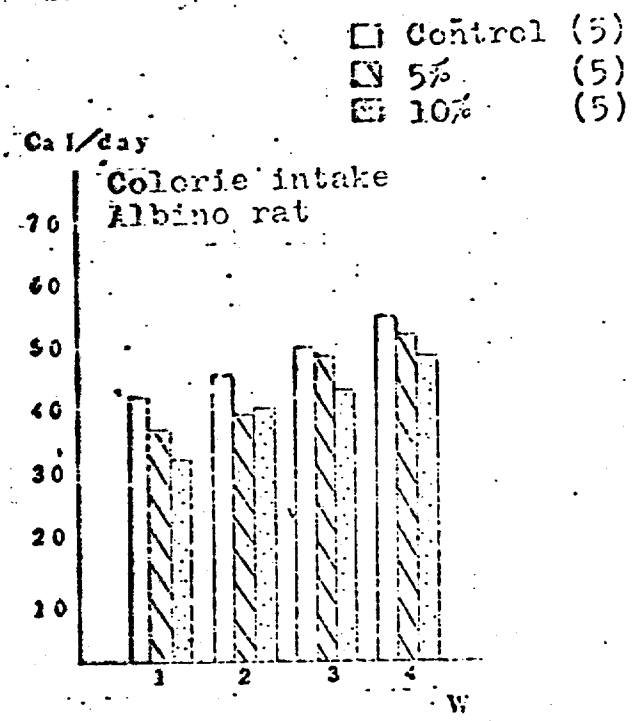


Fig. 2

The average caroly intake for the first through fourth weeks was 45.8 cal for the control subgroup, 41.6 cal for the 5% Avicel subgroup and 39.1 cal for the 10% Avicel subgroup respectively. Thus, the purpose of reducing caroly intake could be achieved in the 10% Avicel subgroup merely by allowing individual rats of this subgroup to take the feed as they pleased.

The body weight increase in these albino rats during the first through fourth weeks averaged at 3.8 g/day for the control subgroup, 3.6 g/day for the 5% Avicel subgroup, and 2.9 g/day for the 10% Avicel subgroup. The increase was slightly less in the 10% Avicel subgroup than in the other subgroups. Comparison of the 8-week averages for the three subgroups indicated substantially the same trend.

After 8 weeks of dietary test, the rats were sacrificed for the observation of peripheral blood picture (amount of blood pigment, number of red-blood corpuscle, number of white-blood corpuscle, total plasma cholesterol, total serum protein, and its demarcation picture), with no significant difference recognized among the three subgroups.

Growth Curve

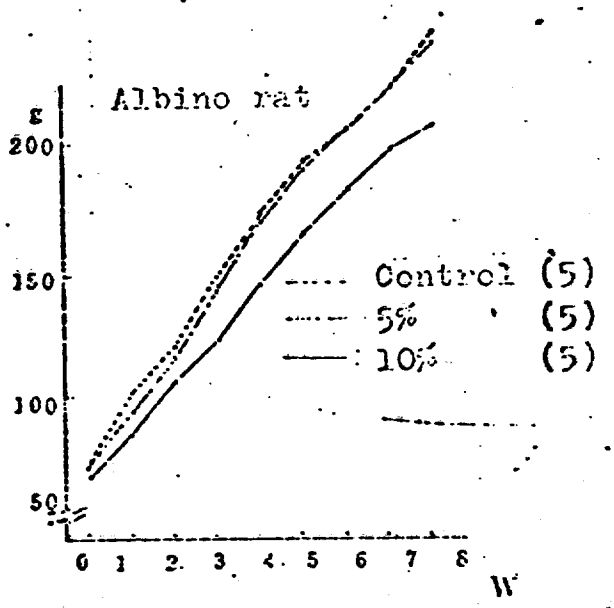


Fig. 3

Table 2.

	Hb	R	W	TC	TP	Alb	α_1	α_2	β	γ
Control	84 %	690 x 10^4	57 x 10^2	65 mg/dl	6.3 g/dl	48.8	10.8	9.0	20.8	10.6
5% Avicel	83	672	60	69	6.1	45.0	11.2	12.1	17.6	14.1
10% Avicel	82	761	54	67	6.3	52.1	8.8	8.3	17.3	13.5

These rats were also subjected to determination of B_1 concentration in the blood, liver, and heart. (The figures in parentheses are those of free B_1). No significant difference was recognized in any concentration among the three subgroups.

Table 3. Vitamin B_1 concentration in blood and organs

	Blood	Liver	Heart
Control	23.1γ (2.5%)	223.4γ (36.5%)	225.2γ (13.3%)
5% Avicel	23.0 (9.8)	180.9 (37.9)	218.7 (15.8)
10% Avicel	24.5 (8.4)	247.0 (51.8)	232.0 (30.8)

All the values given in the table are averages for the corresponding subgroups. The discharge of B_1 in the urine was observed throughout the duration of dietary feeding, with no marked difference recognized among the three subgroups.

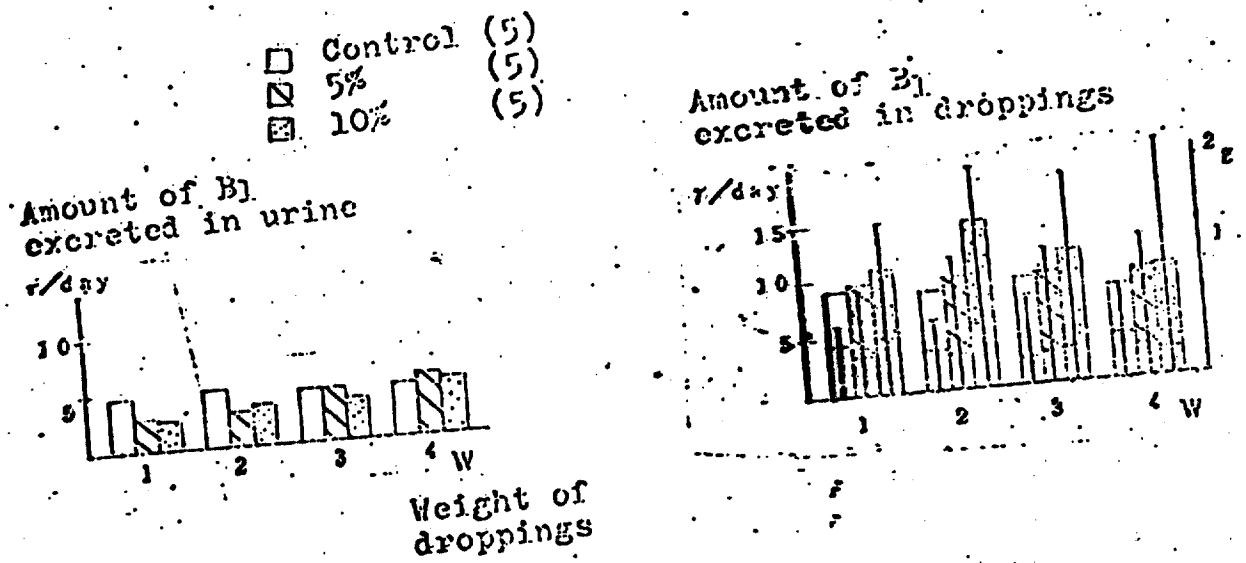


Fig. 4

The discharge of B_1 in feces increased to reach the peak in the second week and thereafter returned to the original level in the 10% Avicel subgroup, the observation suggesting the change due mainly to the increase of complex B_1. (Thick bar graphs in Fig. 4) By contrast, the amount of feces (Thin bar graphs in Fig. 4) increased to a degree and remained substantially unchanged.

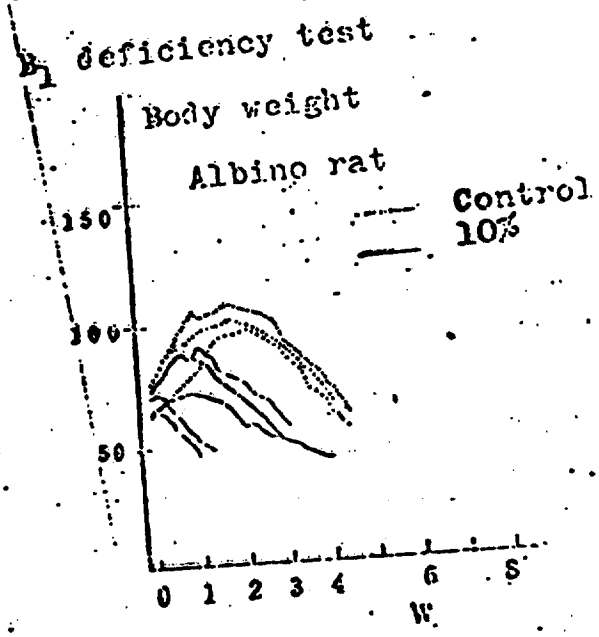


Fig.5

Under the same dietary feeding conditions as used in the preceding experiments, B_1 deficiency test was performed on the control subgroup and 10% Avicel subgroups, which were followed as to body weight curve and development and growth of B_1 deficiency disease. In the control subgroup, the body weight ceased increasing in about 2 weeks and, after four weeks of dietary feeding, declined to substantially the same level as in the initial stage of the feeding. In the 10% Avicel subgroup, the body weight ceased increasing as early as about one week and, in the third week, declined to substantially the same level as in the initial stage. As concerns the development and progress of B_1 deficience disease, no marked difference was recognized between the two subgroups of surviving rats.

(II) Oral administration of Avicel-added bread to human

As the subjects of this experiment, 7 healthy males were selected. Measurement of B_1 concentration in the blood was carried out on each subject while on an empty stomach to confirm that his B_1 concentration was in the normal range. During the period of experiment, use of vitamin-added food and drugs was prohibited.

Vitamin B_1 concentration in blood of subject who received Avicel diet

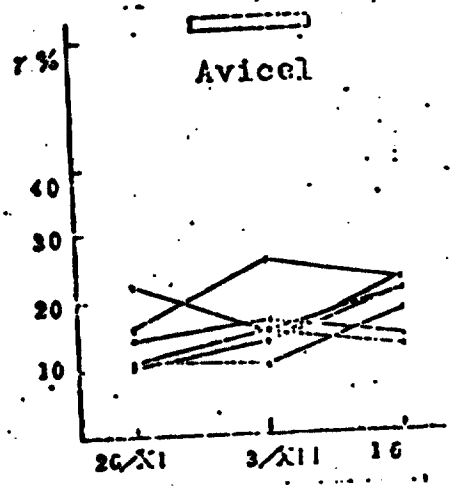


Fig. 6

In the subjects who accepted Avicel-added bread, the B_1 concentration in the blood as determined in early hours while on empty stomach averaged at 14.1 γ%, 16.2 γ% and 19.7 γ% for the predist phase, the diet phase (on the 7th day of diet) and the post diet phase (one week after the conclusion of the diet phase), respectively.

Similarly, the B_1 concentration in the 24-hour urine as determined in the pre-diet phase, during the diet phase (on the 14th and last days of the diet) and in the post-diet phase (one week after the completion of diet) averaged 302 γ/day, 392 γ/day and 333 γ/day respectively.

The B_1 discharge in feces was determined only twice, namely, during the diet phase (the 3rd and fifth days of diet) and in the post diet phase (on the 1st to 3rd days following the completion of diet). The B_1 24-hour discharge in feces (averaged for 3 days) was thus found to be greater in the former phase.

Table 4: Vitamin B_1 discharge in urine of Avicel-administered subjects

	Pre-diet	Avicel diet	Post-diet
No. 1	431 γ/day	565	539
2	373	762	452
3	231	127	157
4	198	258	197
6	201	153	545
7	153	432	255
8	526	447	185
Average	302	392	333

Table 5: Vitamin B_1 discharge in feces of Avicel-administered subjects

	Avicel diet	Post-diet
	γ/day	
No. 1	1336 (8)	854 (72)
2	4510 (2550)	572 (148)
3	1262 (12)	465 (110)
4	3688 (1731)	1276 (14)
6	329 (80)	1550 (1016)
7	8540 (5810)	1793 (862)
8	10560 (5210)	562 (34)
Average	4332 (2200)	1014 (322)

A study of the results of the preceding dietary experiments on both rats and humans has failed to recognize any clear variation on the B_1 discharge in blood, principal organs, and urine attributable to the administration of Avicel. This leads to a conclusion that the ingestion of Avicel has no effect on the assimilation of B_1 through the intestional canal. There is a report that when B_1 is absorbed on acid terra alba and ingested orally by humans, the resulting B_1 discharge in urine is the same as when B_1 crystals are ingested. Another existing report has it that the ingestion of Tween 80, C.M.C. and the like does neither impede the assimilation of B_1 through the intestional canal. The temporary increase of B_1 discharge in feces is principally caused by the increase of B_1 complex and therefore is judged to originate from the increased quantity of B_1 synthesized by the intestional flora. However, because the synthesis of B_1 by the intestional flora takes place mostly within the large intestine, little effect is exerted on B_1 concentration inside the intestional canal at the upper section of the small intestine where intestional ssimilation of B_1 prodeoinates. Therefore, ingestion of B_1 is not concluded to increase the intestional assimilation of B_1 remarkably.

The conclusion is that the ingestion of Avicel does not seem to have any definite influence upon the assimilation of B_1 through the intestional canal, and that in the administration of Avicel-added food to patients requiring the control of caloric intake, there will be no hindrance with respect to the assimilation and metabolism of B_1.

Nagao Shibata
Kazuto Yasuda
Manabu Yamamoto
Yoshitoshi Internal Seminar
Tokyo University

3. Change of intestinal flora due to ingestion of Avicel-added food products

The following experiment was carried out for the purpose of observing the change of intestinal flora due to ingestion of Avicel-added food products.

Procedure of experiment:

To each subject, Avicel-added food (containing 10 g of Avicel per meal) was supplied three times daily. The determination of intestinal flora was performed three times in the pre-diet phase, on the first, sixth and thirteenth days in the diet phase, and on the third and seventh days in the post-diet phase.

By the medium of E.P.B. (Enterococcus Presumptive Broth), the most probable number (M.P.N) of enterococcus was determined to confirm E.C.A. (Enterococcus conformatory agar) and count the number of cells.

G(-) rods were measured for M.P.N. in the B.G.L.B. broth, followed by the identification of microbes and the counting of cell number.

Staphylococcus, fungas, and G(+) rods were measured for the cell number by means of mixed-dilution process, with Staph 110 broth and GS broth diluted as 1/10 solutions.

Results:

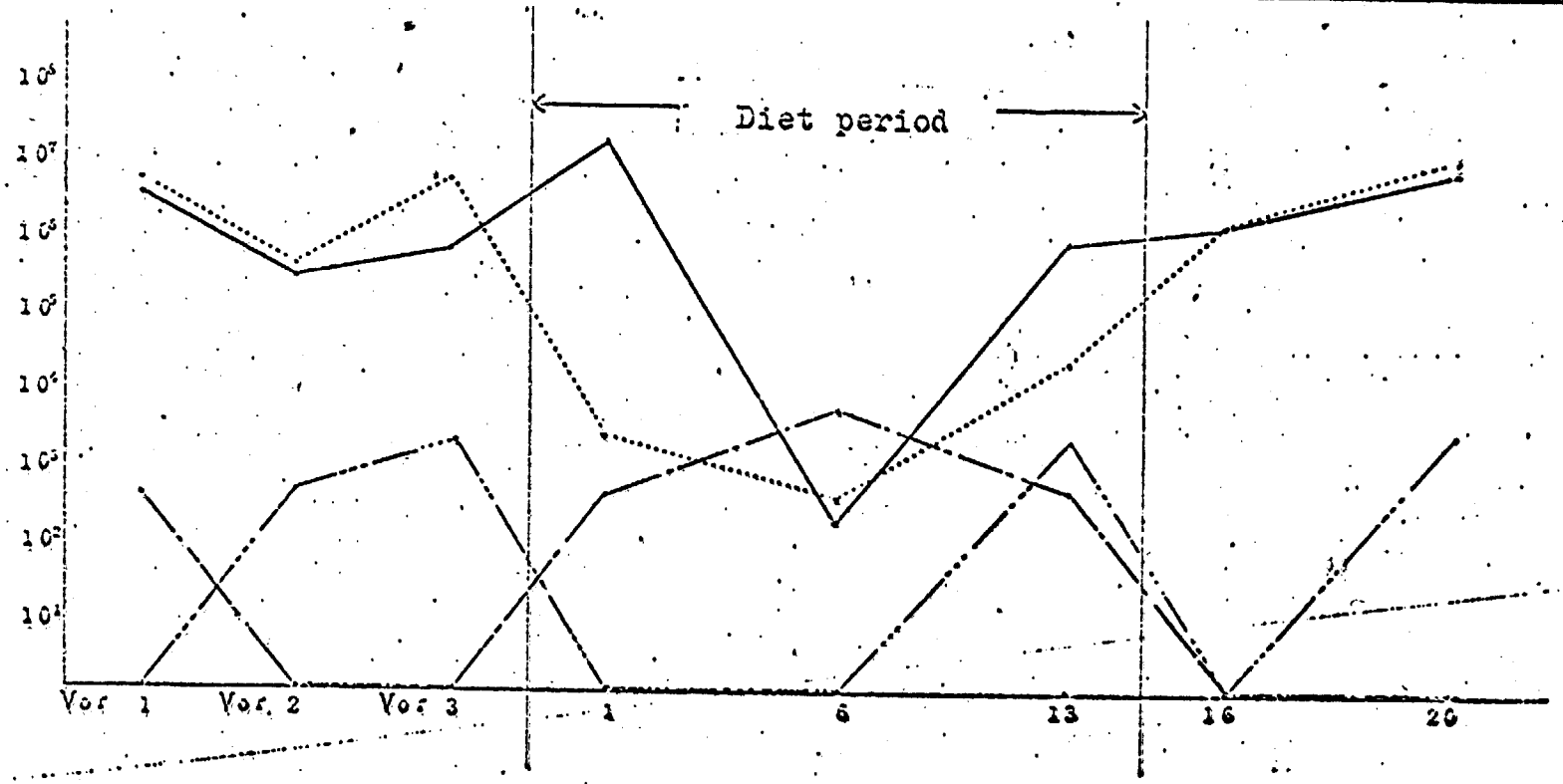
In 8 subjects of this experiment, substantially no change of cell number and intestinal flora was recognized, with the exception of one subject in which was observed a slight decline of G(-) rods and Enterococcus. From these results, it is concluded that ingestion of Avicel-added food products has virtually no effect on the intestinal flora.

In the diet phase of Avicel-added food, no conspicuous change was recognized even by unaided inspection, except that loose passage was suffered by 3 subjects.

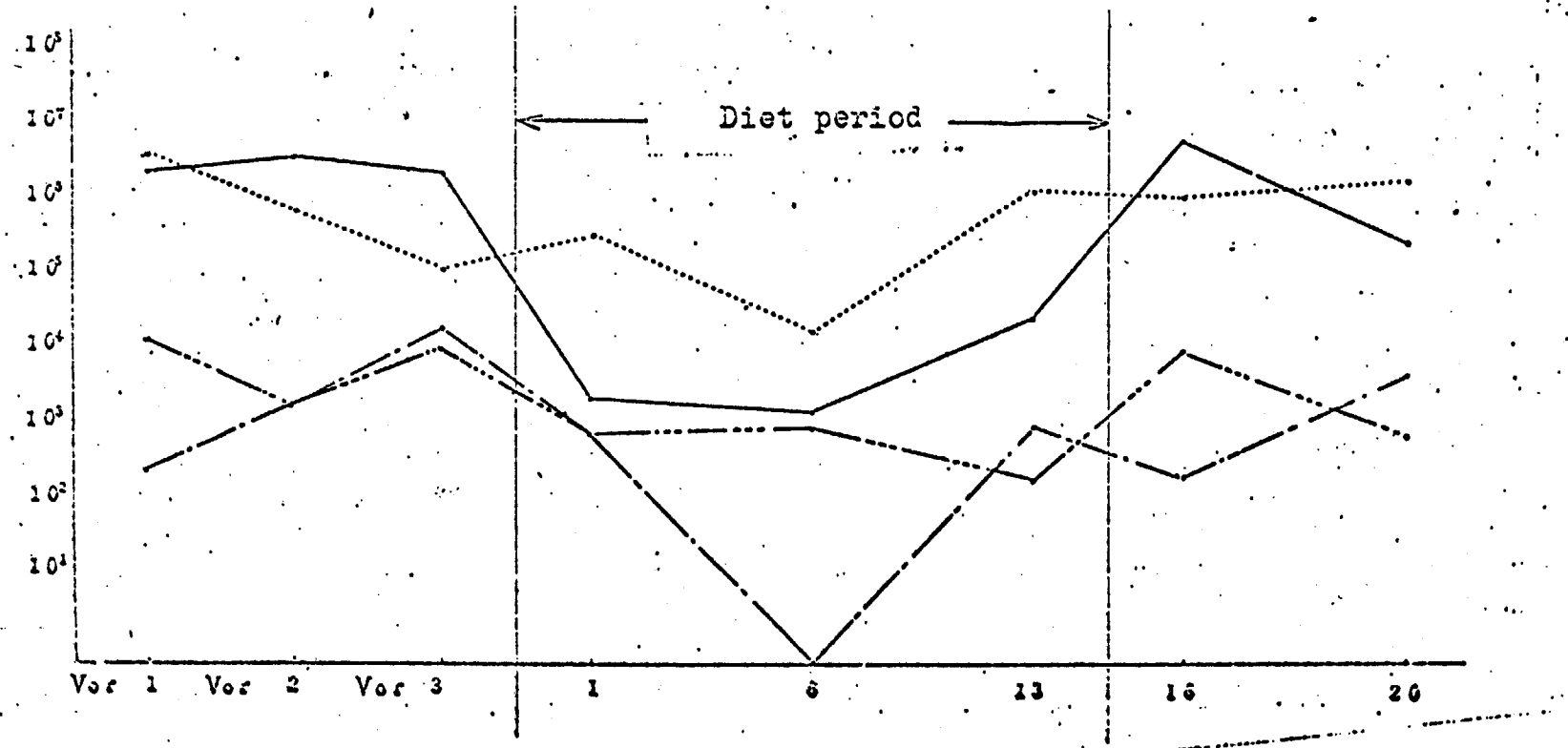
These results lead to a conclusion that the ingestion of Avicel-added food has no effect upon the intestinal flora.

Kihachiro Shimizu
Yoshitoshi Internal Seminar
Tokyo University

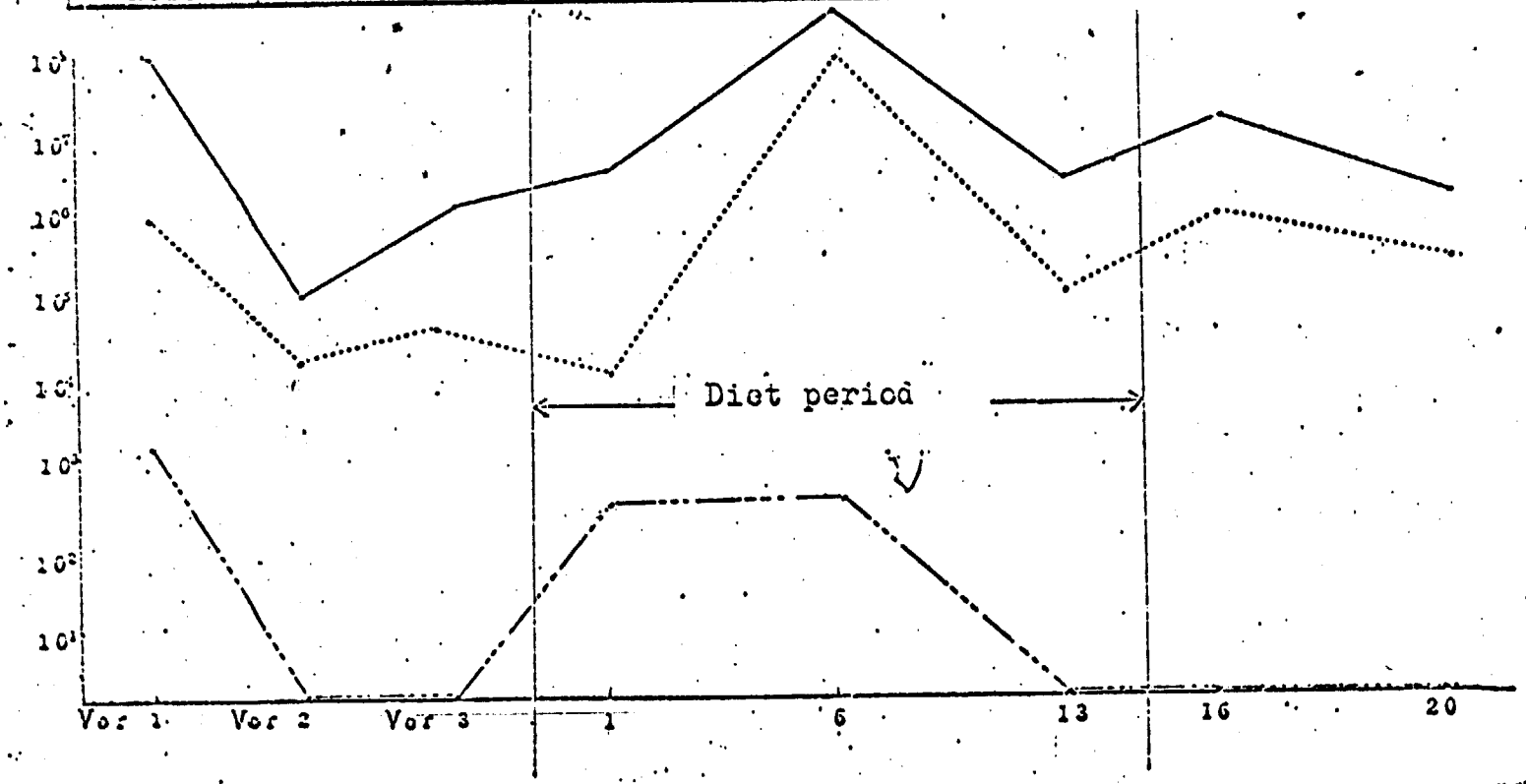
	Vor 1 (22/×1)	Vor 2 (24/×1)	Vor 3 (26/×1)	day of diet 1 (29/×1)	day of diet 6 (3/×11)	13 (10/×11)	each 1 16 (13/×11)	each 2 20 (17/×11)
Enterococcus	4.9×10^6	3.5×10^5	5.4×10^6	2.3×10^3	4.5×10^2	1.3×10^4	9.2×10^5	7.0×10^6
Gram (-) Rods	2.3×10^6 E·coli	2.4×10^5 E·coli	5.4×10^5 E.coli	9.2×10^6 E·coli	2.0×10^2 E·coli	7.0×10^6 E·coli	9.2×10^5 E·coli	5.4×10^6 E·coli
Staphylo	5.0×10^2	(-)	(-)	5.0×10^2	5.5×10^3	5.0×10^2	(-)	(-)
(Fungas) C S	(-)	5.0×10^2	1.5×10^3	(-)	(-)	1.5×10^3	(-)	2.5×10^3
Gram (+) Rods	(-)	(-)	(+)	(-)	(+)	(+)	(-)	(+)



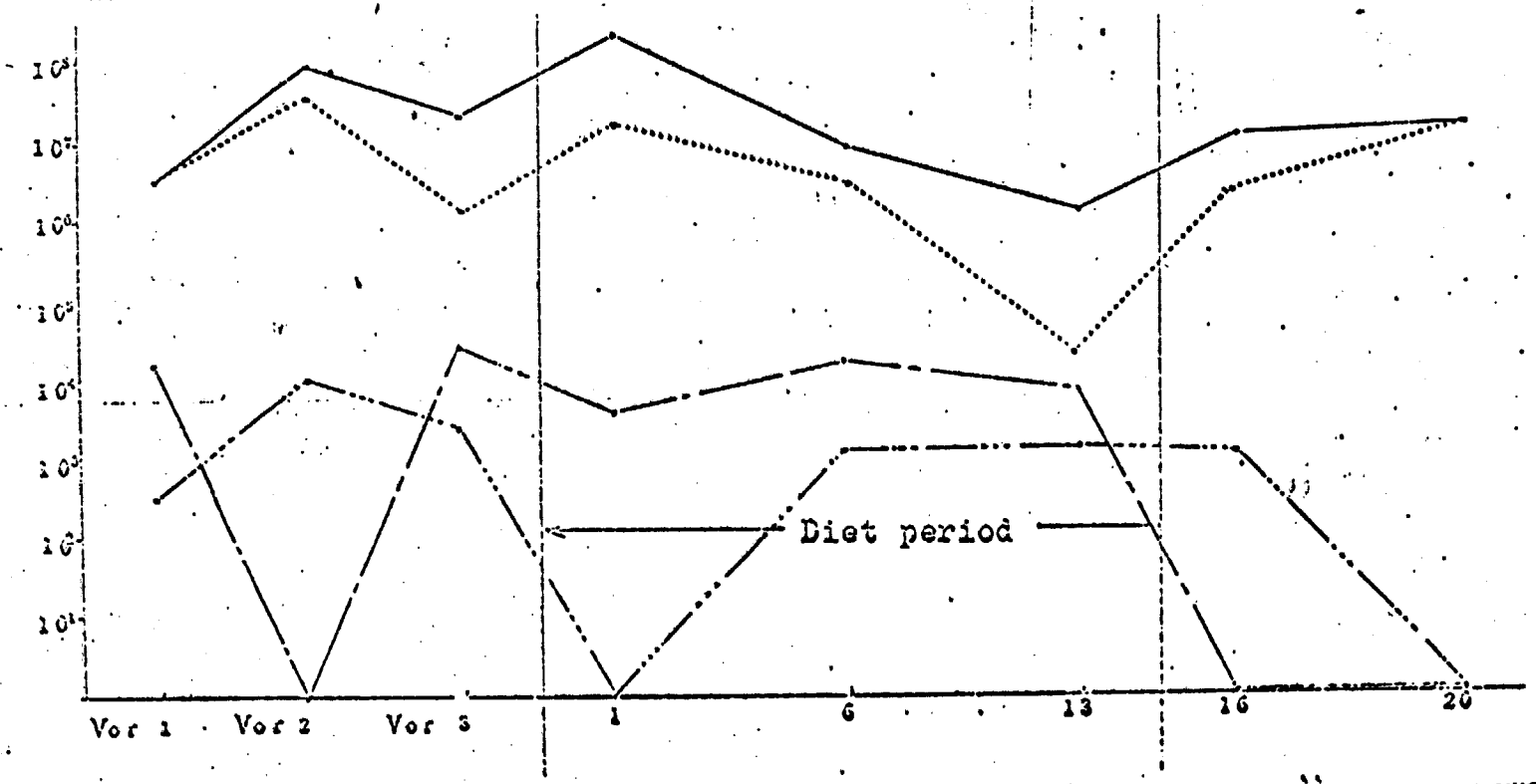
	Vor 1	Vor 2	Vor 3	1	6	13	nach 1 16	nach 2 20
Enterococcus	6.8×10^6	9.2×10^5	1.6×10^5	8.1×10^5	3.5×10^4	2.2×10^4	1.0×10^6	3.5×10^6
Gram (-) Rods	4.9×10^6 Paracolon	5.8×10^6 E·coli Citrobacter	4.6×10^6 E·coli	4.9×10^5 E·coli	3.3×10^3 E·coli	5.4×10^4 E·coli	9.2×10^6 E·coli Paracolon	5.4×10^5 E·coli
Staphylo	5.0×10^2	4.5×10^3	4.3×10^4	1.0×10^3	(-)	1.5×10^3	5.0×10^2	3.5×10^3
G S (Sungas)	2.3×10^4	4.0×10^3	1.3×10^4	1.0×10^3	1.5×10^3	5.0×10^3	1.5×10^4	1.0×10^3
Gram (+) Rods	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(-)



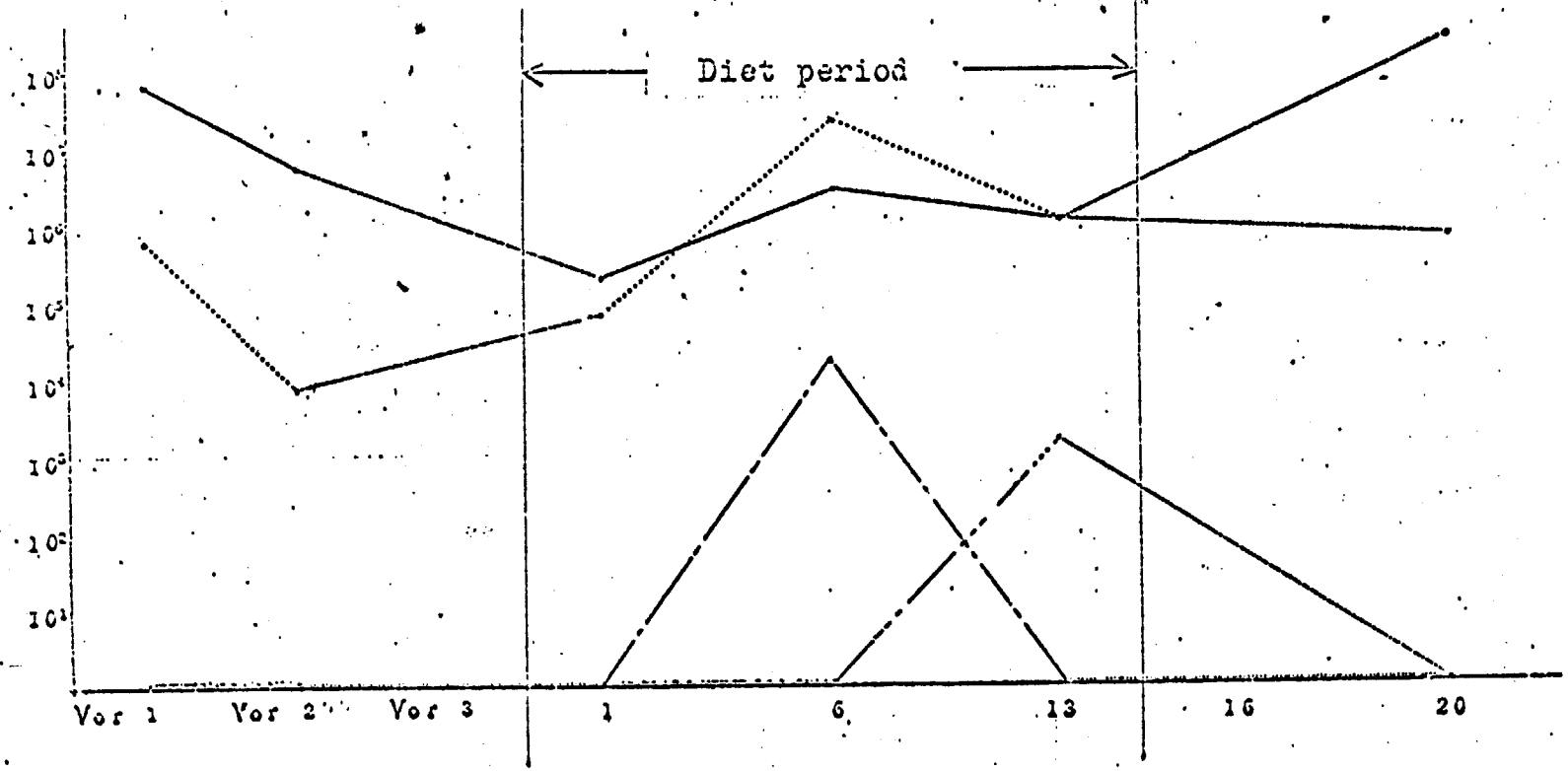
	Vor 1	Vor 2	Vor 3	1	6	13	nach 1 16	nach 2 20
Enterococcus	1.1×10^6	3.1×10^4	1.7×10^5	1.7×10^4	2.3×10^5	1.1×10^5	1.7×10^6	5.4×10^5
Gram (-) Rods	1.6×10^5 E.coli	1.1×10^5 E-coli	2.1×10^6 E-coli	7.0×10^4 E-coli	7.0×10^4 E-coli	5.4×10^6 E-coli Paracolon	3.5×10^7 E-coli Cloacn	3.5×10^6 E-coli
Staphylo	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
(Fungas) C. S	2.0×10^3	(-)	(-)	5.0×10^2	5.0×10^2	(-)	(-)	(-)
Gram (+) Rods	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)



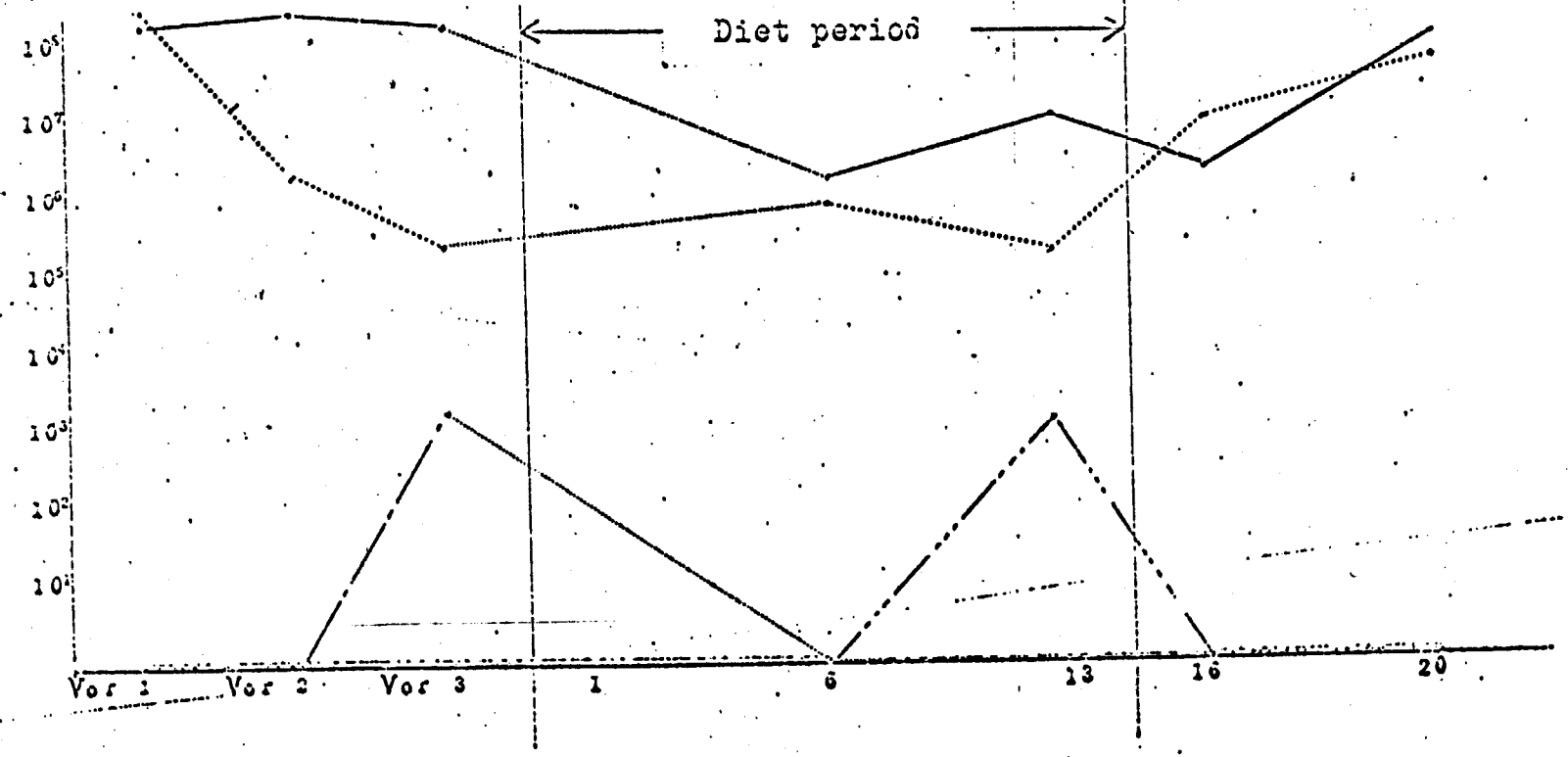
	Vor 1	Vor 2	Vor 3	1	6	13	nach 1 16	nach 2 20
····· Enterococcus	4.6×10^6	5.4×10^7	1.4×10^6	2.2×10^7	4.6×10^6	3.5×10^4	4.6×10^6	2.4×10^7
—— Gram (-) Rods	4.9×10^6 E.coli	9.2×10^7 E.coli	3.5×10^7 E.coli	3.5×10^8 E.coli	9.2×10^6 Hafnia Paracolon	1.6×10^6 Paracolon	1.6×10^7 E.coli	2.4×10^7 E.coli
—— Staphylo	1.8×10^4	(-)	4.3×10^4	6.0×10^3	2.5×10^4	9.0×10^3	(-)	(-)
---- (Fungas) G S	5.0×10^3	1.0×10^4	4.0×10^3	(-)	1.6×10^3	2.0×10^3	1.5×10^3	(-)
Gram (+) Rods	(+)	(+)	(+)	(+)	(+)	(-)	(-)	(+)



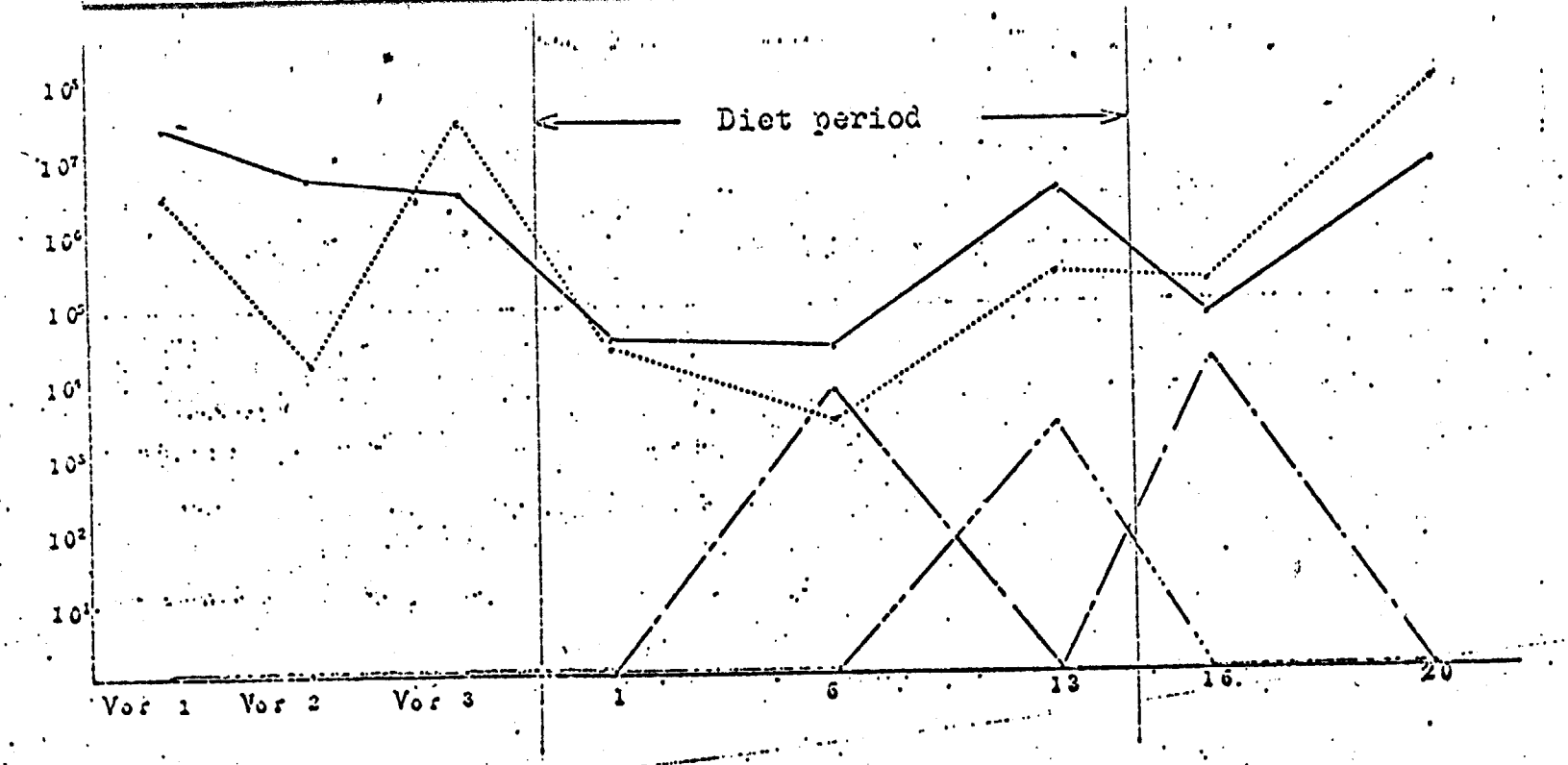
	Vor 1	Vor 2	Vor 3	1	6	13	nach 1 16	nach 2 20
Enterococcus	6.1×10^5	7.0×10^3	/	7.4×10^4	1.6×10^7	9.2×10^5	/	1.7×10^4
Gram (-) Rods	5.4×10^7 E.coli	5.4×10^9 E.coli	/	1.4×10^5 E.coli	3.5×10^6 E.coli	9.2×10^5 E.coli	/	7.0×10^5 E.coli
Staphylo	(-)	(-)	/	(-)	1.5×10^4	(-)	/	(-)
(Fungas) C S	(-)	(-)	/	(-)	(-)	2.0×10^3	/	(-)
Gram (+) Rods	(+)	(+)	/	(+)	(+)	(+)	/	(+)



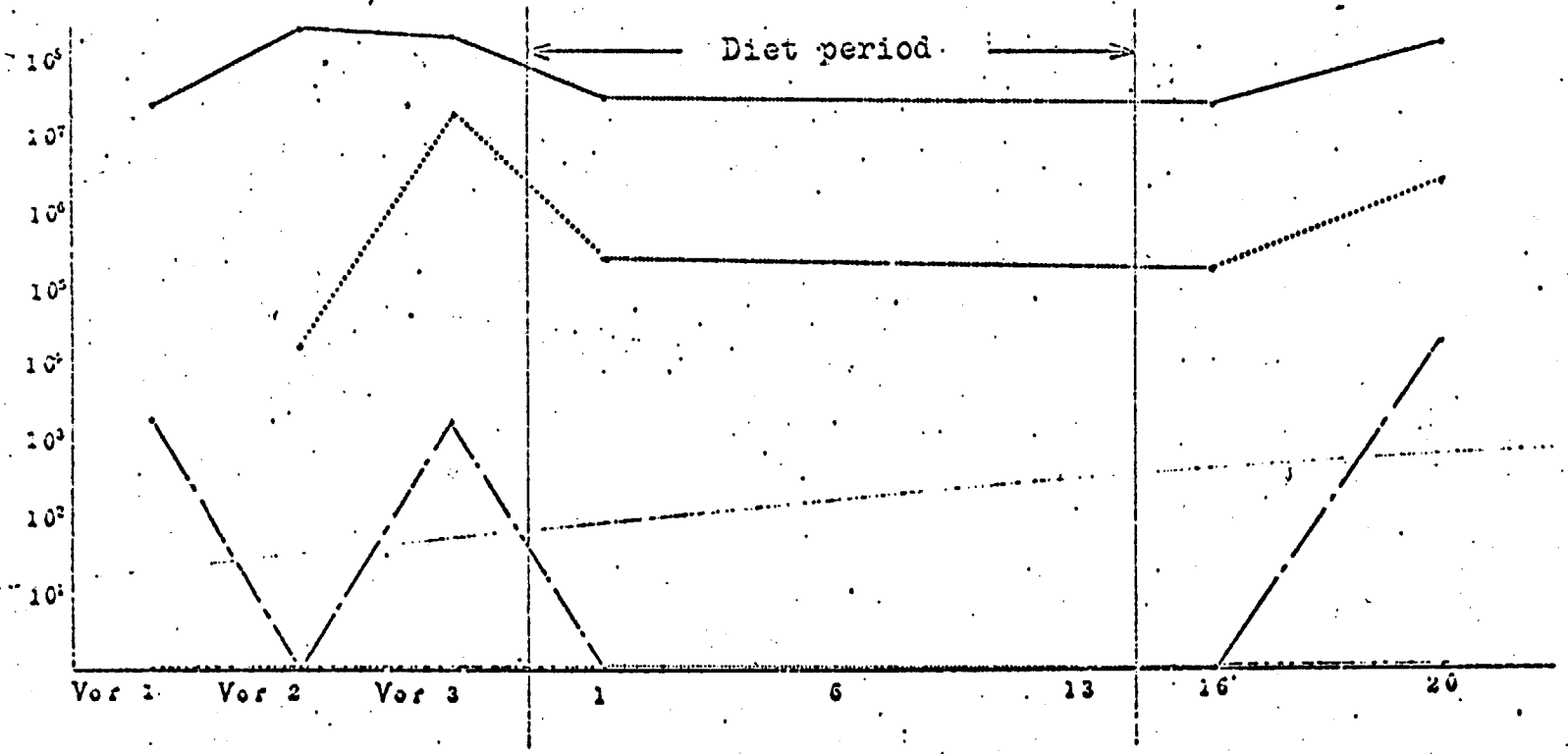
	Vor 1	Vor 2	Vor 3	1	6	13	nach 1 16	nach 2 20
Enterococcus	3.6×10^5	2.8×10^6	3.5×10^5	/	9.2×10^5	2.4×10^5	9.2×10^6	6.8×10^7
Gram (-) Rods	1.1×10^5 E-coli	3.5×10^5 Paracolon	1.7×10^5 E-coli	/	2.4×10^6 E-coli	9.2×10^6 E-coli	2.4×10^6 E-coli	9.2×10^7 E-coli
Staphylo	(-)	(-)	2.0×10^3	/	(-)	(-)	(-)	(-)
(Fungus) G S	(-)	(-)	(-)	/	(-)	1.0×10^3	(-)	(-)
Gram (+) Rods	(+)	(-)	(+)	/	(+)	(+)	(-)	(+)



	Vor 1	Vor 2	Vor 3	1	6	13	nach 1 16	nach 2 20
Enterococcus	3.3×10^6	1.7×10^4	2.1×10^7	2.8×10^4	3.3×10^3	2.4×10^3	1.7×10^5	7.0×10^7
Gram (-) Rode	2.4×10^7 E·coli	5.4×10^6 E·coli	3.5×10^6 Paracolon	3.5×10^4 E·coli Paracolon	3.5×10^4 E·coli Cloaca	2.4×10^6 E·coli Cloaca Paracolon	7.0×10^4 E·coli	5.4×10^6 E·coli
Staphylo	(-)	(-)	(-)	(-)	7.5×10^3	(-)	1.0×10^4	(-)
(Fungus) G S	(-)	(-)	(-)	(-)	(-)	3.0×10^3	(-)	(-)
Gram (+) Rods	(+)	(+)	(+)	(-)	(-)	(+)	(+)	(+)



	Vor 1	Vor 2	Vor 3	1	6	13	nach 1 16	nach 2 20
Enterococcus	(-)→10^{-5}	1.3×10^{6}	1.1×10^{7}	2.4×10^{5}	/	/	1.7×10^{5}	3.5×10^{9}
Gram (-) Rods	1.1×10^{7} E-coli	1.6×10^{8} E-coli	1.1×10^{5} E-coli	2.2×10^{7} E-coli	/	/	1.6×10^{7} E-coli	9.2×10^{7} E-coli Paracolon
Staphylo	1.5×10^{3}	(-)	1.5×10^{3}	(-)	/	/	(-)	1.3×10^{6}
(Fungas) (L S	(-)	(-)	(-)	(-)	/	/	(-)	(-)
Gram (+) Rods	(+)	(-)	(+)	(-)	/	/	(+)	(-)



4. Effect of Avicel-added food products on chemical composition of blood and ureal blood

Two criteria are required for analyzing the change of chemical composition of blood before and after the ingestion of Avicel-added bread. The one is the normal range of objective chemical composition, and the other is the range of physiological variation in individual subjects.

Table 1 enumerates such criteria, which are data obtained by the Biochemical Department of Toranomon Hospital in accordance with the method employed for practical investigation.

Under the heading "normal range" are given average values ±2 S.D. obtained for 200 healthy males and females of age range from 20 to 40. Under the heading "range of physiological variation", there are listed averages allowing of 2 S.D. which were obtained of the variations determined with respect to the blood collected weekly for 3 months from 10 healthy males and females.

The range of physiological variation is such that 60% of the values measured of healthy individuals during the normal conduct of everyday life will fall within it, i.e., that any variations occurring within this range are of physiological nature and ought not to be thought of in terms of morbid phenomenon. Variations rarely (by less than 5%) occur beyond twice this range (4 = ±2 S.D.) in healthy conditions and therefore must be regarded as associable with morbid causes.

When the results of chemical analysis conducted in the pre-diet phase and the post-diet phase are examined in the light of the criteria discussed above, all the variations are found to fall within the healthy range and to involve neither morbid trend nor fixed inclination of ascent or descent.

As regards the uroscopy, unusual conditions of sugar, urabilinogen, and cellular composition were observed occasionally which were in no way related with the ingestion of Avicel-added bread. Considering that the conditions of urine collection were indefinite and considerable amount of effort preceded the urine collection in some of the subjects, such unusual conditions could not be judged abnormal. No peculiar trend could be recognized either in the pre-diet phase or in the post-diet phase.

Also with respect to blood test, no definite trend of variations possible of posing any particular problem was recognized. However, unavailability of normal values and range of their variations prevented this worker from drawing a definite

conclusion. As to the blood platelet test, because the method itself is problematic in point of measuring accuracy, the data obtained leave room for considerable criticism in their interpretation.

Motoshi Kitamura
Head of Biochemical Department
Toranomon Hospital

Table 1. Measuring method for normal values in chemical analysis

	Normal range (average)	Range of physiological variations (2)	Method
Total protein	6.8 - 8.7 (7.7)	0.6 g/dl	Hitachi refractometer
A/G	1.2 - 2.2 (1.7)	0.3 "	Yoshikawa & Saito's modification
TTT	2 - 7 (3)	1.1 unit	Shank-Hoagland
ZST	4 - 12 (7)	1.4 "	Kunkel
Ureal N	8 - 20	3.8 mg/dl	DAM-autoanalysis
Uric acid	♂3.7 - 6.9 (5.3)	0.8 mg/dl	Na_2CO_3-autoanalysis
AlP	2.6 - 10 (5.3)	1.2 unit	Kind-King
Cholesterol	133 - 255 (186)	20 mg/dl	Zak-Henly
Cholester	70 - 80	2.2 %	Kiliani-Digitonide
GOT	5 - 19 (10)	3.6 unit	Gastroenterologic Society's modified Reitman-Frankel method
GPT	0 - 18 (4)	3.9 "	"

Serochemical test results

Total protein

Date	A	B	C	D	E	F	G	H
Nov. 24					7.0			
Nov. 26	7.8	7.1	8.2	7.2		7.2	8.2	7.3
Dec. 3	7.4	8.0	7.1	7.4	7.2	7.0	8.2	7.5
Dec. 10								
Dec. 17	8.2	7.7	7.2	7.5	7.0	7.2	7.6	7.4

A/G

Nov. 24					1.8			
Nov. 26	1.7	1.4	1.6	1.6		2.1	1.3	2.1
Dec. 3	1.7	1.5	1.9	1.8	1.7	2.1	1.4	1.8
Dec. 10	1.8	1.7	2.0	1.9	1.7	2.2	1.4	
Dec. 17	1.8	1.6	1.8	1.7	1.6	2.1	1.4	1.8

Thymol

Nov. 24					2.0			
Nov. 26	4.3	1.8	2.3	2.9		1.7	3.4	2.0
Dec. 3	2.6	1.7	1.7	1.6	2.6	1.0	2.7	2.1
Dec. 10	2.0	1.6	1.7	2.6	3.3	1.0	3.6	
Dec. 17	2.0	1.5	1.2	1.7	2.6	1.0	2.3	1.8

Zinc sulfate turbidity test

Date	A	B	C	D	E	F	G	H
Nov. 24					5.7			
Nov. 26	6.7	6.2	5.7	4.7		3.8	8.3	4.5
Dec. 3	6.9	6.6	5.7	4.5	5.7	3.8	8.0	4.5
Dec. 10	6.6	6.6	6.0	4.7	6.0	3.8	37.8	
Dec. 17	5.7	5.9	5.0	3.9	6.3	3.7	6.7	3.8

Urea-N

Nov. 24					14.5			
Nov. 26	16.2	10.0	12.7	12.7		10.5	12.7	11.0
Dec. 3	12.7	11.7	14.5	14.0	13.8	16.5	16.2	13.5
Dec. 10	15.0	12.5	13.5	13.2	16.0	12.8	13.5	
Dec. 17	14.0	12.5	11.0	14.0	15.0	15.0	12.2	16.7

Uric acid

Nov. 24					5.0			
Nov. 26	4.4	5.9	6.9	6.4		5.5	5.9	5.1
Dec. 3	4.1	6.7	5.7	7.0	4.8	6.1	5.9	5.0
Dec. 10	4.3	7.5	*	6.8	6.3	6.6	6.7	
Dec. 17	3.8	5.7	5.1	6.5	5.2	7.0	6.7	4.9

* Insufficient quantity

G C T

Date	A	B	C	D	E	F	G	H
Nov. 24								
Nov. 26	12	10	12	13		19	12	10
Dec. 3	12	11	10	12	7	13	9	7
Dec. 10	7	8	9	10	8	15	7	
Dec. 17	11	11	8	13	8	15	9	8

G P T

Date	A	B	C	D	E	F	G	H
Nov. 24					4			
Nov. 26	13	4	7	6		9	5	4
Dec. 3	13	5	6	4	0	3	1	3
Dec. 10	7	1	8	1	4	6	1	
Dec. 17	6	5	3	3	5	5	2	1

Al - P

Date	A	B	C	D	E	F	G	H
Nov. 24					4.6			
Nov. 26	4.4	6.6	9.0	7.2		8.0	7.1	6.4
Dec. 3	4.0	8.1	8.2	7.5	4.0	7.1	7.6	7.6
Dec. 10	3.7	6.0	7.1	8.4	5.0	7.8	6.5	
Dec. 17	4.0	6.3	7.6	9.5	4.4	6.3	6.2	6.6

Blood sugar

Date	A	B	C	D	E	F	G	H
Nov. 24								
Nov. 26	82	82	76	99		80	73	76
Dec. 10	84	100	69	77	74	82	82	
Dec. 17	89	89	83	81	98	92	83	80

Ureal test

Outward appearance

Date	A	B	C	D	E	F	G	H
Dec. 3	Yellow	Yellow	Strow	Yellow	Yellow	Strow	Yellow	Yellow
Dec. 10	"	Strow	"	"	"	Yellow	"	"
Dec. 17	"	Yellow	"	"	Strow	"	"	"
Dec. 24	Slightly turbid	Strow	Yellow	"	"	"	"	"

Reaction

Dec. 3	Neutral	Alkali	Acid	Acid	Alkali	Acid	Acid	Acid
Dec. 10	Acid	Neutral	Acid	Acid	Weakly acid	Neutral	Acid	Neutral
Dec. 17	Neutral	Weakly alkali	Alkali	Neutral	Neutral	Acid	Acid	Acid
Dec. 24	Weally alkali	Neutral	Weakly alkali	Neutral	Weakly alkali	Weakly alkali	Weakly alkali	Neutral

Specific gravity

Dec. 3	1.022	1.026	1.027	1.026	1.019	1.035	1.028	1.026
Dec. 10	28	25	30	21	20	28	30	26
Dec. 17	22	17	22	15	22	22	27	17
Dec. 24	19	24	24	15	25	21	22	17

Red blood corpuscle

Date	A	B	C	D	E	F	G	H
Dec. 3	-	4-5/10 flds*	2-3/every fld	5-6/total flds	2-3/10 flds	4-5/total flds	-	-
Dec. 10	-	2-3/10 flds	3-4/several flds	5-6/total flds	2-3/several flds	-	-	-
Dec. 17	-	5-6/total flds	1/several flds	-	1-4/every fld	-	-	-
Dec. 24	-		1-2/10 flds	-	2-3/10 flds	-	-	-

White blood corpuscle

Dec. 3	1-2/10 flds	3-4/total flds	1-2/every fld	2-3/10 flds	2-4/total flds	4-5/total flds	-	2-3/total flds
Dec. 10	5-6/total flds	-	-	-	5-6 several flds/total flds	5-6/total flds	-	-
Dec. 17	-	5-6/total flds	1-10 flds	-	1/10 flds	-	1/10 flds	-
Dec. 24	-		2-3/several flds	1-2/10 flds	2-3/10 flds	-	1-2/10 flds	-

Pavement epitholium

Dec. 3	2-3/total flds	4-5/total flds	3-4/10 / flds	5-6/total flds	3-4/total flds	4-5/total flds	/-	4-5/total flds
Dec. 10	2-3/total flds	2-3/total flds	-	2-3/10 flds	2-3/10 flds	-	5-6/total flds	5-6/total flds
Dec. 17	-	-	-	-	1/10 flds	-	-	-
Dec. 24	-	-	-	-	-	-	-	-

By "fld" is meant a x 300-magnification field.

Protein

	A	B	C	D	E	F	G	H
Dec. 3	-	-	-	-	±	±	-	-
Dec. 10	-	-	-	-	-	-	-	-
Dec. 17	-	-	-	-	±	-	-	-
Dec. 24	-	-	-	-	-	-	-	-

Sugar (Benedict/Testape)

Dec. 3	-/-	-/-	-/-	-/-	-/-	±/-	-/-	-/-
Dec. 10	++/+	+/-	++/+	++/-	++/+	+/+	+++/+	++/+
Dec. 17	-/-	-/-	±/+	-/-	-/-	-/-	-/-	-/-
Dec. 24	-/-	-/±	-/-	-/-	-/±	-/-	-/-	+/+

Acetone

Dec. 3	-	-	-	-	-	-	-	-
Dec. 10	-	-	±	-	-	±	-	-
Dec. 17	-	-	-	-	-	-	-	-
Dec. 24	-	-	-	-	-	-	-	-

Bilirubin

Date	A	B	C	D	E	F	G	H
Dec. 3	-	-	-	-	-	-	-	-
Dec. 10	-	-	-	-	-	-	-	-
Dec. 17	-	-	-	-	-	-	-	-
Dec. 24	-	-	-	-	-	-	-	-

Urobilinogen

Dec. 3	+	+	+	+	+	+	+	+
Dec. 10	+ ~ ++	+	+	+	+	+	+	+
Dec. 17	+	+	+	+	+ ~ ++	+	+	+
Dec. 24	+	+	+	+	+	+	+	+

Others

Dec. 3	Bact(+)	Bact(+)	Bact(+) Calcium oxalate (+)	Bact(+)	Bact(+) Mucous thread (+)	Bact(+)	Bact(+)
Dec. 10		Bact(+)	Bact(+) Uric acid crystal (+)			Bact(+)	Bact(+) Uric acid crystal (+)
Dec. 17	Calcium oxalate (+)		Calcium oxalate (+)		Uric acid (+) Sperm(+)		Calcium oxalate (+) Bact(+) Mucous thread (+)
Dec. 24	Bact (+++)		Calcium oxalate (+)	Calcium oxalate (+)			Calcium oxalate (+)

Blood test results

Blood pigment g/dl — Pare	A	B	C	D	E	F	G	H
11.26	16.0	13.5	16.0	15.2	13.8	*	15.2	14.9
12. 3	14.1	13.5	14.8	14.1	13.5	14.5	14.1	14.1
12.10	13.8	13.5	14.8	*	14.1	14.5	14.5	
12.17	15.1	13.5	14.5	15.5	13.8	15.1	14.1	14.8

Number of red blood corpuscles x 10^4								
11.26	480	420	434	470	413	*	487	421
12. 3	435	438	406	448	421	488	474	424
12.10	426	436	419	*	445	493	476	
12.17	464	427	416	487	438	504	467	433

Pigment index								
11.26	1.04	1.00	1.15	1.01	1.04	*	0.97	1.09
12. 3	1.01	0.98	1.13	0.98	1.00	0.93	0.93	1.04
12.10	1.01	0.97	1.10	*	0.99	0.92	0.94	
12.17	1.01	1.00	1.08	0.99	0.98	0.93	0.75	1.06

* Coagulation

Hematocrit (%)	A	B	C	D	E	F	G	H
11.26	46	40	46	43	40		46	44
12.03	41	40	45	44	40	45	42	43
12.10	41	40	46	*	42	44	43	
12.17	43	41	44	46	41	46	43	44

Reticulacyte count (%)	A	B	C	D	E	F	G	H
11.26	1.8	1.0	0.4	0.4	0.4	*	0.8	0.6
12.03	1.2	1.2	0.6	1.0	0.4	1.2	0.8	1.2
12.10	1.2	1.0	0.4	*	0.8	1.2	1.0	
12.17	1.0	1.0	1.8	0.8	0.2	2.4	1.6	1.0

Number of white blood corpuscles	A	B	C	D	E	F	G	H
11.26	6000	4800	3000	4200	4000	*	4000	4000
12.03	5800	4600	6000	6200	6700	6300	7000	6000
12.10	4500	5000	3500	*	4700	4400	8500	
12.17	5200	4200	5500	4700	3400	6000	6000	4800

* Coagulation

Percentage / Date	N		Eos	Bas	Mon	Lymph
	St	Sg				
Nov. 26	0	56	0	0	3	41
Dec. 3	1	65	1	1	1	31
Dec. 10	3	37	2	1	2	55
Dec. 17	1	47	2	1	2	47
Nov. 26	1	50	1	0	2	46
Dec. 3	2	55	2	2	1	38
Dec. 10	0	50	1	0	1	48
Dec. 17	1	34	1	1	11	52
Nov. 26	0	51	5	0	2	42
Dec. 3	1	46	8	0	1	44
Dec. 10	0	54	3	1	9	33
Dec. 17	1	63	6	1	2	27
Nov. 26	0	45	6	0	1	48
Dec. 3	4	44	10	1	1	40
Dec. 10	*	*	*	*	*	*
Dec. 17	0	40	7	0	1	52
Nov. 26	0	51	2	0	2	45
Dec. 3	2	65	3	0	1	29
Dec. 10	2	55	2	0	6	35
Dec. 17	0	59	0	1	1	39
Nov. 26	*	*	*	*	*	*
Dec. 3	3	52	4	1	1	39
Dec. 10	1	45	1	1	2	50
Dec. 17	0	53	1	0	1	45
Nov. 26	0	46	3	1	2	48
Dec. 3	0	56	4	0	1	39
Dec. 10	2	69	1	0	7	21
Dec. 17	1	61	0	1	1	36
Nov. 26	0	47	2	0	2	49
Dec. 3	8	60	0	0	1	31
Dec. 10						
Dec. 17	1	74	0	1	1	23

* Coagulation

(Though the number of blood platelets is considerably varied, relatively unsatisfactory accuracy of the measuring method and daily occurrence of deviation seem to prevent definite interpretation of such variation.)

Number of blood platelets ($\times 10^4$)

11.26	32.6	46.2	23.2	24.4		*	26.4	33.8
12.03	24.4	14.7	16.4	11.6	16.8	25.4	20.8	23.8
12.10	33.3	19.2	20.0	*	30.2	25.6	26.6	
12.17	55.7	42.7	21.7	30.2	24.5	22.1	46.9	62.5

* Coagulation

Careful analysis of these results has failed to detect any definite trend of variation of dubious nature.

Clinical Blood Examination Department
Toranomon Hospital

December 20, 1965

5. Change of body weight before and after ingestion of Avicel-contained food products

The body weight of each subject was measured at an early hour on the first day (November 27, 1965) of diet phase (for the ingestion of Avicel-added food) and on the morning of the day (December 11, 1965) following the completion of diet phase. Comparison of weights showed no marked difference in each subject.

	Nov. 27	Dec. 11	Increase or decrease
A	57.5 kg	57.5 kg	0
B	57	57.5	+ 0.5
C	53.5	54	+ 0.5
D	59.5	61.5	+ 2.0
E	64.5	63.5	- 1.0
F	55.2	54.5	- 0.7
G	54.0	55.0	+ 1.0
H	44.0	45.0	+ 1.0

C O N C L U S I O N

In the present study, Avicel diet study (daily supplementary ingestion of 30 g of Avicel) was conducted on 8 healthy males, who were followed in the pre- and post-diet phases as to digestion, assimilation test, chemical composition of blood, urine, blood test, influence upon intestinal becterial flora, and influence upon vitamin B_1 assimilation. Results obtained in these phases indicated no particular trend, namely, no morbid trend, lending support to a conclusion that ingestion of Avicel to patients requiring lower calory intake produces no adverse effect.

(Referential Example)

Management of obesity with use of Avicel bread

For the management of obesity, the only resort is generally the dietary control to reduce the calory intake, though it is quite difficult to carry through. Incidentally, Avicel bread seems to serve the purpose of alleviating the pain of obesity to a fair extent when it is used in a well-arranged dietary program. The author prepared a dietary table on the basis of "Food Substitution Schedule for Treatment of Diabetes" as shown in Table 1, and has been using it effectively for the practical treatment of patients. Fig. 1 represents one typical case of such treatment.

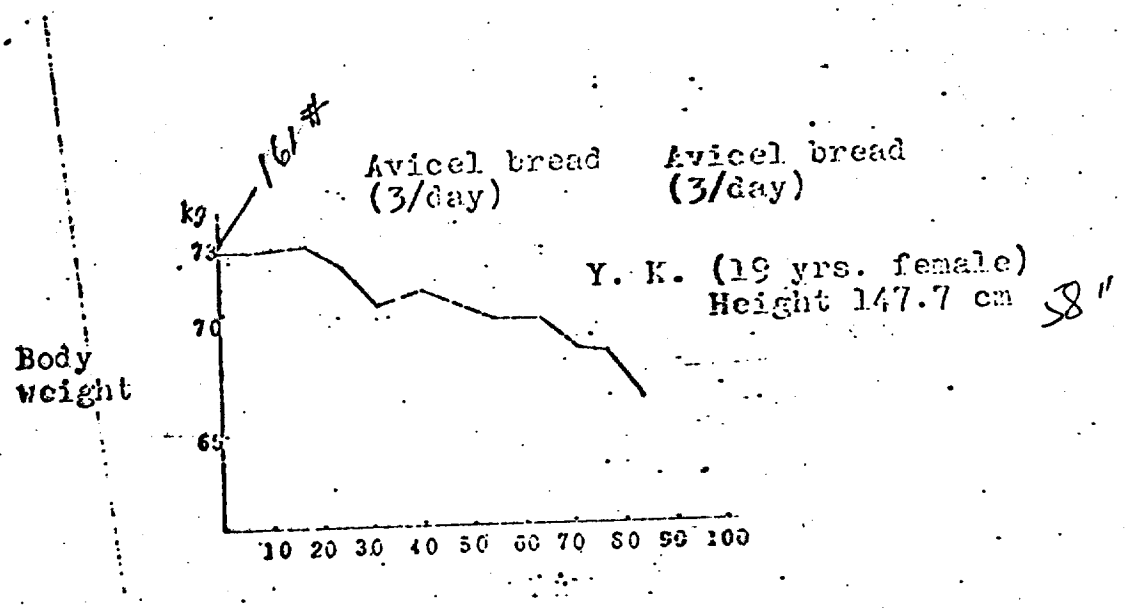


Fig. 1

Table 1. (Food Substitution Schedule for Treatment of Diabetes, recommended by Japan Diabetes Society)

(Daily diet)

(Unit)	1000 cal	1200 cal	1500 cal
I_{-1}	1	1	2
-2	1	2	2
II_{-1}	3	4	4
-2	1.3(2)	1.3(2)	2.6(2)
III	0.5(4)	0.5(4)	2(4)
IV_a	0.5(5)	0.5(5)	0.5(5)
Avicel bread	3 pcs	3 pcs	3 pcs

Remarks: (1) The food will be taken in equal amount in 3 meals daily as far as practical.

(2) One bottle of milk

(3) Two bottles of milk

(4) Use of vegetable oil is desirable.

(5) At least 100 g will be taken in the form of colored vegetable per day. Another 50 g will be in the form of vegetable having a slightly greater content of sugar. The remainders may be taken in the form of vegetable having a smaller content of sugar.

Chikayuki Naito
Yoshitoshi Internal Seminar
Tokyo University

MICROCRYSTALLINE CELLULOSE

Biological data

Biochemical aspects

Four rats were fed ^{14}C-labelled microcrystalline cellulose at 10 and 20 per cent. of their diet. No evidence of degradation or digestion was noted. Faecal recoveries of radioactivity ranged from 96-104 per cent. and were complete for all labelled material. No radioactivity appeared in the urine (Baker, 1966). One human subject received 150 g of ^{14}C-labelled microcrystalline cellulose (47.6 µc) in two portions on one day and 150 g unlabelled microcrystalline cellulose daily for the subsequent 10 days. Twenty-four hour faecal and urine collections were examined for radioactivity. No radioactivity appeared in the urine or in the expired CO_2. All administered radioactivity was recovered from the faeces within 2 days. (Baker, 1968.) Examination of the stools of 1 male and 1 female patient given 30 g microcrystalline cellulose as dry flour or gel for 5-1/2 weeks showed the presence of undegraded material of the same birefringence as the original microcrystalline cellulose administered. No significant effects on the human gastro-intestinal tract were noted during the administration (Rusing et al., 1964).

Acute toxicity

Animal	Sex	Route	mg/kg body-weight	Reference
Rat	male	oral	>3160	Hazleton Labs., 1959
Rat	male	i.p.	>3160	" " "

Short-term studies

Rat

Groups of 4 male rats were kept on diets containing 0.25, 2.5 or 25 per cent. of various edible celluloses for three months. No differences were observed among the groups with regard to growth and faecal output. Histopathology of the gastro-intestinal tract revealed no treatment-related abnormalities (Frey et al., 1928).

Three groups of 5 male rats received 0, 5 or 10 per cent. microcrystalline cellulose in their diet for 8 weeks. Growth was comparable to the controls but the 10 per cent. group showed slightly lower body-weights. Haematology, serum chemistry and vitamin B_1 levels in blood and faeces showed no differences from the controls (Yoshitoshi Internal Seminar, 1966).

Long-term studies

Rat

Three groups of 50 male and 50 female rats received in their diet for 72 weeks either 30 per cent. ordinary cellulose or dry microcrystalline cellulose or microcrystalline cellulose gel. Appearance and behaviour was comparable in all groups. No adverse effects were noted. The body-weights of males given microcrystalline cellulose gel were higher than those of the controls. Food efficiency, survival and haematology were comparable in all groups. The liver and kidney weights of males receiving microcrystalline cellulose gel were higher than the controls. Gross and histopathology showed some dystrophic calcification of proximal renal tubules in females on microcrystalline cellulose but all other organs appeared unremarkable. Tumour incidence did not differ between the groups (Hazleton Laboratories, 1963).

Reproduction studies

Rat

Groups of 8 male and 16 female rats were used to produce a P, F_{1A}, F_{1B}, F_2 and F_3 generation after having been fed on diets containing 30 per cent. of microcrystalline cellulose flour or gel or ordinary cellulose as a control. The presence in the diet of such an amount of non-nutritious material, which contributed no calories had an adverse effect on reproduction. Fertility and numbers of live pups were relatively depressed and lactation performance in all three generations, as well as survival and the physical condition of the pups, were unsatisfactory throughout the study. The new-born pups appeared smaller, weak and showed evidence of disturbed motor co-ordination. Liver weights were increased in the group receiving microcrystalline cellulose gel in all generations but other organ weights showed no consistent patterns. Gross and histopathology revealed renal changes similar to those seen in the feeding study in females of all generations. Other organs showed no consistent changes. No teratological deformities were seen (Hazleton Laboratories, 1964).

Observation in man

A number of clinical studies using refined cellulose as roughage in human diet for the treatment of constipation showed no deleterious effects. Groups of 18 children received regular amounts of edible cellulose instead of normal cereal for 3 months. The only effect noted was an increase in bowel movements but no diarrhoea or other gastro-intestinal disturbances were seen (Frey et al., 1928).

Eight male and 8 female volunteers supplemented their normal diet with 30 g microcrystalline cellulose per day either as dry powder or gel (15 per cent. aqueous) for 6 weeks followed by 2 weeks without supplementation. No adverse findings were reported regarding acceptance or body-weight but most subjects complained of fulness and mild constipation. Haematology was normal in all subjects. Biochemical blood values showed no differences between treatment and control periods, nor was there evidence of liver or kidney function disturbance. Urinalysis produced normal findings. The faecal flora remained unchanged. The cellulose content of faeces increased 5-8 times during the test period. Microscopy revealed the presence of microcrystalline cellulose (Hazleton Laboratories, 1962).

In another study 8 healthy males received 30 g microcrystalline cellulose daily as supplement to their diet for 15 days. D-zylose absorption varied between pre-test, test and post-test periods being lower during microcrystalline cellulose ingestion. The absorption of I^{131}-triolein was unaffected by microcrystalline cellulose ingestion. No change was noted in the faecal flora nor was there any significant effect on blood chemistry during ingestion of microcrystalline cellulose. Examination of urine, blood and faecal levels of vitamin B_1 during microcrystalline cellulose ingestion showed no difference from control periods (Yoshitoshi Internal Seminar, 1966).

Comments

The animal and human studies including the use of radio-labelled material show complete absence of digestion or absorption. Microcrystalline cellulose is structurally very close to naturally occurring cellulose. Doses up to 30 g per day appear to be tolerated therapeutically as bulk laxative. The adverse effects reported in the long-term studies are probably attributable to the inadequacies of a diet containing a large amount of indigestible material.

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A METHOD OF IMPROVING FUNCTION OF THE BOWEL: THE USE OF METHYLCELLULOSE

J. ARNOLD BARGEN, M.D.,
Division of Medicine, Mayo Clinic, Rochester, Minnesota

Many digestive and intestinal dysfunctions, which in themselves are not serious, may cause untold misery and unrest. Among these the syndrome of irritable bowel associated with constipation or diarrhea holds a prominent place. There are also serious lesions, which may be associated with symptoms of great discomfort; for example, neoplasms for which intestinal stomas become necessary, regional ileitis associated with mild diarrhea, ulcerative colitis of the distal segments of the large intestine, segmental colitis, some functional disturbances for which ileosigmoidostomy may be needed, and operations and disease associated with paralysis of the anal sphincters. These conditions, in addition to their more serious aspects, often may produce great discomfort.

Physicians speak of a normal gradient in the gastrointestinal tract by which food passes in an orderly fashion from the stomach to the rectum. This intestinal gradient may be upset in many ways. Although diseases of the gastrointestinal tract (malignant and inflammatory) represent far and away the most serious causes of such upsets, they are by no means the most common. Nervous tension and improper dietary habits are common causes of digestive upsets. These and many other unusual, although inconsequential happenings, may so interfere with the intestinal gradient that anorexia, vomiting, diarrhea or constipation results. With the recurrence of one or another upset the distressing symptoms become habitual. From these habitual intestinal disturbances, the syndrome of irritable bowel associated with diarrhea or at least frequent stools, or the more common disorder, constipation, may result.

Constipation is probably the most common of all physical complaints. It may result from improper habits of living and eating. In general, the factors which either singly or in combination, play their role in causing constipation are as follows: (1) nervous fatigue and nervous tension; (2) improper intake of fluid; (3) improper dietary habits; (4) failure to respond to the call to stool; (5) lack of physical exercise, and (6) abuse of the intestinal tract through the excessive use of laxatives.

If then, these are common causes of constipation, it would seem logical that the correction of constipation lies in the suitable adjustment of these factors.

The plan behind any diet for the relief of constipation is to supply material which will be absorbed in the small intestine to only a limited extent and will largely pass on into the colon and add bulk. Thus, the diet should include adequate amounts of fruits and vegetables. In addition, the intake of adequate amounts of fluids, at least 2.5 to 3.5 liters daily, is of the greatest importance. Since it is so difficult for many individuals to include adequate amounts of fruits and vegetables to provide bulk, the tendency in recent years has been to add a variety of hydrophilous colloids to the diet. Such bulk substances include agar-agar, kelp, acacia, tragacanth, karaya gum, psyllium seeds and many others.

It was natural then that a search should be undertaken for a substance of this type which could be taken by mouth and which would have little or no effect in the stomach and small intestine. Methylcellulose* seems to answer these criteria.

METHYLCELLULOSE

Methylcellulose under the trade name of "cellothyl" has been found to be of particular help to patients who have the syndrome of irritable bowel, associated with either obstinate constipation or frequent stools, patients who have overactive intestines in the presence of intestinal stomas, and patients who have malfunctioning intestines from one cause or another.[1]

Methylcellulose is a white, fluffy, cotton-like material dissolving in water to form a colloidal solution. A 0.5 gm. tablet placed in 1 ounce (30 cc.) of lukewarm water dissolves in five to ten minutes, leaving innumerable tiny translucent gelatinous particles 0.5 mm. or less in diameter. It will dissolve in a somewhat similar manner in tap water at room temperature in one and a half hours but in water of this temperature the substance will not actually disintegrate, but will remain as a fluffy substance and when only $\frac{1}{2}$ cup water is used very little of it will disintegrate in a period of one and a half hours. It has been found to pass through the digestive tract unchanged.[2] Studies on animals suggest that ingested methylcellulose remains in the liquid state throughout most of its passage through the digestive tract so that there is no bulk in the stomach or upper part of the small intestine and bulk does not begin to form until it gets to the lower part of the ileum and colon.[3]

A number of patients with the syndrome of irritable bowel associated with diarrhea have taken 4 methylcellulose tablets every four hours by mouth with resultant abdominal comfort and reduction of the number of stools. A large number of patients with the syndrome of irritable bowel associated with constipation have received a similar number of tablets of methylcellulose

* Cellothyl tablets are manufactured by Chilcott Laboratories, Division of the Maltine Company, and contain 0.5 gm. of methylcellulose.

with relief of symptoms. These patients were not afflicted with any ordinary form of constipation, for they had taken large quantities or as some of them said "barrels of laxatives" of one kind or another. The results achieved are all the more striking because the patients whose cases are reported as cases 1 to 5 all felt that there was no hope for the relief of their obstinate constipation.

RESULTS OF TREATMENT

Case 1.—A woman, aged 57 years, from Mississippi came to the clinic in August, 1939. She gave a history of having had dyspepsia, gaseous bloating, belching and obstinate constipation since early childhood. The usual anticonstipation program was instituted with some relief. However, the patient had recurrent difficulty. Finally on September 1, 1948, she resumed the anticonstipation diet and took 4 tablets of methylcellulose before each meal and at bedtime. Complete relief of the indigestion and abdominal discomfort occurred and she continued to pass normal soft, formed stools.

Case 2.—A nun, aged 69 years, complained of obstinate constipation of lifelong duration. The usual anticonstipation program had not been helpful. She had been taking laxatives almost daily for as long as she could remember. Various bulk-producing substances had been given without avail. She was given 4 tablets of methylcellulose before meals and at bedtime and in about a week she began having soft stools and two weeks later she reported that she was having the most satisfactory bowel function that she could ever remember.

Case 3.—The patient, a man, aged 44 years who had severe diabetes, had had trouble with abdominal discomfort, gaseous dyspepsia and constipation for many years. He had used various bulk-producing substances without satisfactory results. Four tablets of methylcellulose by mouth every four hours resulted in normal soft, formed stools at the end of the first week and he continued to have these during the period of observation, which lasted for at least a month.

Case 4.—The patient, a woman aged 62 years, had had a carcinoma removed from the sigmoid in January, 1945. As a result of scarring at the site of the resection narrowing occurred and she had great difficulty with evacuation. After 4 tablets of methylcellulose were added every four hours to the usual diet, the stools were changed from hard pellets to a soft, tapioca-like substance and she passed her stools without discomfort.

Case 5.—A woman, aged 19 years, came to the clinic November 1, 1948, because of lifelong indigestion and obstinate constipation. Her mother stated that she had taken a laxative nearly every day of her life since early childhood. The proctoscopic examination showed a rather large rectum and the roentgenologic examination of the colon suggested the presence of a macrocolon. There was no evidence of real megacolon.

An anticonstipation program was instituted and 6 tablets of methylcellulose were given every four hours. At the end of three days the patient complained of most uncomfortable abdominal distention and examination revealed some abdominal distention. As the patient had no other complaints, the program was continued. At the

end of two weeks she was passing stools daily. The stools were fairly large and of a soft texture. Frequent reports from her since then, up to March 1, 1949, indicate that this happy state of affairs has continued.

In these 5 cases of obstinate constipation of long duration, abdominal distention, and discomfort a striking change for the better followed the administration of methylcellulose.

Case 6.—The patient, a man 50 years of age, had undergone ileosigmoidostomy for constipation at a time when this procedure was used. It had resulted in increased dysfunction rather than relief. The patient had suffered for many years before and after the ileosigmoidostomy. After several weeks on using a suitable diet with the addition of 4 tablets of methylcellulose every four hours, he passed daily a formed, soft stool. Improvement of intestinal function began six days after administration of these tablets was begun and continued for ten days thereafter, during which time he was under our observation. He also reported satisfactory intestinal function during the months immediately after he left the clinic.

Besides the 6 patients whose cases I have reported in detail many others who had diarrhea or similar complaints have been given methylcellulose. Brief mention of them will be made.

Twenty patients who had stomas of the sigmoid and who had loose to watery fecal discharges which caused them considerable discomfort were given 6 tablets of methylcellulose every four hours. Twenty-four hours after this treatment was started, the number of stools decreased and the stools took on a soft jelly-like consistency.

Six patients who had had stomas in the distal portion of the ileum because of chronic ulcerative colitis had numerous liquid discharges from the ileac stoma. Six tablets of methylcellulose, given four times a day, resulted in a definite change from a watery to a jelly-like or gummy discharge. One patient who had been passing fifteen to twenty watery stools daily had a reduction after seventy-two hours of treatment to eight stools in twenty-four hours. The discharges were of a mucoid consistency.

Four patients with high ileac stomas are of especial importance because in these the jelly-like particles could be seen floating in an otherwise liquid stool without any evidence of jellying of the discharge as a whole. This observation tends to confirm the findings in animals; namely, that the solution changes to a jelly as it passes down the digestive tract. In other words, this substance is transformed as water is removed and the concentration of the methylcellulose is increased into a colloidal solution.

In several cases of regional ileitis, the rectal stools changed from loose, watery discharges to soft, jelly-like discharges. However, in cases of advanced ulcerative colitis when much of the large intestine was damaged, the change was

not noteworthy. In fact, in some cases, there was no change whatsoever. The reason for this may have to do with the rapid transport of the food residue through the intestinal tract.

Some individuals who were unhappy because of anal incontinence, or a sphincter paralysis, the result of a spinal injury, were made much more comfortable through the addition of methylcellulose to their intake of food.

COMMENT

Function of the bowel can be greatly improved by the addition of methylcellulose, appropriately prepared, in such conditions as the syndrome of irritable bowel associated with either constipation or diarrhea, intestinal stomas and the milder forms of intestinal infections associated with diarrhea. It is of little value in the severe forms of diarrhea, such as that of extensive ulcerative colitis, possibly because the transport of intestinal content is too rapid to allow the necessary change in the methylcellulose.

It is likely that after the initial control of the intestinal activity, a smaller amount of the methylcellulose will continue the early satisfactory results. Schweig[1] has found that as little as 2 tablets per day will maintain normal function.

The use of methylcellulose does not constitute a so-called cure for the several intestinal dysfunctions discussed. It represents a valuable addition to a well-ordered program of the medical care for the conditions mentioned, which often constitutes the difference between happiness and unhappiness of the individual afflicted with that symptom.

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Fed. Proc.
3:65, 1944

Chronic toxicity of an alkyl ether or cellulose methyl cellulose. ROBERT BAUER (by invitation), ARNOLD J. LEHMAN and FREDERICK F. YONKMAN. *Wayne Univ. College of Medicine, Detroit, Mich.*

Methyl cellulose is a water soluble derivative of cellulose synthesized in the cold by the interaction of methyl chloride and an alkaline solution of cellulose. Purified methyl cellulose produces a clear colloidal jell when combined with water. It was by the use of this jell that the experimental diets described below were mixed, dried and ground. For chronic studies four groups of five white rats were employed, three groups of females and one of males. These animals weighed between 45 and 50 grams each at weaning. Male rats were included for the purpose of studying reproduction and effects of methyl cellulose on the second generation under similar experimental conditions. One group of females served as the control and had free access to Steenbach's whole artificial diet, and the remaining groups were placed on this basic diet in which 1.66 per cent (Dow Chemical Company), 1.66 per cent (Hercules Powder Company) and 5 per cent (Hercules) methyl cellulose had replaced carbohydrate. Each rat, then, in respective groups consumed per day of these experimental diets 0.17, 0.17 and 6.2 grams/kgm. of methyl cellulose. The experiment lasted for one hundred and eighty-four days.

Growth and food intake curves showed that the experimental groups as compared to the controls gained weight more rapidly, attained greater average weight at the conclusion of the experiment, and consumed more food per day. Gross examination of the feces of rats on the methyl cellulose diet showed a mineral oil type of stool. Pathologic examination of one rat from each group revealed no gross or microscopic effects.

It would seem, then, from this experiment that:

(1) methyl cellulose as synthesized by domestic chemical industries is nontoxic in the quantities used, (2) to secure sufficient nutrition the experimental rats must eat more bulk, and that, (3) due to the increased bulk and probably a better utilization of food by improved large bowel elimination, a more rapid growth and greater body weight is attained.

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Fed. Proc.
4:112, 1945

Methyl cellulose: its laxative action and effects of chronic feeding on growth and reproduction. ROBERT O. BAUER (introduced by A. J. Lehman). *Wayne Univ. College of Medicine, Detroit, Mich.* In young rats the time of passage of food through the gastrointestinal tract was the same in the animals fed methyl cellulose as in the controls. With advancing age, the passage time in the methyl cellulose fed rats was greater than in the controls. Two dogs which were fed 2 to 100 grams of this compound daily for one month showed no significant increase in the weight of the stools for the different levels of intake. The increase was almost exactly that of the dry weight of the agent administered. In man 2.5 to 5.25 grams of methyl cellulose taken orally as gels in 250 c.c. of water had a tendency to be mildly obstipating.

Rats fed 5 per cent of methyl cellulose in a basal diet through three generations showed a definitely greater average increase in body weight than did the controls. Reproductive functions were not impaired. Paired feeding experiments in which 50 per cent of the food intake consisted of the compound, indicated that some loss of weight occurred This effect could be ascribed entirely to the inability of the rats to ingest sufficient quantities of the diet to maintain normal development.

Formic acid was determined in the urines of the dogs and paired rats. Only minute quantities were found which were within the normal variations of the method employed.

It is concluded that methyl cellulose is non-toxic per os, but is a poor substitute for the accepted colloidal laxatives. Methanol is probably not formed as a degradation product after oral ingestion of methyl cellulose.

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Chronic Toxicity Studies on Methylcellulose in Rats*

By ROBERT O. BAUER† and A. J. LEHMAN‡,§

A standard diet of 5 per cent methylcellulose fed to rats for thirty-two weeks (averaging 690–775 mg./Kg./day/rat during this period) has no deleterious effect on growth, and significantly increased dietary intake. Also, it neither inhibited reproduction nor left evidence of pathologic change in the tissue. Fifty per cent of the diet replaced by methylcellulose significantly depresses growth through nutritional want. Methylcellulose is not hydrolyzed to cellulose and methanol in the intestinal tract of rats to any detectable extent. Methylcellulose *per se* does not appear to be absorbed from the intestinal tract of rats.

THE RECENT application of derivatives of methylcellulose as laxatives in clinical medications suggested that it would be worth while to publish chronic toxicity data obtained eight years ago. The purposes of this study were to determine any alteration of growth, food intake, fecal output, urinary formic acid production, or reproduction produced by feeding various amounts of methylcellulose to albino rats.

METHOD

Chronic Oral Toxicity.—Young albino rats of the Sprague-Dawley strain, thirty-five to forty days old and weighing 55–84 Gm., were divided into three groups of ten (five males and five females) and fed diets containing, respectively: (*a*) 5% methylcellulose (high MC diet); (*b*) 0.17% methylcellulose (low MC diet) (changed on the forty-second day of the experiment to 0.5% methylcellulose); and (*c*) no added ingredient (standard diet). The experimental diet was prepared by mixing an aqueous solution of high viscosity methylcellulose (1) with Steenbock's diet (2), spreading the resulting mixture on metal sheets, drying at room temperature, and grinding to a fine powder. The rats were segregated according to sex. They were kept in cages containing groups of five, with water and food *ad libitum*. Group body weights, and food and water consumption were recorded weekly. After about eight months on the experimental diet, the rats were mated according to the method described by Farris (3). The young of the f_1,f_2, generations were divided as above, placed on 5% methylcellulose diet, and later mated in the same manner as the f_0 generation. Representative animals from each group of the first generation were sacrificed at eight months of age for macroscopic and microscopic examination.

In the second experiment, nine young Sprague-Dawley rats, litter mates of the f_1 generation, divided according to sex into three males and six females and weighing between 55 and 116 Gm., were placed on a modified paired feeding regimen. This system was employed in order to determine the effect of massive dosage and the "bulk" producing effects of the questionably inert substance in contrast to one (cellulose flour) shown to be inert and to possess only a "bulk" effect when included in the diet (4). The nine animals were divided into three groups. Rat 1 in each of the three groups received, *ad libitum*, a diet of equal parts of the nutrient and of methylcellulose (50% methylcellulose

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† Present address: Department of Pharmacology, Boston University School of Medicine, Boston, Mass.
‡ Present address: Division of Pharmacology, Food and Drug Administration, Washington 25, D. C.
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diet). Rat 2 received, *ad libitum*, a diet of equal parts of the nutrient and cellulose flour (forming the "bulk" control), and rat 3 was given the nutrient alone (nutritive control), but in an amount equivalent to the nutrient consumed by rat 1 of the respective group the preceding day. The basic nutrient consisted of a modified Steenbock's diet as follows: 36% yellow ground corn meal, 20% linseed cake meal, 20% casein, 10% lard, 6% cod liver oil, 4% powdered alfalfa, 3% bone ash and 1% sodium chloride.

Each animal was housed in an individual metabolism cage, and daily records were kept of the urine volume, feces weight, and food and water consumption. Pooled seven-day urine samples from each rat were subjected to quantitative formic acid determinations by the method of de Eds (5).

RESULTS

The results of the chronic eight-month methylcellulose rat feedings are tabulated in Table I. These results were obtained by treating the data by the Zucker "Weight-age Relation" (6) as modified by Gray and Addis (7). These workers demonstrated that if one plots the log of the weight in grams ($\log_{10} W$) against the reciprocal of age in days ($1/d$), a straight line is obtained and k is the slope of this line which characterizes the normal rate of growth [$k = (\log W_2 - \log W_1)/(1/d_1 - 1/d_2)$]. Their formula for the straight line is: $\log_{10} W = -k(1/d) + \log A$, where A is the body weight approached asymptotically during growth. The rat continues to grow asymptotically until death, chiefly due to unclosed epiphyses. If external variables are superimposed on the normal growth pattern so as to depress growth, the k and A values become smaller. The k and A values of either sex fed 5% or less of methylcellulose do not differ significantly from their respective controls. The k and A values were considered significantly different if experimentals varied from controls by more than two standard deviations (7).

Both experimental groups ate significantly more food than did the controls. The same general trend held for succeeding generations. The final average intake of methylcellulose for males and females, respectively, was 690 and 775 mg./Kg./day given the high MC diet, and 61 and 57 mg./Kg./day given the low MC diet (see Table II).

TABLE II.—AVERAGE DAILY FOOD CONSUMPTION PER RAT FED METHYLCELLULOSE

Group of 5 Animals	Sex	F_0 Generation Mean and S. D.[a]	p Value
Control	F	8.0 ± 1.1	...
Low MC diet	F	11.4 ± 2.0	<0.02
High MC diet	F	15.5 ± 6.3	<0.01
Control	M	10.5 ± 3.2	...
Low MC diet	M	12.2 ± 1.5	<0.02
High MC diet	M	13.8 ± 3.3	<0.01

[a] Mean and Standard Deviation in Gm.

All three generations of mated rats produced litters of 8 to 11 vigorous, healthy pups. All animals were weaned at one month of age, by which time each had reached a weight of 55–60 Gm. The animals of the f_1 and f_2 generation, following weaning, were fed the high MC diet for four months. The animals of the f_1 generation grew at a rate comparable to that of the f_0 generation when fed this diet (Table I).

Macroscopic and microscopic pathologic examination of representative animals of each group of the first generation revealed essentially normal tissues. Deposition of abnormal material in the tissues was not observed.

In order to obtain still higher intake of methylcellulose, the paired feeding experiments were conducted.

Table III presents the results of this experiment. It is clear that animals on *ad libitum* intake of nutrient diet grew much more than did animals on 50% methylcellulose. However, animals on restricted diet (nutrient limited to that taken by the methylcellulose group) grew indistinguishably from: (*a*) the test animals, or (*b*) the cellulose flour group. There is no statistical difference between nutrient intake of the three groups during the test period.

At the end of ninety days the three special diets were terminated. The animals were placed on standard diet *ad libitum* for forty additional days. The k values, during this time, increased fivefold and A exceeded the control value (Table IV). Figure 1 demonstrates the plotted data for the male

TABLE I.—WEIGHT-GROWTH SLOPES AND ASYMPTOTES OF METHYLCELLULOSE-FED RATS

Groups of 5 Animals	Weight, Gm. Start	Weight, Gm. Finish	Age, Days Start	Age, Days Finish	k	A
			F_0 Generation Females			
Controls	76	183	39	272	17.2	210
High MC diet	76	212	39	238	20.6	256
Low MC diet	79	211	40	226	20.3	254
			F_1 Generation Females			
Control diet	55	170	36	163	22.6	234
High MC diet	55	200	36	163	25.9	292
			F_0 Generation Males			
Control diet	56	258	33	210	30.0	572
High MC diet	60	310	35	176	31.1	461
Low MC diet	84	291	41	267	26.7	379
			F_1 Generation Males			
Controls	55	258	36	163	31.0	428
High MC diet	55	305	37	163	34.4	496

k = Slope of growth curve.
A = Estimated asymptote or limit approached by body weight in Gm.

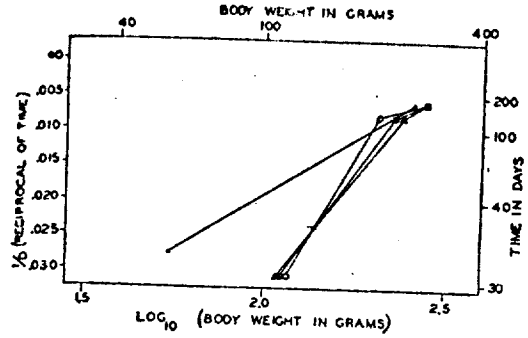

Fig. 1.—Male weight-growth curves methylcellulose-paired feedings.

Control ●——●, nutritive control ⊙——⊙, 50% methylcellulose ○——○, 50% cellulose flour ▲——▲, paired rats continued on standard diet ■——■. The greater the slope, small k value, the greater is the growth depression, and, likewise, a resulting small A value at infinity.

animals given in Tables III and IV. It is concluded that the weight-growth curve depression of the bulk-containing diet is the result of nutritional want, and not the result of specific toxicity of the methylcellulose or cellulose flour.

The fecal weights from the animals whose daily diet consisted of 50% cellulose flour were 60 ± 2% of their dietary intake, whereas the fecal weights of the animals fed 50% methylcellulose were significantly higher (p = <0.02) than the former, being 73 ± 5% of the respective dietary intake. The fecal texture of the cellulose flour-fed animals was hard and dry, whereas those animals fed methylcellulose produced fecal pellets which were larger than average, soft and translucent. This translucent character is observed in highly concentrated "solutions of methylcellulose." The control rats' feces were scanty, soft, and mushy. The daily weights of the latter's feces ranged from 0.1 to 1.5 Gm. with a mean of 1.16 ± 0.23 Gm., in contrast to the 7.9 ± 1.82 Gm. average of the experimentals, or 7.9 ± 1.85 Gm. average of the cellulose flour rats' feces. It is apparent from the foregoing that the bulk-producing portions of the nutritive control diet had been reduced to a minimum, and this diet was essentially all nutritionally available to the rat.

The urinary output of the methylcellulose (3.1 ± 0.8 cc.) and cellulose flour (4.2 ± 0.8 cc.) rats was about 50% that of the nutritive controls (7.3 ± 2.1 cc.). The lower average renal fluid excretion of the experimental group probably reflects the amount of fluid excreted with the bulk of the feces, due to the hydrophilic property of methylcellulose. In contrast, the control animals required small amounts of fluid to facilitate defecation and thus eliminated most of the fluid by way of the kidneys.

The values of urinary formic acid obtained from weekly pooled urine specimens were not constant. In several specimens formic acid was not detectable. The most notable instances of the absence of formic acid in the urine were at the beginning and end of the experiment when the weather was cool. The highest weekly levels were obtained during the periods of hot, humid weather. The methylcellulose rats were less consistent in their production of formic acid than the other groups, having detectable levels in only eight out of thirteen weeks during which the experiment was conducted. The cellulose flour group of animals was the most consistent in their production of formic acid and showed no sex difference (see Table V).

In spite of the irregularity of formic acid detection of the urine, the differences between means ($\bar{x}$)

TABLE V.—PAIRED RAT URINARY FORMIC ACID LEVELS

Rat Group	Formic Acid[a]	p Value
Female controls	3.71 ± 2.80	...
Female 50% cellulose flour	2.07 ± 1.73	0.05
Female 50% methylcellulose	1.84 ± 1.24	0.02
Male control	0.56 ± 0.46	...
Male 50% cellulose flour	2.19 ± 2.60	0.01
Male 50% methylcellulose	0.81 ± 0.86	0.5

[a] Mean and standard deviation in mg. of thirteen weekly observations.

TABLE III.—WEIGHT-GROWTH SLOPES AND ASYMPTOTES OF PAIRED METHYLCELLULOSE FEEDINGS

	No.	Weight, Gm. Start	Weight, Gm. Finish	Age, Days Start	Age, Days Finish	k	A
Female Groups:							
Control	5	55	170	36	163	22.6	234
Nutritive control	2	95	155	36	131	10.3	184
50% Cellulose flour	2	95	164	36	131	11.7	201
50% Methylcellulose	2	76	131	36	131	12.6	164
Male Groups:							
Control	5	55	258	36	163	31.0	428
Nutritive control	1	113	229	32	127	13.0	290
50% Cellulose flour	1	110	240	32	127	14.4	312
50% Methylcellulose	1	116	205	32	127	10.5	249

TABLE IV.—PAIRED METHYLCELLULOSE RATS RETURNED TO STANDARD DIET

	No.	Weight, Gm. Start	Weight, Gm. Finish	Age, Days Start	Age, Days Finish	k	A
Males	6	149	191	131	171	58.95	422.5
Females	3	224	280	127	167	50.29	559.6

as well as paired data were in two instances significant. The female 50% methylcellulose rats had a mean detectable level about one-half that of the female controls, whereas there was no significant difference between the male formic acid values of these two groups. The male 50% cellulose flour animals produced about four times more formic acid than did the male controls. No difference could be found between the male control and the male 50% methylcellulose rats.

Mated females of the paired feeding experiment produced litters of 6 to 12 healthy young pups which were fed Steenbock's diet containing 5% methylcellulose for the four months following weaning. No abnormalities were noted in the animals' growth.

DISCUSSION

Deichmann, *et al.* (8), using the oral route have reported methylcellulose harmless to albino rats when given 440 mg./animal/day for eight months. Although the chronic-fed rats of this experiment consumed about one-third as much methylcellulose as Deichmann's animals, the dosage was continued in the present work through three generations without evidence of impaired growth or reproduction. A recent publication by Hodge, *et al.* (9), using longer chronic oral methylcellulose feedings, bears out this contention. Pathologic examination failed to reveal deposition of the material in body tissues. By contrast, Hueper, *et al.* (10), reported that intravenous administration of 5% solutions led to retention and deposition in various organs. Therefore, it is unlikely that the methylcellulose in these experiments was absorbed from the gastrointestinal tract.

On the basis of urinary formic acid studies, evidence was sought to show that methylcellulose was degraded in the intestinal tract. Conclusive evidence was not obtained. In our experiments, formic acid appeared in urines of both control and methylcellulose-fed animals. It seems likely that the appearance of formic acid in the urine was due to substances other than methylcellulose. In addition, the inconsistency of detection of formic acid does not permit sound conclusions as to the source of this substance. Both methanol and formic acid have been found in the urine following the ingestion of small amounts of methanol arising from the hydrolysis of the methyl esters of pectins present in many common foods (11, 12). Components of Steenbock's diet have small amounts of pectins as part of their composition. The highest urinary formic acid levels were detected during the hot, humid weather. Increased bacterial activity of intestinal flora acting on the pectins with production of free methanol could explain the elevated formic acid values during the hot weather. It is conceivable that absorption of the formic acid precursor, methanol, by the colloidal substance methylcellulose could block the absorption of methanol by the digestive tract. The significantly lower mean urinary formic acid values of the female 50% methylcellulose-fed rats compared to the female controls support this interpretation. The data for the corresponding two animals in the male group do not conform. Similarly, the fate of methylcellulose in the human digestive tract as discussed by Machle, *et al.* (13), concluded that practically all of the methoxyl ingested in a single dose of methylcellulose could be recovered from the feces, irrespective of the nature of the diet of the subject. On a normal or low-roughage diet the agent passes the digestive tract, in large part, unaltered. Urinary formic acid did not rise in these experiments after methylcellulose was fed to the rats, but remained at levels less than or the same as the control animals.

Individual differences, both as to sex and variety, in the formation and excretion of formic acid apparently depend in part on the individual's ability to detoxify methanol by other routes than catabolism to formic acid. The variability is well illustrated by the sex differences of the paired feedings.

CONCLUSIONS

Methylcellulose, fed to rats over three generations in quantities amounting to slightly less than 5.0 and 0.5 Gm. per Kg. body weight, has no deleterious effect on growth, and significantly increased dietary intake. Also, it neither inhibits reproduction nor leaves evidence of pathologic changes in the tissues. Fifty per cent of the diet replaced by methylcellulose significantly depresses growth through nutritional want.

Methylcellulose is not hydrolyzed to cellulose and methanol in the intestinal tract of rats to any detectable extent.

Methylcellulose *per se* does not appear to be absorbed from the intestinal tract in rats.

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IDENTIFICATION BY PAPER CHROMATOGRAPHY OF SWELLING AGENTS IN FOODSTUFFS

Testing for swelling agents or thickeners in pure form presents no difficulties. A compilation by Letzig (1) lists a number of precipitating reactions which permit distinguishing between the several thickeners in simple manner. However, these compounds seldom exist in such pure form that the use of such precipitating reactions, presented in synoptic form, permits non-ambiguous conclusions. Even the occurrence of two different swelling agents simultaneously can make a clear identification difficult; when there are more than two, this is true even more so. However, not only a mixture of these substances complicates the tests but also the presence of other compounds among which the proteins are predominant. Letzig's reagents are mainly protein precipitants so that precipitation will always occur in the presence of proteins. It is easy, of course, to determine the presence of proteins but this does not yet allow us to decide whether, in the absence of proteins, precipitation will have been due to thickeners. Prior to testing, proteins must therefore be removed and Wyler (2) has already described a possibility for this, specifically for meat products. This suggested removal of proteins with the aid of Carrez precipitation is so thorough, however, that not only are many swelling agents precipitated for which we must thus test prior to precipitation but the possibility of identifying those still remaining in solution is also restricted to a very few. A method of complete removal of proteins without interfering with subsequent testing and identification of swelling agents is not yet known so that the Letzig reagents in practice unfortunately have only a limited importance. As seductive as such tests are in their simplicity, they alone very rarely furnish non-ambiguous

indications of the kind of the swelling agents present. There is a possibility of precipitating the proteins with alcohol which can subsequently be easily evaporated (e.g. in vacuum) but this has the disadvantage that the proteins are not always completely eliminated and that some thickeners, specifically carob-seed flour, are also precipitated.

These disadvantages raise the question of different testing methods and Letzig already indicates in the report cited that the application of paper chromatography promises success. Since most thickeners are constituted by carbohydrates, no difficulties exist from the point of view of paper chromatography; it is merely necessary to convert the test substance into a form suitable for paper-chromatographic testing. It may happen, however, that with several swelling agents present, a non-ambiguous conclusion on the kind of the individual substances is not possible since a number of thickeners have a similar composition and/or furnish similar cleavage products after hydrolysis. In that case, however, a combination with the precipitating reagents of Letzig would permit us to continue further since the decision will lie between the few still possible thickeners. Whereas the proteins do not interfere with such paper-chromatographic tests, we must first test for the presence of carbohydrates not originating from swelling agents. Admixtures of simple sugars easily determinted with the aid of paper chromatography, can generally be removed by extracation with 50-% alcohol which does not affect the swelling agents. It is sufficient to let the substance stand with the alcohol for several hours under occasional shaking. The extract and/or the filtrate can serve to test for sugar (3) whereas the thickeners can be determined from the residue.

Hydrolysis of Swelling Agents

Since thickeners do not directly respond to paper chromatography, it is necessary to convert them to a form which is accessible to this method of testing. Among the possibilities of hydrolytic cleavage, that using enzymes is excluded and,

since carbohydrates are involved, acid hydrolysis is indicated primarily in order to prevent decomposition of cleavage products. The following conditions have been shown to be suitable and have been steadily employed for some time:

10 g of pure swelling agent and/or test substance are diluted with 50 ml of water and 50 ml of a 10-% sulphuric acid solution. If the mixture is too thick so that the retort may burst under direct heating, it should be placed in a hot water bath for a short time. When fluid, the solution is boiled in a reflux condenser where duration is a function of the kind of the probable thickeners since each substance is hydrolyzed at different rates of speed. If no indication of the type of swelling agent exists, the solution is kept at boiling temperature for three hours. It can then be directly chromatographed. However, it is preferable to concentrate if there is only a small amount of test substance; sulphuric acid also interferes somewhat, specifically with uronic acids, so that it is preferable to remove the former. For this purpose, 30 g barium hydroxide (with 8 mol water) are dissolved in 100 ml of water under heating and dripped, under constant shaking, through a filter into the hot hydrolytic solution until the latter reaches a p_H of 7. With these generally known conditions for removal of the sulphuric acid, it is easy to adjust the p_H-value as above by spotting Merck indicator paper. The precipitated barium sulphate can be rapidly filtered out, after settling, through a standard filter. Since only small amounts are required, it is sufficient if a part of the filtrate is processed further. This part is concentrated under vacuum to 50% and thus reaches again the starting concentration. If only small amounts of swelling agent are available, these can be further concentrated as desired. This solution is now suitable for paper chromatography. It is not at all necessary to keep within the quantitative relations indicated above and they can all be reduced correspondingly which is of value when only small amounts of test substance are available.

Paper-Chromatographic Conditions

Paper chromatography always takes place under the conditions already described earlier (3 & 4). The fluxing agent is n-butanol/pyridine/water (3:2:1.5) and staining is done with phtaleic acid/aniline and/or naphthoresorcinol/trichloroacetic acid. The last-named staining for non-reducing sugars plays a subordinate role in this connection. The chromatograms thus obtained with the ascending method are shown in the figure at the end of the article. The parallel obtained test solutions permit non-ambiguous identification so that even closely adjacent spots cannot be confused with each other which may easily occur when working exclusively with R_f-values.

Evaluation

Fig. 1 shows two chromatograms of some frequently occurring swelling agents. It will be clearly seen that, after hydrolysis of carob-seed flour, only the spots of galactose and mannose appear. Although mannose is nearly equal in R_f-value to mannose, it can be distinguished from the reddish arabinose by its brown color tone but these colors cannot be recognized in a black-and-white photo. With agar-agar, only galactose is clearly seen whereas xylose is manifested only as a weak spot. Gum arabic shows galactose in addition to arabinose and rhamnose. Although rhamnose is recognized only vaguely, it is characteristic since it is only infrequently found in thickeners. In the sample examined by us, there occurs further a spot immediately below the starting point which we were never able to identify. Pectin shows mainly the spot of uronic acid which can be recognized from the typical light-brown color after staining with phthalate/aniline as well as by staining with naphthoresorcinol/trichloroacetate when it turns slightly blue after some time (cf. 3 for details). We know that this involves galacturonic acid which paper chromatography cannot demonstrate since sufficient separation from glucuronic acid does not take place. The R_f-values of the two uronic acids do differ slightly and we might therefore assume that differentiation would be possible after sufficient time. Here we must take into account that the R_f-values of the two

acids are to a considerable extent a function of the concentration of these acids and of the kind of an eventually existing cation. Something similar has already been shown for some other organic acids (5). The variations so caused are greater, however, than the difference between the two R_f-values. In addition to the spot of galacturonic acid, a weak spot of glucose also occurs with pectin. It must be remembered further that a duration of hydrolysis of three hours must be strictly kept, in contrast to most other swelling agents. Whereas a duration of hydrolysis of one to two hours generally produces complete cleavage in the latter, galacturonic acid in pectin shows only weakly after one hour but increases with continued hydrolysis. Tragacanth gum shows the spots of galactose, arabinose and xylose. Comparison with the test solutions which were run parallel on the left chromatogram, makes possible rapid identification of the individual spots. The first test solution on the left shows the upper spot as galactose and the lower spot as mannose; the second test solution indicates the upper spot as arabinose and the lower as rhamnose. We already mentioned that arabinose and mannose cannot be differentiated in the figure since it is not possible to see the differences in color. The chromatogram makes it impossible to mix up the red color of arabinose with the brown color of mannose. The last test solution shows galacturonic acid at the top and, below this, xylose.

The chromatogram on the right of the figure shows "tylose" (methyl cellulose) on the left side which shows as expected the spot of glucose and below this non-hydrolyzed methylated glucoses. The occurrence of these methylated glucoses was in several preparations and appears to be characteristic for most of the tylose preparations. It is possible, however, that this is a function of manufacture and that these spots may therefore be absent some times. The adjacent cellulose glycolate also shows the spot of glucose and, in the upper part of the chromatogram, two and occasionally three unidentified spots which always have a characteristic semilunar form. Alginate exhibits only the spot of a uronic acid. The adjacent test solutions show in the

first case from top to bottom the spots of lactose, maltose and glucose whereas glucuronic acid is shown on the left.

When several thickeners are present, identification on the basis of the chromatogram can become difficult. It then seems preferable to make identification less through the spots that do show but initially through those absent. The absence of uronic acid permits a definite conclusion that neither pectin nor alginate exist. However, we believe sufficient concentration of the hydrolyzed solution must be made in order to avoid that a uronic acid does not show by reason of excessive dilution. The absence of galactose excludes the presence of carob-seed flour, agar-agar, gum arabic and tragacanth. Absence of glucose means that neither tylose nor cellulose glycolate, generally also no pectin and obviously no swelling starch can be present. Lack of mannose would exclude carob-seed flour and that of arabinose would eliminate gum arabic and tragacanth. Here we must take into account, however, that the presence of one of the two last-named spots does not permit a conclusion on the absence of the other since a possible superposition may exist which does not allow this.

With this method of selection, most of the known swelling agents can easily be excluded so that we do obtain in general a non-ambiguous picture of the composition of the mixture of swelling agents. Where in complicated cases several interpretations are possible, we can turn to the precipitating reactions Letzig mentioned above for clarification of any remaining doubts, in spite of the interferences indicated. When direct interpretation is not possible, the method of selection has proved itself also for the Letzig reactions. In most cases, however, paper chromatography should be sufficient or at least give decisive indications. As in most investigative methods, a certain intuitive judgment will develop with practice.

As described, such testing for swelling agents is obviously restricted only to those whose composition includes carbohydrates. Although these represent the greater number, this does not allow us to neglect others, e.g., gelatine. Testing for such substances

by specific paper chromatography would evidently have to be carried out from other viewpoints.

Conclusion

1. The precipitating reactions in testing for swelling agents are excellent for pure compounds but rarely furnish non-ambiguous findings for mixtures and in the presence of other substances, e.g. proteins.

2. Since most of the swelling-agents are constituted by carbohydrates, paper chromatography of the sugars and/or sucroid compounds offers the possibility of identifying swelling agents by hydrolytic cleavage.

3. Hydrolysis is made with sulphuric acid and boiling at an average duration of 3 hours. After removal of sulphuric acid by barium hydroxide and concentration of the clear filtrate under vacuum, the test is made with a method described in an earlier communication.

4. Evaluation is best made by a method of selection in which all swelling agents are excluded which cannot exist due to the absence of a spot. However, it is necessary to take into account the ratios of concentration.

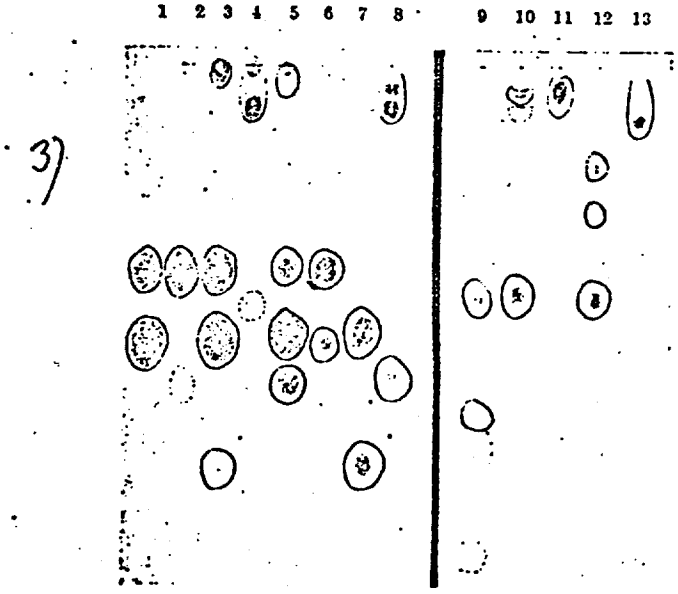

Abb. 1. *Mit Schwefelsäure hydrolysierte Quellstoffe und entsprechende Testlösungen.* Fließmittel: n-Butanol/Pyridin/Wasser (3:2:1,5). Färbung: Phthalsäure/Anilin. *Linkes Chromatogramm:* (von links) *1* Johannisbrotkernmehl, *2* Agar-Agar, *3* Gummi-arabicum, *4* Pektin, *5* Tragantb.Testlösungen: *6* Galaktose + Mannose, *7* Arabinose + Rhamnose, *8* Galakturonsäure + Xylose. *Rechtes Chromatogramm:* (von links) *9* Tylose, *10* Celluloseglykolat, *11* Alginat. Testlösungen: *12* Lactose + Maltose + Glucose, *13* Glucuronsäure

Fig. 1 - Chromatogram of swelling agents hydrolyzed with sulphuric acid and the resulting test solutions. Fluxing agent: n-butanol/pyridine/water (3:2:1.5). Staining: Phthaleic acid/aniline. Left side: 1= carob-seed flour; 2 = agar-agar; 3 = gum arabic;

4 = pectin; 5 = tragacanth; test solutions: 6 = galactose + mannose; 7 = arabinose + rhamnose; 8 = galacturonic acid + xylose. Right side: 9 = tylose; 10 = cellulose glycolate; 11 = alginate; test solutions: 12 = lactose + maltose + glucose; 13 = glucuronic acid.

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EFFECT OF SODIUM CARBOXYMETHYLCELLULOSE ON THE BLOOD CELLS*

IRVING B. BRICK, M. D.** Washington, D. C.

IN VIEW OF the recent therapeutic usage of sodium carboxymethylcellulose (sodium cellulose glycolate) in such diverse clinical conditions as constipation (1, 2, 3), peptic ulcer (4, 5), in the modification of digitoxin action (6), and in controlling nausea and vomiting caused by terramycin (7), a thorough study of the effects of sodium carboxymethylcellulose on the blood cells was thought to be of value. Since this substance has been used as a thickener for foodstuffs since 1924 (5) and is finding increased use in the food industry as a stabilizer, a study in patients that could be given large doses of the drug and could be carefully followed with a serial blood studies seemed particularly desirable.

*From the Department of Medicine, Georgetown University Hospital and School of Medicine, and the Home of Incurables, Washington, D. C.

**Assistant Professor of Medicine, Georgetown University School of Medicine.

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METHOD OF STUDY

The subjects were selected from among the patients of an institution for chronic diseases. The patients chosen did not have anemia or constitutional disease affecting the hematopoietic system with one exception, a patient with polycythemia of moderate degree. Some of the patients were ambulatory, others were bedridden. There were in constant attendance either registered or practical nurses who assisted in the distribution of the medication, in addition to their regular duties. In this way a careful check of drug administration and ingestion was possible. In the control or foreperiod of two weeks two complete blood counts per

TABLE I

Case No.	*Sex*	*Age*	*Disease*	*Control Period* R.B.C. X10⁶	Het. VOL %	W.B.C. X10³	*End of Study* R.B.C. X10⁶	Het. VOL %	W.B.C. X10³	*Total Dose (GMS)*	*Remarks*
1.	M	71	Paget's Disease & Osteoarthritis	5.2	42.0	11.2	5.3	44.5	10.85	1940	Constipation relieved
2.	M	42	Birth Injury	5.3	44.0	8.7	5.5	45.5	6.25	1940	
3.	M	60	Cerebral Atrophy	5.5	47.5	8.6	5.3	43.5	10.2	1940	
4.	M	49	Parkinson's Disease	5.2	43.0	6.9	5.4	47.5	6.8	1940	
5.	F	56	Multiple Sclerosis	4.7	40.0	4.6	4.5	40.0	8.2	1940	
6.	F	80	Fractured Femur	5.2	42.0	7.5	5.4	43.5	7.1	1940	
7.	F	79	Osteoarthritis	5.1	47.0	6.6	5.8	45.0	7.6	1940	
8.	M	82	Arteriosclerosis	5.2	44.1	9.0	4.8	40.0	7.8	1940	
9.	F	75	Arteriosclerosis	4.7	35.0	7.3	4.3	38.0	5.2	1940	
10.	F	68	Parkinson's Disease	4.7	39.1	6.1	5.1	42.0	8.2	1940	Constipation relieved
11.	F	68	Metastatic Carcinoma of uterus	4.4	40.0	5.0	4.7	40.5	9.6	1940	
12.	M	67	Diabetes Mellitus	5.9	47.0	6.9	6.0	47.5	6.0	1806	Transient diarrhea
13.	F	61	Poliomyelitis	5.1	40.6	5.0	5.2	41.0	7.6	1346	Diarrhea—dose reduced
14.	F	70	Osteoarthritis	4.8	40.0	7.2	5.3	44.5	7.5	1268	Diarrhea—dose reduced
15.	M	63	Birth Injury	5.1	44.0	8.7	5.4	42.5	7.6	1262	Dose reduced
16.	M	27	Friedreich's Ataxia	5.7	44.0	5.8	5.4	45.0	11.2	1110	Intermittent diarrhea
17.	F	58	Multiple Sclerosis	5.3	42.0	5.8	5.4	42.0	8.5	982	Diarrhea—dose reduced
18.	F	80	Transverse Myelitis	5.1	39.5	6.7	5.2	41.5	9.6	866	Abdominal discomfort —dose reduced
19.	M	79	Polycythemia Vera and Buerger's Disease	7.5	59.5	10.9	7.1	62	12.1	786	Transferred to another hospital
20.	F	56	Osteoarthritis	5.2	43.0	9.9	5.2	22.0	7.4	694	Refused to continue
21.	F	80	Osteoarthritis	4.6	41.0	5.8	5.1	41.5	8.0	460	Refused to continue
22.	M	78	Parkinson Disease	5.1	41.5	7.7	5.3	42.5	7.8	440	Refused to continue

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week were done. A similar number of urinalyses were performed. After the beginning of drug administration, a complete blood count was done every two weeks until the cessation of the test period. In selected patients, bone marrow study was done early in the period of drug administration and at the completion of the study.

A complete blood count consisted of red blood cell count (two counts were done and averaged for the result), white blood cell count and differential smear, hematocrit, hemaglobin and buffy coat determinations. Red and white blood cell pipettes certified by the United States Bureau of standards, a Fisher Electro-Photometer Hemoglobinometer, and Wintrobe hematocrit tubes were used. All of the laboratory work performed was supervised by Dr. Charles E. Rath, Director, Hematology Laboratory, Georgetown University Hospital. Each of the patients had been examined prior to the institution of drug administration and diagnosis recorded.

A daily dose of 10 grams of sodium carboxymethylcellulose given in the form of wafers each containing 2 grams of the drug was started in each patient. Eleven patients continued this dose without change for the test period of six months. In other cases reductions in dose had to be made, usually because of diarrhea, loose bowel movements, or lack of cooperation on the part of the patient in continuing prolonged therapy with a daily large dose.

PREVIOUS STUDIES ON THE BLOOD IN ANIMALS AND HUMANS

Rowe, Spencer, Adams and Irish (8) studied the effect of feeding sodium carboxymethylcellulose in varying dosages on rats for a period of eight months. No discernible ill effects from large amounts could be found as determined by growth curves, organ weights, gross observations and histopathological examinations. While the blood cells were not specifically studied, the spleen was not affected. Shelanski and Clark (9) examined the blood of 10 rats and 10 guinea pigs during and

TABLE 2

Case No. 2 **Age 42**

Diagnosis: Injury at birth, muscle incoordination, eyes and hands.

Blood

Date	Medication	HGB Gms.	RBC Millions	WBC	Hematocrit	Buffy Coat	Diff-Smear % Lymphs	Mono.	Eos.	Baso.	PMN Mature	PMN Bands
5-2-51	Control	16.6	5.35	9,200	46	.6	12	1	2		75	
5-15-51	Control	16.4	5.34	8,100	42	.5	23	1			77	
5-25-51	Control	15.8	5.25	8,750	44	.6	25		3		72	
5-5-51	Control	14.8	5.46	7,400	47	.5	26	1	2		71	
7-20-51	10 g/day	17	5.61	7,700	46.5	.5	24	3			73	
8-1-51	10 g/day	16.2	5.60	8,500	45	.5	21	3	2	1	71	2
8-21-51	10 g/day	16.5	5.79	8,050	48	.6						
9-11-51	10 g/day	16.5	5.85	7,750	47	.6	18				82	
9-25-51	10 g/day	16.4	5.65	7,500	45	.6	20	2	3	2	72	1
10-8-51	10 g/day	15.2	5.40	7,800	46.5	.5	22	1	1		76	
10-22-51	10 g/day	15.8	5.39	7,250	45.5	.5	21	2	1	1	75	
11-5-51	10 g/day	15.5	5.40	8,250	46.5	.5	26		3	1	70	
11-22-51	10 g/day	16	5.46	7,500	45	.5	28	2	1	1	68	
12-6-51	10 g/day	15.4	5.39	5,500	43	.5	16	3			81	
12-20-51	10 g/day	15.2	5.60	8,000	43.5	.5	24	4	2		70	
1-3-52	10 g/day	15.6	5.55	6,250	45.5	.5	20	3	1		77	

Urine

Date	Color.	Sp. Gr.	React.	Alb.	Sugar	Per HPF Centrifuged Clumps Wbc	Rbc.	Wbc.	Casts	Crystals
5-17-51		1.025	5	+ —	—		1-2	occ.	occ.	hyaline
5-24-51		1.020	5	0	—		1-2	1-2	occ.	hyaline
5-29-51		1.022	5	0	—		1-2	1-2	occ.	hyaline
1-11-52		1.020	5	0	0			0-2	occ.	1 hyaline cast.

Total Dose 1940 Grams

ADDITIONAL REMARKS—Dosage, etc. Patient started July 5th 10 g/day through January 15th—no complications.
BONE MARROW 6-25-51 Sternum—Cellular preparation which shows an essentially normal distribution of cells. Megakaryocytes numerous. Plasma cells slightly increased. No abnormal cells found. Impression: Essentially normal marrow. 1-16-52 Sternum—Cellular preparation which shows no change from previous marrow. Grade II marrow iron. Impression: Normal bone marrow.

after a six month feeding study and no evidence of pathology was found. Urinalyses also showed no changes. In white rats fed one gram of sodium carboxymethylcellulose daily per kilogram of body weight for over two years, no deviations from the control monthly blood studies of hemoglobin determinations, red and white blood cell counts were noted. The study also indicated that sodium carboxymethylcellulose was not absorbed from the intestinal tract and was eliminated almost completely.

Blake (2) reported the use of two to four grams of sodium carboxymethylcellulose daily in 17 human subjects who had determinations of hemoglobin, red blood cell count, white blood cell count and differential smear before and after treatment which extended from four to nine months. No deviations that could be considered physiologically significant were noted. This report is apparently the only one available in which a specific study of the effect of the drug on the blood cell constituents of human patients was undertaken.

RESULTS

Table I lists in brief form the results of the study. The complete protocol of a typical case is shown in Table II. The values of the red blood cell count, white blood cell count and hematocrit showed no significant variations from the control period after administration of sodium carboxymethylcellulose for six months. The total dose ranged from 440 to 1940 grams. Differential smears of the white blood cells also revealed no abnormal changes. No patients on the drug experienced either an anemia or a leukopenia. Bone marrow studies of three patients during the early part of the test period as compared to similar studies at the end of six months of drug administration revealed no changes. An additional three patients had bone marrow studies at the end of the six month test period and the results of these studies were within normal limits.

It is to be noted in Table I that despite the assorted types of diseases that these chronic patients had, the control blood studies were essentially all normal. We attribute this to the excellent dietary management and abundance of food offered these chronically ill patients in the institution in which the study was done. One patient with polycythemia vera received the drug without any change in his blood picture. The urinalyses at the completion of the study revealed no significant changes in comparison with those done during the control period.

In seven of the patients it was necessary to reduce the dose of sodium carboxymethylcellulose usually because of diarrhea, abdominal discomfort, or a feeling of urgency to have a bowel movement. The diarrhea consisted of an increase in the number of bowel movements, varying from two to six movements daily. The stools were noted to be loose without any blood, pus, or mucous being observed. It was also found that ambulatory patients did not seem to be troubled by this difficulty whereas the bedridden ones were. Most of these patients were able to tolerate doses of six to eight grams daily without further difficulty. This is not surprising in view of the 10 gram daily dose used when it is recognized that the usual dose used for laxative effect, due to its hydrophilic properties varies from two to four grams daily.

Since many of these patients had been chronically constipated, it was interesting to observe, incidentally, the satisfactory relief obtained by some patients. Tolerance for the larger doses was much better in the previously constipated patients. No changes in any of the diseases that the patients had, as listed in Table I, could be discerned clinically.

SUMMARY

1. A large daily dose of sodium carboxymethylcellulose was given to a group of patients for a six month period.

2. Careful study of the effect on the blood cell constituents was made.

3. No significant effect on the blood cell constituents could be ascertained as resulting from the administration of sodium carboxymethylcellulose. Bone marrow was also unaltered in a small group of patients in which such study was done.

4. Many of the patients tolerated much larger doses than are ordinarily used therapeutically without ill effect.

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Company Code:
Sub. No. 427

Question 55

Biochemical Research Laboratory

The Dow Chemical Company

June 11, 1964

RESULTS OF TWO-YEAR DIETARY FEEDING STUDIES OF METHOCEL MC IN RATS

PROBLEM

Methocel MC (Dow methylcellulose) is an old established product covered by the Food Additives Regulations as a substance "generally recognized as safe". This status was achieved by toxicological data obtained many years ago and safety under conditions of use as a food additive. At the present time, long term feeding studies are usually required of new food additives. Therefore it was believed desirable to acquire these data in rats to substantiate the status of Dow methylcellulose.

SUMMARY AND CONCLUSIONS

Groups of male and female rats were maintained for a period of two years on diets containing 5.0% or 1.0% of 15 cps., 400 cps., or 4000 cps. Methocel MC without evidence of adverse effect as judged by general appearance and behavior, growth, mortality, incidence of tumorous growths, food consumption, hematological studies, serum urea nitrogen and alkaline phosphatase determinations, final average body and organ weights, and gross and microscopic examination of the tissues.

DESCRIPTION OF TEST MATERIAL

C.R.I. Name:	Cellulose: Methyl ether
Common Name:	Methylcellulose
Trade Name:	Methocel MC
Appearance:	White powder
2% Viscosity (Dry Weight):	15 cps. 14.8 400 cps. 375 4000 cps. 3740
Apparent Density:	15 cps. 0.49 400 cps. 0.41 4000 cps. 0.36

pH 2% Solution:	15 cps. 7.2	
	400 cps. 7.2	
	4000 cps. 6.6	
% Methoxyl:	15 cps. 29.5	
	400 cps. 30.0	
	4000 cps. 30.3	
Lot Numbers:	15 cps.	50% lot number 8052 (15 sec.pulp)
		50% lot number 7847 (Rayofilm pulp)
	400 cps.	50% lot number 8077 (600 sec. pulp)
		50% lot number 7775 (Acetacell pulp)
	4000 cps.	lot number 8158 (500 sec. pulp)
Source:	M. J. Roberts, Cellulose Production	

EXPERIMENTAL DATA

Procedure

Weanling rats from the stock colony of the Biochemical Research Laboratory were placed on a diet of ground Purina Chow and observed for a period of about four weeks before being divided into well-matched groups according to body weight of 20 of each sex per group. Two separate control groups were established. Additional groups of ten males and ten females for each dietary level were set up and designated to be sacrificed for examination after 12 and 18 months on the experiment. When approximately 50 days of age, the groups of rats were started on diets containing 5.0% or 1.0% of 15 cps., 400 cps., or 4000 cps. of Methocel. Experimental diets were prepared by thoroughly mixing the test material with the basic diet of ground Purina Laboratory Chow. The rats were caged individually and allowed food and water ad libitum.

During the course of the experiment, the rats were observed frequently for any changes in appearance or behavior. Each rat was weighed twice a week for the first month and weekly to the end of the experiment. Whenever possible, failing animals were autopsied when moribund in an effort to ascertain the cause of impending death. In addition, records were kept of mortality, and food consumption was recorded for the first two months of the experiment.

Hematological studies, including hematocrit, hemoglobin content, white blood cell count, and differential white cell count were made on five male and five female rats from the control, and each 5.0% level of the 15, 400, or 4000 cps. groups of Methocel. These studies were done at 12, 18 and 24 months.

At the end of the two-year period, all surviving rats were fasted overnight, sacrificed by decapitation and examined grossly at autopsy. The lungs, heart, liver, kidneys, spleen, and testes were removed and weighed. Portions of each organ, as well as adrenal and pancreas were preserved in formalin, and hematoxylin-eosin stained sections were prepared for microscopic examination. Samples of blood serum were obtained for the determination of urea nitrogen content and alkaline phosphatase activity using the Technicon Auto-Analyzer.

This same procedure was followed for the interim sacrifices after 12 and 18 months.

When appropriate, the Fisher "t" test was used in comparing the mean values obtained on the experimental groups with those of the controls; in general, probability values (P) of less than 0.05 were interpreted as indicating a significant difference.

RESULTS

Groups of male and female rats were maintained for a period of two years on diets containing 5.0% or 1.0% of 15 cps., 400 cps., or 4000 cps. of Methocel. On a body weight basis for adult rats, the test substance was consumed in amounts of 2.5 or 0.5 g/kg/day, respectively. During the first few months of the experiment when the rats were young and growing rapidly, these dietary levels were equivalent to the administration of at least twice these figures on a g/kg/day basis.

In general, all the groups of rats appeared normal in appearance and behavior throughout the experimental period. There was no significant difference in food consumption between the control rats and those receiving the test material in their diets (Table 1). Records of mortality (Tables 2 and 3) and incidence of tumorous growths (Tables 4 and 5) show no relationship to the inclusion of Methocel in the diet of rats. Growth was within normal limits for all the groups of rats (see charts).

There was no evidence of adverse effect observed in any of the groups of rats maintained on diets containing Methocel when compared with the controls as judged by hematological examinations (Tables 6 through 13) and determination of urea nitrogen content and alkaline phosphatase activity (Tables 14 through 16).

The findings from the final average body and organ weight tabulations and the gross and microscopic examination of the tissues after 12, 18 and 24 months are outlined below.

Twelve Months - Final average body and organ weight ratios showed no significant changes attributable to the inclusion of Methocel in the diet of the rats (Table 17). The statistically significant increase in the spleen/body weight ratio in the male 5.0% 15 cps. group is believed to be an artifact because no pathological changes were seen upon microscopic examination. Gross and microscopic examination of all of the tissues revealed no significant pathological findings in the animals that received the test substance in feed for one year.

Eighteen Months - No evicence of any adverse effect was observed in the animals which were sacrificed after 18 months as judged by final average body and organ weights (Table 18), and gross and microscopic examination of the tissues. The statistically significant decreases in lung/body weight ratios in two of the male groups and the increase in the liver/body weight ratio in the female 1.0% 15 cps. level are artifacts not due to the test chemical.

Twenty-four Months - Upon gross and microscopic examination of the tissues, no significant changes were seen in the animals receiving Methocel in their diets for two years. None of the statistically significant variations in organ/body weight ratios found at this time have any practical relationship to the ingestion of methylcellulose (Table 19). The significant decrease in body weight of the 5.0% 4000 cps. males developed after 18 months and is therefore believed due to variations in the group average by random mortality (see growth chart); this decrease in final body weight is also responsible for increased kidney and testes/body weight ratios in this group. The statistically significant decreases seen on the 1.0% 15 cps. male level (lung and heart ratios) and the 5.0% 4000 cps. females (liver ratio) are also artifacts of the experiment.

By: D. D. McCollister
J. R. Copeland

TABLE 1

Average Food Consumption for Male and Female Rats Maintained on Diets Containing Methocel

Percent in Diet	Average Food Consumption grams/rat/day*	
	Males	Females
Control	21	19
15 CPS 5%	21	17
15 CPS 1%	22	23
400 CPS 5%	22	19
400 CPS 1%	22	17
4000 CPS 5%	23	20
4000 CPS 1%	23	17

* Calculated from food consumption recorded for first two months of study.

TABLE 2

Mortality Record for Male Rats Maintained

For Two Years on Diets Containing Methocel

Percent in Diet	No. of Rats Left	No. not Surviving	Principal Pathological Findings
Control(A)	9	11	Lung infection and/or pneumonia (5) Dead in cage (4)? Upper respiratory infection ++, lung +, liver +++, kidney ++ (1) Liver +++, kidney ++, gaseous distention of G-I tract with inflammation of cecum (1)
15 CPS 5%	13	7	Lung infection and/or pneumonia (1) Dead in cage (1)? Fibroma on side of jaw bone, kidney +++ (1) Liver ++++, Kidney +++, Spleen ++++, enlarged heart ++ (1) Abscess in prescapular area, liver ++, kidney ++, severe anemia (1) Large testicular tumor 5x3 cm, metastasis to lung 2x2 cm (1) Bleeding tumor in pleural cavity ++++ (1)
15 CPS 1%	9	11	Lung infection and/or pneumonia (2) Dead in cage (3)? Ear infection (1) Liver +++, kidney ++ (1) Liver ++, kidney + (1) Liver +, kidney ++ (1) Peritonitis with adhesions, liver ++, kidney ++ (1) Lymphosarcoma with metastasis to liver, liver ++++, enlarged spleen studded with tumors, fluid in chest cavity, kidney ++ (1)
400 CPS 5%	10	10	Lung infection and/or pneumonia (4) Liver +, kidney ++ (1) Liver +++, kidney +++, enlarged adrenal (1) Liver ++++, kidney ++++, testes small (1) Lung ++, Liver +++, kidney +++, tumor +++ (1) Pneumonia ++, liver ++, kidney +++, enlarged adrenal (1) Lymphosarcoma of intestine and lymph nodes (1)
400 CPS 1%	10	10	Dead in cage (4)? Nephritis + (1) Liver +, kidney +++ (1) Liver +++, kidney ++ (1) Liver +++, kidney +++, peritonitis (1) Reticulum cell sarcoma of lymph nodes and spleen (1) Seminoma of the left testis, liver ++++, kidney ++++, hemorrhagic adrenals +, jaundice (1)

TABLE 2 Continued

Percent in Diet	No. of Rats Left	No. not Surviving	Principal Pathological Findings
Control(B)	10	10	Lung infection and/or pneumonia (5) Dead in cage (1)? Liver ++, Kidney ++(2) Pneumopericarditis +++ (1) Massive lymphosarcoma of gut with extensive metastasis (1)
4000 CPS 5%	9	11	Lung infection and/or pneumonia (3) Liver +++, kidney +++(3) Pneumopericarditis +++, liver ++, kidney ++ (1) Pericarditis, liver +++, kidney ++ (1) Ear infection, pneumonia, liver ++, kidney +++ (1) Kidney +, hemorrhagic fibroma in the anterior neck region(1) Reticulum cell sarcoma of mesentery, with metastasis in liver (1)
4000 CPS 1%	8	12	Lung infection and/or pneumonia (6) Dead in cage (2)? Kidney +++, testes +++, gaseous distention of G-I tract (1) Pneumopericarditis ++++, liver ++ (1) Carcinoma in adrenal cortex, subcutaneous sarcoma, liver +++, kidney +++, lung ++ (1) Lymphosarcoma of liver, kidney, spleen, and lymph nodes (1)

? Death suspected due to acute upper respiratory infection or pneumonia.

TABLE 3

Mortality Record for Female Rats Maintained

For Two Years on Diets Containing Methocel

Percent in Diet	No. of Rats Left	No. not Surviving	Principal Pathological Findings
Control(A)	11	9	Dead in cage (2)? Liver ++, kidney ++ (1) Mesenteric tumor with metastasis to the liver, lymphocytic leukemia, liver ++++, kidney ++++ spleen ++++, adrenals +++, pancreas ++, enlarged heart (1) Prolapsed uterus, liver ++, kidney + (1) Mammary fibroma (2) Mammary fibroma, kidney ++, liver + (1) Uterine fibroma, liver ++, kidney ++ (1)
15 CPS 5%	11	9	Lung infection and/or pneumonia (2) Dead in cage (2)? Pneumonia +++, pericarditis +++ (1) Lung ++, liver +++, kidney ++++, bladder distention ++++ (1) Pneumonia ++, liver ++, kidney +++, cystic ovary (1) Unclassified vaginal tumor, liver +, kidney +(1 Mammary fibroma (1)
15 CPS 1%	10	10	Lung infection and/or pneumonia (2) Dead in cage (4)? Ear infection (1) Liver ++, kidney +++, enlarged heart ++ (1) Large putrified abscess on leg, liver ++++, spleen large; enlarged heart, kidney ++ (1) Reticular carcinoma of the uterus (1)
400 CPS 5%	9	11	Lung infection and/or pneumonia (2) Dead in cage (5)? Liver +++, kidney +++ (1) Liver ++, kidney ++, subcutaneous hematoma (1) Chronic pneumonia, lung ++++ abscessed, liver ++, kidney +, abscess of left ovary (1) Subcutaneous fibroma (1)
400 CPS 1%	11	9	Lung infection and/or pneumonia (3) Dead in cage (3)? Pneumonia ++, emphysema (1) Mammary fibroma, liver ++, kidney ++ (1) Carcinoma of the pancreas with metastasis to the colon, liver +++, kidney +++ (1)

? Death suspected due to acute upper respiratory infection or pneumonia.

TABLE 3 Continued

Percent in Diet	No. of Rats Left	No. not Surviving	Principal Pathological Findings
Control(B)	11	9	Lung infection and/orpneumonia (2) Dead in cage (6)* Lung - advanced pneumonia ++++ - filled with abscesses, pericarditis - liver ++, kidney +++ (1)
4000 CPS 5%	10	10	Lung infection and/or pneumonia (3) Dead in cage (5)* Liver +++, kidney +++, ovarian cysts, enlarged heart (1) Mammary adenocarcinoma with secondary tumors in lung, pancreas, and ovary (1)
4000 CPS 1%	12	8	Lung infection and/or pneumonia (4) Dead in cage (2)* Liver ++, kidney ++ (1) Uterine carcinoma - about 1 1/2 inches in diameter (1)

* Death suspected due to acute upper respiratory infection or pneumonia.

TABLE 4

Tumorous Growths observed in Groups of Male Rats Maintained on Diets Containing Methocel

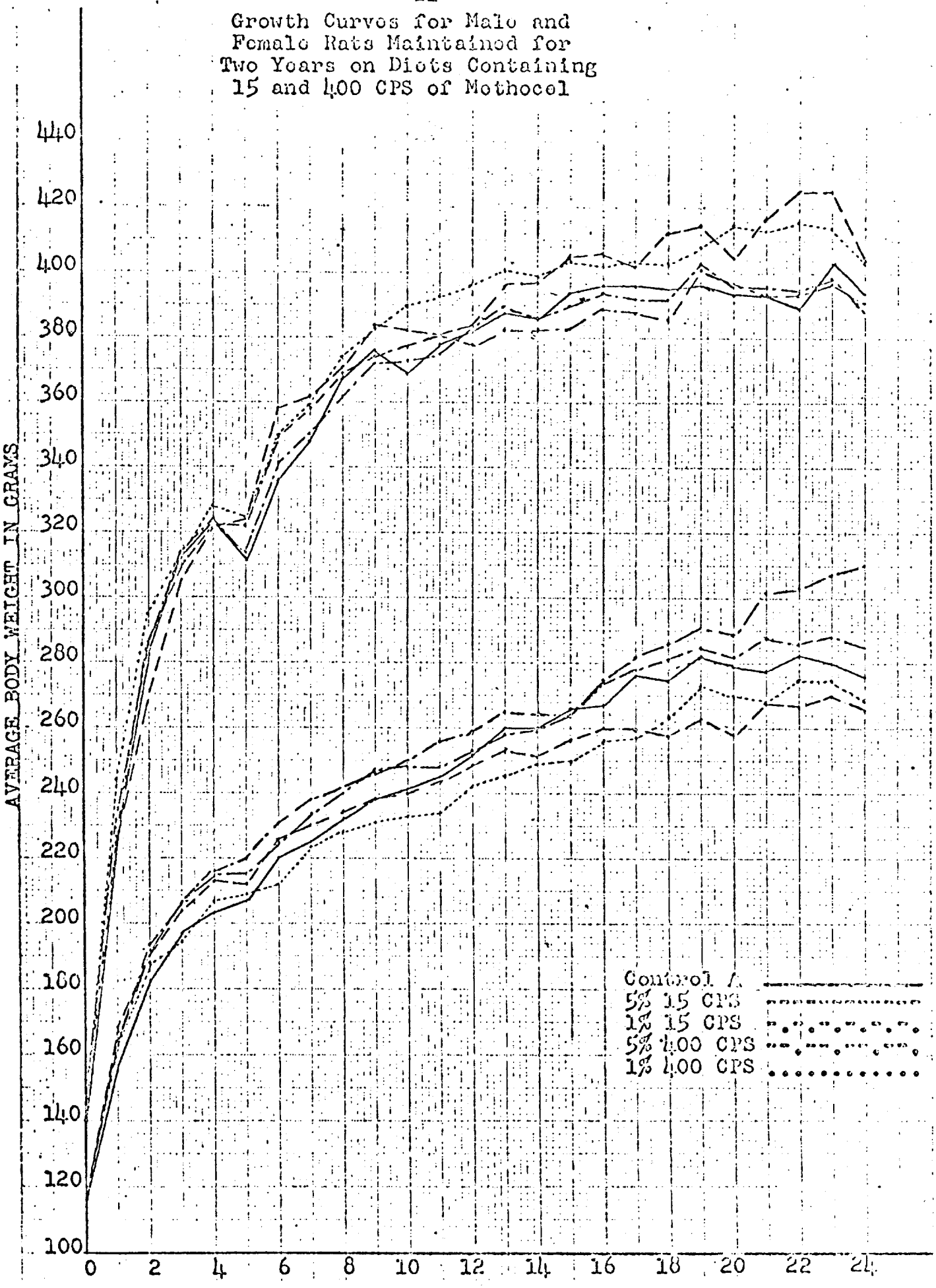
Dietary Concentration	Tumor
Control	1. Lymphosarcoma with metastasis (autopsied 477th day) 2. Heart tumor (discovered at 2-year autopsy) 3. Interstitial cell tumor (discovered upon microscopic examination after 2 years)
15 CPS 5%	1. Fibroma on jaw bone (autopsied 635th day) 2. Testicular tumor with metastasis to lung (autopsied 395th day) 3. Tumor in ploural cavity (autopsied 264th day)
15 CPS 1%	1. Lymphosarcoma with metastasis to liver and spleen (autopsied 663rd day)
400 CPS 5%	1. Lymphosarcoma of intestine and lymph nodes (autopsied 459th day)
400 CPS 1%	1. Reticulum cell sarcoma of lymph nodes and spleen (autopsied 347th day) 2. Seminoma of left testis (autopsied 579th day) 3. Unclassified testes tumor (discovered upon microscopic examination after 2 years) 4. Unclassified testes tumor (discovered upon microscopic examination after 2 years)
4000 CPS 5%	1. Hemorrhagic fibroma in anterior neck region (autopsied 549th day) 2. Reticulum cell sarcoma of mesentery with metastasis to liver (autopsied 329th day)
4000 CPS 1%	1. Carcinoma in adrenal cortex, subcutaneous sarcoma (autopsied 642nd day) 2. Lymphosarcoma of liver, kidney, spleen, and lymph nodes (autopsied 527th day) 3. Unclassified testes tumor (discovered at 2-year autopsy)

TABLE 5

Tumorous Growths observed in Groups of Female Rats Maintained on Diets Containing Methocel

Dietary Concentration	Tumor
Control	1. Mesenteric tumor with metastasis to liver, lymphocytic leukemia (autopsied 670th day)
	2. Mammary fibroma (autopsied 586th day)
	3. Mammary fibroma (autopsied 684th day)
	4. Mammary fibroma (autopsied 649th day)
	5. Uterine fibroma (autopsied 531st day)
	6. Thymoma (discovered at 2-year autopsy)
15 CPS 5%	1. Unclassified vaginal tumor (autopsied 579th day)
	2. Mammary fibroma (autopsied 545th day)
	3. Mammary gland sarcoma (discovered at 2-year autopsy)
15 CPS 1%	1. Reticular carcinoma of the uterus (autopsied 501st day)
	2. Mammary adenocarcinoma (discovered at 2-year autopsy)
400 CPS 5%	1. Subcutaneous fibroma (autopsied 684th day)
400 CPS 1%	1. Mammary fibroma (autopsied 684th day)
	2. Carcinoma of pancreas with metastasis to colon (autopsied 691st day)
	3. Lipoma of kidney (discovered at 2-year autopsy)
	4. Mammary gland adenoma (discovered at 2-year autopsy)
	5. Thymoma (discovered at 2-year autopsy)
4000 CPS 5%	1. Mammary adenocarcinoma with metastasis to lung, pancreas, and ovary (autopsied 676th day)
4000 CPS 1%	1. Uterine carcinoma (autopsied 469th day)
	2. Fibro-adenoma (discovered at 2-year autopsy)

Growth Curves for Male and Female Rats Maintained for Two Years on Diets Containing 15 and 400 CPS of Methocel



Growth Curves for Male and Female Rats Maintained for Two Years on Diets Containing 4000 CPS of Methocel

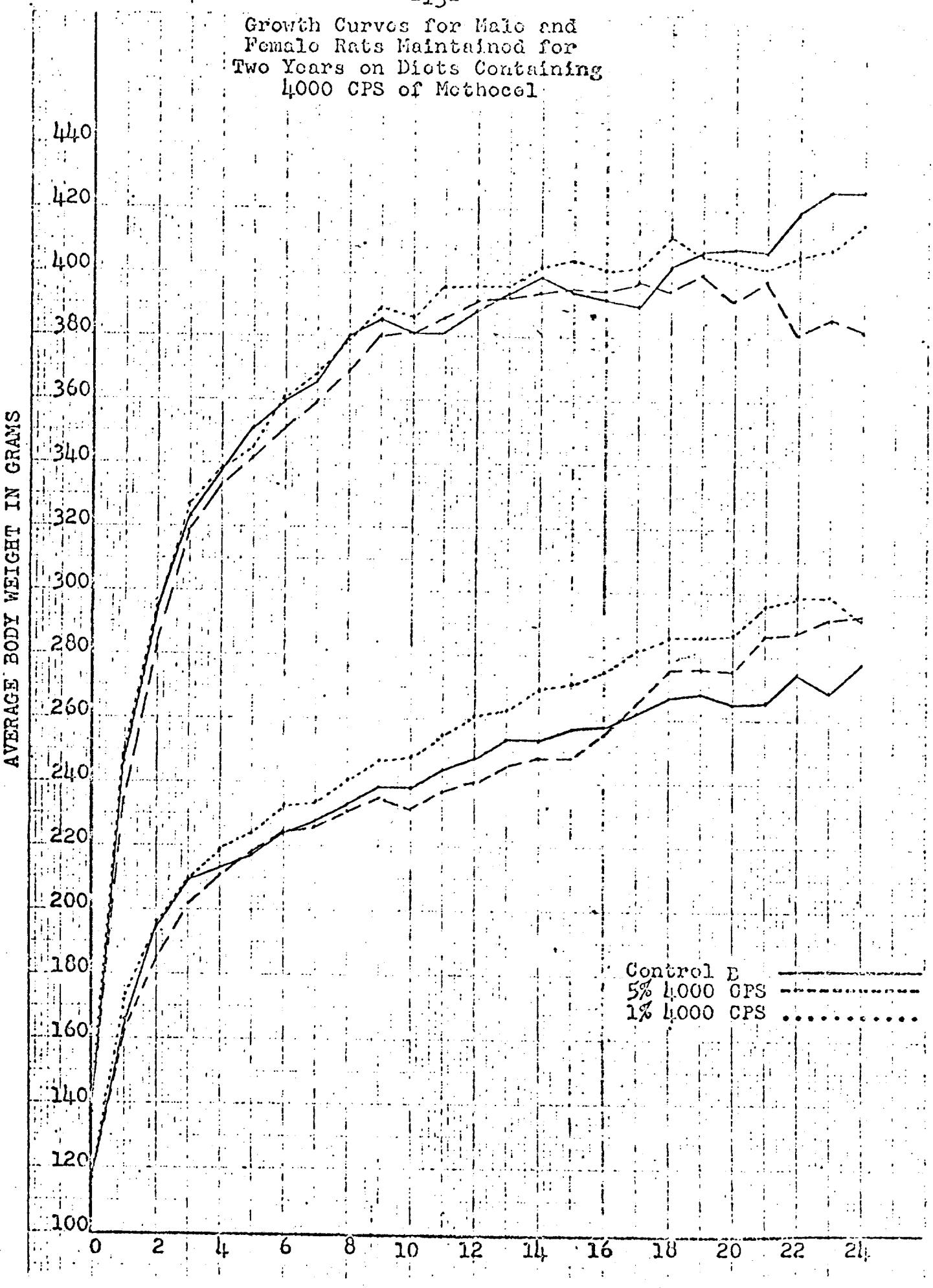


TABLE 6

Average Hematological Values from Groups of Five Male Rats Maintained on Diets Containing Methocel

Dietary Level and Time of Examination	Hgb (g/100cc)	HCT (%)	WBC_3 X10^3	Differential Count (%)				
				Neut.	Lymph.	Mono.	Eo.	Baso
Control								
12 Months	13.4	46	18.6	37	60	1	3	0
18 Months	10.1	38	17.5	37	60	2	1	0
24 Months	9.4	39	25.7	56	39	5	0	0
15 CPS (5.0 Percent)								
12 Months	13.8	47	24.0	35	62	0	3	0
18 Months	13.5	47	23.8	40	57	2	1	0
24 Months	11.4	42	24.5	42	51	4	3	0
400 CPS (5.0 Percent)								
12 Months	14.6	50	21.4	29	69	1	2	0
18 Months	14.4	52	22.7	40	57	2	1	0
24 Months	9.0	36	19.1	31	62	6	1	0
4000 CPS (5.0 Percent)								
12 Months	14.3	49	21.8	45	54	1	1	0
18 Months	14.0	50	23.3	39	58	1	2	0
24 Months	11.7	42	23.8	36	54	8	2	0

TABLE 7

Average Hematological Values From Groups of Five Female Rats Maintained on Diets Containing Methocel

Dietary Level and Time of Examination	Hgb (g/100cc)	HCT (%)	WBC_3 X10^3	Differential Count (%)				
				Neut.	Lymph.	Mono.	Eo.	Baso
Control								
12 Months	13.6	46	14.3	24	75	0	1	0
18 Months	13.8	47	9.3	26	72	1	2	0
24 Months	13.4	48	12.9	49	48	3	1	0
15 CPS (5.0 Percent)								
12 Months	13.4	48	17.6	39	59	0	2	0
18 Months	14.6	52	13.8	44	55	0	1	0
24 Months	14.7	52	15.0	33	63	2	1	0
400 CPS (5.0 Percent)								
12 Months	13.8	48	23.7	32	65	1	2	0
18 Months	13.9	47	14.2	37	60	2	1	0
24 Months	13.5	49	18.1	41	55	3	1	0
4000 CPS (5.0 Percent)								
12 Months	14.4	49	16.8	32	64	2	2	0
18 Months	14.4	49	15.7	33	66	0	1	0
24 Months	13.3	48	16.8	37	59	0	1	0

TABLE 8

Individual Hematological Values for Male Rats Maintained for Twelve Months on Diets Containing Methocel

Percent in Diet	Rat No.	Hgb (g/100cc)	HCT (%)	WBC $X10^3$	Differential Count (%)				
					Neut.	Lymph.	Mono.	Eo.	Baso.
Control	1	13.5	48	16.9	38	61	0	1	0
	2	14.5	49	17.4	30	67	2	1	0
	3	12.5	43	20.0	33	60	1	6	0
	4	14.0	48	16.6	35	61	0	4	0
	5	12.5	44	22.1	48	50	0	2	0
	Average	13.4	46	18.6	37	60	1	3	0
15 CPS (5.0%)	1	14.5	49	15.4	19	79	0	2	0
	2	14.5	49	34.4	50	47	1	2	0
	3	12.5	42	14.1	23	71	0	6	0
	4	13.5	47	28.3	55	42	1	2	0
	5	13.5	46	28.0	29	70	0	1	0
	Average	13.8	47	24.0	35	62	0	3	0
400 CPS (5.0%)	1	15.5	53	19.8	25	73	0	2	0
	2	15.0	51	24.5	41	55	2	2	0
	3	13.5	48	24.7	27	70	0	3	0
	4	14.5	49	21.8	26	73	1	0	0
	5	14.5	50	16.0	26	73	0	1	0
	Average	14.6	50	21.4	29	69	1	2	0
4000 CPS (5.0%)	1	14.5	49	21.6	37	62	1	0	0
	2	15.0	50	18.8	38	60	0	2	0
	3	14.5	50	26.6	41	58	1	0	0
	4	13.5	48	20.6	58	42	0	0	0
	5	14.0	48	21.3	49	48	1	2	0
	Average	14.3	49	21.8	45	54	1	1	0

TABLE 9

Individual Hematological Values for Female Rats Maintained for Twelve Months on Diets Containing Methocel

Percent in Diet	Rat No.	Hgb (g/100cc)	HCT (%)	WBC_3 X10	Differential Count (%)				
					Neut.	Lymph.	Mono.	Eo.	Baso.
Control	1	14.0	47	11.9	14	86	0	0	0
	2	13.5	46	14.3	38	60	0	2	0
	3	13.0	46	16.4	22	78	0	0	0
	4	14.0	46	16.8	27	72	0	1	0
	5	13.5	45	12.2	20	77	0	3	0
	Average	13.6	46	14.3	24	75	0	1	0
15 CPS (5.0%)	1	13.5	47	18.1	50	46	1	3	0
	2	12.5	46	14.7	36	63	0	1	0
	3	14.0	49	17.3	45	50	1	4	0
	4	14.0	51	15.2	32	66	0	2	0
	5	13.0	45	22.7	31	68	0	1	0
	Average	13.4	48	17.6	39	59	0	2	0
400 CPS (5.0%)	1	13.0	47	36.9	34	63	1	2	0
	2	12.5	45	21.0	32	67	0	1	0
	3	13.5	48	21.6	34	61	3	2	0
	4	14.5	49	17.1	31	67	0	2	0
	5	15.5	51	22.0	30	69	0	1	0
	Average	13.8	48	23.7	32	65	1	2	0
4000 CPS (5.0%)	1	14.0	48	17.9	32	68	0	0	0
	2	14.5	49	11.5	40	57	1	2	0
	3	15.5	53	14.3	19	79	1	1	0
	4	13.5	46	15.7	28	66	5	1	0
	5	14.5	50	24.9	42	52	1	5	0
	Average	14.4	49	16.8	32	64	2	2	0

TABLE 10

Individual Hematological Values for Male Rats Maintained for Eighteen Months on Diets Containing Methocel

Percent in Diet	Rat No.	Hgb (g/100cc)	HCT (%)	WBC $X10^3$	Differential Count (%)				
					Neut.	Lymph.	Mono.	Eo.	Baso.
Control	1	13.5	47	26.1	57	43	0	0	0
	2	>7.0	27	15.0	32	65	3	0	0
	3	7.5	31	15.7	40	55	2	3	0
	4	9.0	36	13.6	20	77	2	1	0
	5	13.5	48	17.3	35	62	1	2	0
	Average	10.1	38	17.5	37	60	2	1	0
15 CPS (5.0%)	1	13.0	46	33.0	53	45	1	1	0
	2	14.0	48	20.5	37	57	4	2	0
	3	13.5	46	16.1	26	69	2	3	0
	4	14.0	50	21.7	33	67	0	0	0
	5	13.0	46	27.9	49	48	3	0	0
	Average	13.5	47	23.8	40	57	2	1	0
400 CPS (5.0%)	1	14.5	51	32.5	57	36	4	3	0
	2	16.0	58	19.4	37	62	1	0	0
	3	14.0	50	18.2	31	66	1	2	0
	4	14.5	52	20.2	35	61	3	1	0
	5	13.0	48	23.4	40	60	0	0	0
	Average	14.4	52	22.7	40	57	2	1	0
4000 CPS (5.0%)	1	13.0	48	27.1	45	54	0	1	0
	2	15.0	53	20.6	29	70	0	1	0
	3	13.0	47	21.7	52	42	3	3	0
	4	14.0	48	26.8	38	59	0	3	0
	5	15.0	53	20.1	29	67	1	3	0
	Average	14.0	50	23.3	39	58	1	2	0

TABLE 11

Individual Hematological Values for Female Rats Maintained for Eighteen Months on Diets Containing Methocel

Percent in Diet	Rat No.	Hgb (g/100cc)	HCT (%)	WBC $X10^3$	Differential Count (%)				
					Neut.	Lymph.	Mono.	Eo.	Baso.
Control	1	14.5	46	9.8	29	69	1	1	0
	2	13.5	47	10.7	25	74	0	1	0
	3	14.0	47	8.0	24	72	3	1	0
	4	13.5	47	7.2	30	67	1	2	0
	5	13.5	47	10.7	21	76	0	3	0
	Average	13.8	47	9.3	26	72	1	2	0
15 CPS (5.0%)	1	13.5	47	7.6	44	56	0	0	0
	2	14.5	50	14.7	40	59	0	1	0
	3	16.5	64	22.4	57	43	0	0	0
	4	14.0	49	12.3	36	62	0	2	0
	5	14.5	48	12.3	42	56	0	2	0
	Average	14.6	52	13.8	44	55	0	1	0
400 CPS (5.0%)	1	15.0	50	15.8	48	50	1	1	0
	2	13.5	46	17.6	27	69	4	0	0
	3	13.0	45	15.4	38	58	3	1	0
	4	14.5	49	8.1	42	57	1	0	0
	5	13.5	47	14.2	30	67	1	2	0
	Average	13.9	47	14.2	37	60	2	1	0
4000 CPS (5.0%)	1	14.0	49	9.2	32	65	1	2	0
	2	14.0	50	22.4	48	51	0	1	0
	3	14.5	47	10.1	30	69	0	1	0
	4	15.0	51	21.0	22	77	0	1	0
	Average	14.4	49	15.7	33	66	0	1	0

TABLE 12

Individual Hematological Values for Male Rats Maintained for Twenty-four Months on Diets Containing Methocel

Percent in Diet	Rat No.	Hgb (g/100cc)	HCT (%)	WBC $X10^3$	Differential Count (%)				
					Neut.	Lymph.	Mono.	Eo.	Baso.
Control	1	9.5	39	28.1	60	38	1	1	0
	2	9.0	38	22.4	54	38	8	0	0
	3	9.5	39	26.7	57	39	4	0	0
	4	10.5	43	29.7	52	42	6	0	0
	5	8.5	34	21.5	56	37	7	0	0
	Average	9.4	39	25.7	56	39	5	0	0
15 CPS (5.0%)	1	12.5	46	20.7	51	43	3	3	0
	2	8.0	32	22.4	47	48	4	1	0
	3	14.0	48	20.5	35	54	3	8	0
	4	12.5	44	21.0	44	49	4	3	0
	5	10.0	42	37.8	34	62	4	0	0
	Average	11.4	42	24.5	42	51	4	3	0
400 CPS (5.0%)	1	7.0	28	15.9	23	68	5	4	0
	2	9.0	40	23.6	23	65	12	0	0
	3	10.0	40	18.1	30	65	4	1	0
	4	8.0	32	18.7	39	53	6	2	0
	5	11.0	38	19.3	40	58	2	0	0
	Average	9.0	36	19.1	31	62	6	1	0
4000 CPS (5.0%)	1	13.5	44	20.9	40	44	13	3	0
	2	11.0	41	24.3	37	51	11	1	0
	3	13.5	50	22.0	31	63	5	1	0
	4	7.0	30	31.4	41	53	5	1	0
	5	13.5	47	20.1	29	59	8	4	0
	Average	11.7	42	23.8	36	54	8	2	0

TABLE 13

Individual Hematological Values for Female Rats Maintained for Twenty-four Months on Diets Containing Methocel

Percent in Diet	Rat No.	Hgb (g/100cc)	HCT (%)	WBC $X10^3$	Differential Count (%)				
					Neut.	Lymph.	Mono.	Eo.	Baso.
Control	1	13.0	46	14.1	91	9	0	0	0
	2	13.0	45	11.7	31	63	4	2	0
	3	14.0	51	10.0	42	54	4	0	0
	4	12.5	50	13.2	42	52	5	1	0
	5	14.5	50	15.7	37	63	0	0	0
	Average	13.4	48	12.9	49	48	3	1	0
15 CPS (5.0%)	1	15.0	52	11.9	51	47	0	2	0
	2	14.5	50	14.7	30	63	5	1	1
	3	16.0	58	19.4	33	63	3	1	0
	4	12.5	46	14.4	19	79	1	1	0
	5	15.5	56	14.9	34	63	2	1	0
	Average	14.7	52	15.0	33	63	2	1	0
400 CPS (5.0%)	1	13.0	51	9.5	38	60	0	2	0
	2	12.0	44	18.9	64	34	2	0	0
	3	14.5	50	12.4	26	66	7	1	0
	4	14.0	48	17.5	29	66	4	1	0
	5	14.0	50	32.2	46	50	2	2	0
	Average	13.5	49	18.1	41	55	3	1	0
4000 CPS (5.0%)	1	13.0	46	19.1	24	76	0	0	0
	2	13.5	48	11.4	57	39	3	1	0
	3	14.5	52	9.9	29	68	2	1	0
	4	11.5	46	11.2	36	59	5	0	0
	5	13.5	47	32.7	40	54	5	1	0
	Average	13.3	48	16.8	37	59	3	1	0

TABLE 14

Average Serum Urea Nitrogen and Alkaline Phosphatase Values for Male and Female Rats Maintained for Twenty-four Months on Diets Containing Methocel

Dietary Concentration	MALES		FEMALES	
	Urea Nitrogen (Mg %)	Alkaline Phosphatase (King Armstrong Units)	Urea Nitrogen (Mg %)	Alkaline Phosphatase (King Armstr Units)
Control (A)	18	38.7	24	17.3
15 CPS (5.0%)	21	21.9	13	16.5
15 CPS (1.0%)	15	24.9	11	17.0
400 CPS (5.0%)	20	29.7	20	18.8
400 CPS (1.0%)	15	27.0	16	16.9
Control (B)	17	30.9	18	16.2
4000 CPS (5.0%)	18	26.7	18	17.2
4000 CPS (1.0%)	21	25.0	18	16.8

TABLE 15

Individual Serum Urea Nitrogen and Alkaline Phosphatase Values for Male Rats Maintained for Twenty-four Months on Diets Containing Methocel

Dietary Concentration	Animal No.	Urea Nitrogen (Mg %)	Alkaline Phosphatase (King Armstrong Units)
Control (A)	5176	16	46.9
	5177	15	25.5
	5178	17	34.9
	5179	18	34.9
	5180	14	54.6
	5181	18	27.0
	5182	27	58.2
	5183	17	40.7
	5184	18	25.7
	Average	18	38.7
15 CPS (5.0%)	5185	14	16.9
	5186	13	13.3
	5187	16	23.3
	5188	15	14.1
	5189	22	31.0
	5190	16	25.0
	5190A	46	32.5
	5191	40	19.3
	5192	18	24.0
	5193	22	24.8
	5194	14	18.6
	5195	15	22.5
	5196	17	19.7
	Average	21	21.9
15 CPS (1.0%)	5197	13	18.2
	5198	17	13.5
	5199	12	36.0
	5200	14	19.1
	5201	13	11.6
	5202	16	20.3
	5203	18	20.5
	5204	17	43.0
	5205	15	41.5
	Average	15	24.9

TABLE 15 Continued

Dietary Concentration	Animal No.	Urea Nitrogen (Mg %)	Alkaline Phosphatase (King Armstrong Units)
400 CPS (5.0%)	5206	20	29.1
	5207	20	54.8
	5208	20	41.5
	5209	17	17.6
	5210	17	53.9
	5211	18	18.8
	5212	17	14.1
	5213	27	17.3
	5214	17	27.2
	5215	24	23.1
	Average	20	29.7
400 CPS (1.0%)	5216	18	41.7
	5217	13	18.6
	5218	17	39.2
	5219	15	36.4
	5220	18	24.2
	5221	16	21.8
	5222	15	21.0
	5223	12	26.1
	5224	14	16.9
	5225	15	24.4
	Average	15	27.0
Control (B)	5226	18	39.0
	5227	15	28.7
	5228	33	37.0
	5229	18	25.3
	5230	13	22.7
	5231	15	15.2
	5232	16	20.3
	5233	16	46.0
	5234	15	45.4
	5235	15	29.1
	Average	17	30.9
4000 CPS (5.0%)	5236	21	27.8
	5237	14	15.6
	5238	17	55.0
	5240	34	12.6
	5241	16	10.7
	5242	14	30.6

TABLE 15 Continued

Dietary Concentration	Animal No.	Urea Nitrogen (Mg %)	Alkaline Phosphatase (King Armstrong Units)
4000 CPS (5.0%) Continued	5243	18	29.3
	5244	15	39.0
	5245	12	19.9
	Average	18	26.7
4000 CPS (1.0%)	5246	33	55.4
	5247	16	25.3
	5248	15	18.0
	5249	17	15.0
	5250	14	43.0
	5251	39	13.9
	5252	17	13.1
	5253	20	16.5
	Average	21	25.0

TABLE 16

Individual Serum Urea Nitrogen and Alkaline Phosphatase Values for Female Rats Maintained for Twenty-four Months on Diets Containing Methocel

Dietary Concentration	Animal No.	Urea Nitrogen (Mg %)	Alkaline Phosphatase (King Armstrong Units)
Control (A)	5254	10	13.7
	5255	7	6.9
	5256	9	9.6
	5257	18	13.3
	5258	17	15.2
	5259	9	14.8
	5260	8	9.8
	5261	13	11.8
	5262	10	9.0
	5263	143	57.4
	5264	17	28.7
	Average	24*	17.3
*Without Animal No. 5263, the Urea Nitrogen Average is 12 Mg %.			
15 CPS (5.0%)	5265	10	19.3
	5266	23	10.5
	5267	9	16.3
	5268	8	15.4
	5269	15	25.3
	5270	8	11.1
	5271	23	15.0
	5272	12	14.3
	5273	8	13.5
	5274	17	24.8
	5275	9	16.5
	Average	13	16.5
15 CPS (1.0%)	5276	15	31.9
	5277	9	12.6
	5278	8	17.3
	5279	13	11.6
	5280	7	12.8
	5281	8	7.9
	5282	15	15.8
	5283	11	31.2
	5284	11	12.2
	Average	11	17.0

TABLE 16 Continued

Dietary Concentration	Animal No.	Urea Nitrogen (Mg %)	Alkaline Phosphatase (King Armstrong Units)
400 CPS (5.0%)	5285	56	34.5
	5286	10	11.6
	5287	7	14.8
	5288	31	15.6
	5289	13	16.1
	5290	16	16.9
	5292	9	18.8
	5293	18	22.3
	Average	20	18.8
400 CPS (1.0%)	5294	13	11.1
	5295	14	16.1
	5296	15	19.7
	5297	12	18.0
	5298	15	11.3
	5299	11	13.1
	5300	18	25.3
	5301	14	15.4
	5302	14	8.6
	5303	30	12.2
	5304	15	34.7
	Average	16	16.9
Control (B)	5305	15	14.3
	5306	19	16.5
	5307	16	14.3
	5308	17	5.4
	5309	19	7.9
	5310	28	17.8
	5311	16	14.8
	5312	13	18.0
	5313	21	13.3
	5314	16	14.6
	5315	16	41.3
	Average	18	16.2
4000 CPS (5.0%)	5317	16	9.8
	5318	14	17.8
	5319	14	32.5
	5320	17	17.1
	5321	14	9.0
	5322	18	22.3

TABLE 16 Continued

Dietary Concentration	Animal No.	Urea Nitrogen (Mg %)	Alkaline Phosphatase (King Armstrong Units
4000 CPS (5.0%) Continued	5323	19	15.0
	5324	14	14.6
	5325	15	11.8
	5326	39	22.5
	Average	18	17.2
4000 CPS (1.0%)	5327	13	15.4
	5328	14	15.8
	5329	15	9.2
	5330	15	7.5
	5331	23	7.7
	5334	18	12.2
	5335	25	25.3
	5336	15	22.7
	5337	23	23.3
	5339	22	15.8
	5340	13	11.1
	5341	17	36.0
	Average	18	16.8

TABLE 17

Final Average Body and Organ Weights of Male and Female Rats Maintained for Twelve Months on Diets Containing Methocel

Percent in Diet	No. of Rats	Sex	Av. Body Wt. (g)	Av. Organ Weights (Grams and Grams/100 Grams)											
				Lung		Heart		Liver		Kidney		Spleen		Testes	
				g.	g/100g	g.	g/100g	g.	g/100g	g.	g/100g	g.	g/100g	g.	g/100g
Control	5	M	352	1.88	0.53	1.12	0.32	9.28	2.65	2.55	0.72	0.51	0.14	2.92	0.83
15 CPS (5.0%)	3	M	393	1.85	0.47	1.23	0.32	10.17	2.60	2.77	0.71	0.60	0.16*	2.99	0.76
15 CPS (1.0%)	5	M	389	1.93	0.50	1.19	0.31	9.68	2.49	2.70	0.69	0.56	0.14	2.92	0.76
400 CPS (5.0%)	3	M	319	2.36	0.77	1.08	0.34	8.32	2.61	2.45	0.77	0.45	0.14	2.78	0.89
400 CPS (1.0%)	5	M	415*	2.02	0.49	1.22	0.30	11.49	2.77	2.84	0.69	0.67	0.16	3.08	0.75
4000 CPS (5.0%)	4	M	380	1.83	0.49	1.18	0.32	9.67	2.55	2.59	0.69	0.52	0.14	2.67	0.71
4000 CPS (1.0%)	4	M	372	2.36	0.64	1.18	0.32	10.15	2.74	2.66	0.72	0.55	0.15	2.61	0.71
Control	3	F	231	1.34	0.58	0.92	0.40	6.80	2.97	1.95	0.85	0.43	0.19		
15 CPS (5.0%)	5	F	209	1.26	0.60	0.87	0.42	6.24	3.00	1.76	0.85	0.38	0.18		
15 CPS (1.0%)	4	F	222	1.33	0.60	0.92	0.42	7.10	3.20	1.91	0.86	0.40	0.18		
400 CPS (5.0%)	4	F	238	1.58	0.66	0.88	0.37	6.71	2.83	2.09	0.89	0.45	0.19		
400 CPS (1.0%)	4	F	233	1.30	0.56	0.95	0.41	6.93	2.99	2.10	0.91	0.56	0.24		
4000 CPS (5.0%)	5	F	219	1.43	0.67	0.92	0.42	6.90	3.15	1.90	0.87	0.45	0.21		
4000 CPS (1.0%)	3	F	252	1.34	0.53	0.88	0.35	6.91	2.74	1.95	0.77	0.46	0.18		

* P = .01 - .05

TABLE 18

Final Average Body and Organ Weights of Male and Female Rats Maintained for Eighteen Months on Diets Containing Methocel

Percent in Diet	No. of Rats	Sex	Av. Body Wt. (g)	Av. Organ Weights (Grams and Grams/100 Grams)											
				Lung		Heart		Liver		Kidney		Spleen		Testes	
				g.	g/100g	g.	g/100g	g.	g/100g	g.	g/100g	g.	g/100g	g.	g/100g
Control	5	M	405	2.88	0.71	1.34	0.33	10.62	2.62	2.90	0.72	0.71	0.18	3.06	0.76
15 CPS (5.0%)	4	M	364	2.12	0.62	1.22	0.34	9.30	2.61	2.64	0.75	0.48	0.13	2.83	0.79
15 CPS (1.0%)	4	M	414	2.59	0.64	*1.32	0.32	10.51	2.55	2.82	0.68	0.56	0.14	3.18	0.77
400 CPS (5.0%)	2	M	335	3.00	0.89	1.20	0.35	9.13	2.71	2.68	0.80	0.57	0.17	2.51	0.75
400 CPS (1.0%)	3	M	357	2.12	0.60	+1.19	0.34	9.80	2.74	2.66	0.75	0.54	0.15	2.90	0.82
4000 CPS (5.0%)	5	M	379	2.85	0.74	1.32	0.35	9.82	2.60	2.68	0.71	0.60	0.16	2.50	0.67
4000 CPS (1.0%)	2	M	444	2.23	0.50	1.45	0.33	10.94	2.47	4.98	0.66	0.59	0.13	3.00	0.68
Control	3	F	263	2.06	0.78	1.19	0.45	7.33	2.78	2.22	0.84	0.42	0.16		
15 CPS (5.0%)	4	F	260	1.78	0.71	1.12	0.44	7.88	3.05	2.27	0.89	0.52	0.20		
15 CPS (1.0%)	5	F	240	1.47	0.62	1.15	0.48	7.39	3.10	*2.51	1.05	0.57	0.24		
400 CPS (5.0%)	4	F	242	1.69	0.71	1.14	0.47	7.94	3.30	2.35	0.98	0.48	0.20		
400 CPS (1.0%)	4	F	277	1.55	0.56	1.11	0.40	8.48	3.06	2.45	0.88	0.59	0.21		
4000 CPS (5.0%)	5	F	257	2.00	0.78	1.06	0.42	7.39	2.88	2.13	0.83	0.48	0.19		
4000 CPS (1.0%)	4	F	262	1.54	0.60	1.04	0.40	7.71	2.97	2.03	0.78	0.49	0.18		

*P = .01 - .05

+P = .00 - .009

Final Average Body and Organ Weights of Male and Female Rats Maintained for Twenty-Four Months on Diets Containing Methocel

Percent in Diet	No. of Rats	Sex	Av. Body Wt. (g)	Av. Organ Weights (Grams and Grams/100 Grams)											
				Lung		Heart		Liver		Kidney		Spleen		Testes	
				g.	g/100g	g.	g/100g	g.	g/100g	g.	g/100g	g.	g/100g	g.	g/100g
Control (A)	9	M	370	3.04	0.83	1.50	0.41	9.51	2.57	2.85	0.77	0.82	0.22	2.71	0.74
15 CPS (5.0%)	13	M	376	2.44	0.65	1.52	0.40	10.38	2.76	2.94	0.78	0.93	0.25	2.35	0.63
15 CPS (1.0%)	9	M	363	2.13	0.58*	1.32	0.36+	9.30	2.56	2.85	0.79	0.85	0.23	2.82	0.78
400 CPS (5.0%)	10	M	365	2.89	0.81	1.39	0.38	9.63	2.65	2.72	0.75	0.77	0.21	2.66	0.73
400 CPS (1.0%)	10	M	376	2.41	0.65	1.41	0.38	10.01	2.67	2.79	0.75	0.92	0.24	2.64	0.71
Control (B)	10	M	401	2.51	0.62	1.46	0.36	10.15	2.54	2.91	0.73	0.91	0.22	2.78	0.71
4000 CPS (5.0%)	9	M	354*	3.03	0.87	1.42	0.40	9.03	2.55	2.89	0.82*	1.00	0.28	2.88	0.81*
4000 CPS (1.0%)	8	M	390	2.73	0.73	1.35	0.35	9.96	2.56	2.87	0.74	0.79	0.20	2.17	0.56*
Control (A)	11	F	255	1.90	0.79	1.27	0.50	7.52	2.95	2.17	0.85	0.62	0.24		
15 CPS (5.0%)	11	F	242	1.82	0.77	1.25	0.53	7.56	3.16	2.40	1.02	0.60	0.26		
15 CPS (1.0%)	10	F	286	1.65	0.60	1.28	0.46	7.96	2.82	2.32	0.83	0.72	0.26		
400 CPS (5.0%)	9	F	256	2.12	0.84	1.21	0.49	7.56	3.05	2.06	0.93	0.61	0.24		
400 CPS (1.0%)	11	F	250	1.79	0.75	1.26	0.51	7.56	3.06	2.30	0.93	0.62	0.25		
Control (B)	11	F	258	1.59	0.62	1.34	0.52	8.01	3.13	2.38	0.94	0.71	0.26		
4000 CPS (5.0%)	10	F	271	1.84	0.71	1.24	0.47	7.66	2.86*	2.54	0.96	0.58	0.22		
4000 CPS (1.0%)	12	F	269	1.77	0.66	1.31	0.50	8.13	3.05	2.49	0.93	0.71	0.27		

*P = .01 - .05

+P = .001 - .009

Summary of Histopathological Examination of Rat Tissues

Methocel Dietary Feeding Study

This summary includes the results of histopathological examinations of tissues from rats that received Methocel of different viscosities up to a two year period. The tissues examined were lung, heart, liver, kidney, spleen, pancreas, adrenal and testes. All tissues were cut at 7 microns and stained with hematoxylin-eosin. The common pathological conditions usually encountered in rats such as chronic murine pneumonia, kidney nephrosis, granulamatous changes in spleen, hemorrhagic necrosis of adrenal cortex and cyst formation, and myocardial degenerative changes will not be referred to in this summary as they are not related to the compound.

Male and Female Rats - 12 Months

After gross and microscopic observation of lung, heart, liver, kidney, spleen, adrenal, pancreas and gonads there was no evidence of adverse effect in the animals exposed to 5% and 1% respectively of 15 CPS, 400 CPS and 4000 CPS when compared with the control animals.

Male Rats - 18 Months

Methocel 15 C.P.S. - 5% and 1%

There were no pathological effects which could be attributed to the effect of feeding compound.

Methocel 400 C.P.S.

5%

Only two animals were available for examination. The kidney of one animal showed extensive degeneration of tubular epithelium of the convoluted tubules and hyalinization of glomerular tufts. Other animal was normal.

1%

No significant effects were observed in the organs examined.

Methocel 4000 C.P.S. - 5% and 1%

No significant changes.

Female Rats - 18 Months

Methocel 15 C.P.S. - 5% and 1%

No significant changes.

Methocel 400 C.P.S. - 5% and 1%

No significant changes.

Methocel 4000 C.P.S. - 5% and 1%

No significant changes.

Male and Female Rats - 24 Months

Senile degenerative changes in the kidney and liver were more apparent in this group of animals than the group sacrificed at 18 months.

Methocel 15 C.P.S. Males and Females - 5% and 1%

No significant changes.

Methocel 400 C.P.S. Males and Females - 5% and 1%

No significant changes.

Methocel 4000 C.P.S. Males and Females - 5% and 1%

No significant changes.

rat 2953

testes		normal
pancreas		normal
spleen		normal
kidney	±	very slight interstitial nephritis
liver		normal
adrenal		normal
heart		normal
lung	++	moderate bronchial pneumonia

rat 2954

lung	+	interstitial pneumonitis with some emphysema
heart		normal
adrenal		normal
liver		normal
kidney		normal
spleen		normal
pancreas		normal
testes		normal

rat 2955

testes		normal
pancreas		normal
spleen		normal
kidney	++	hydronephrosis with interstitial nephritis
liver	+	central lobular granular degeneration
adrenal		normal
heart		normal
lung	++	moderate interstitial pneumonitis

rat 2956

lung	±	interstitial pneumonitis with congestion with some peri-bronchial inflammatory response
heart		normal
adrenal		normal: cortex; medulla not seen
liver	+	central lobular granular degeneration
kidney	+	interstitial nephritis with cloudy swelling of renal tubular epithelium
spleen		normal
pancreas		normal
testes		normal

rat 2957

testes		normal
pancreas		normal
spleen		normal
kidney	+	interstitial nephritis with cloudy swelling of renal tubular epithelium
liver	±	very slight central lobular granular degeneration
adrenal		normal: cortex; medulla not seen
heart		normal
lung	±	interstitial pneumonitis with peri-bronchial inflammatory response

rat 2958

pancreas		normal
testes		normal
spleen		normal
kidney	+	tubular and interstitial nephritis
liver	+	central lobular granular degeneeration and necrosis with one or two large fat vacuoles on the section
adrenal		normal
heart		normal
lung	±	very slight interstitial pneumnnitis

rat 2959

lung	±	interstitial pneumonitis and congestion
heart		normal
adrenal		normal
liver	+	central lobular granular degeneration and necrosis
kidney	++	interstitial and tubular nephritis
spleen		normal
pancreas		normal
testes		normal

rat 2960

pancreas		normal
testes		normal
spleen		normal
kidney	++	interstitial and tubular nephritis
liver	++	generalized cloudy swelling with some necrosis centrally
heart		normal
adrenal		normal
lung	+	interstitial pneumonitis with emphysema and atelectasis and some peri-bronchial inflammatory response

rat 2961

lung	+	interstitial pneumonitis
heart		normal
adrenal		normal
liver	+	central lobular granular degeneration
kidney	±	interstitial nephritis
spleen		normal
pancreas		normal
testes	+++	degeneration of all the tubules

rat 2962

lung		normal
heart		normal
adrenal		normal
liver	+	central lobular granular degeneration
kidney	±	interstitial nephritis together with some cloudy swelling of renal tubular epithelium
testes		normal
pancreas		normal
spleen		normal

rat 2963

lung	±	slight interstitial pneumonitis and congestion
heart		normal
adrenal		normal
liver	+	central lobular granular degeneration
kidney	±	slight interstitial nephritis
testes		normal
pancreas		normal
spleen		normal

rat 2964

lung	+	interstitial pneumonitis with emphysema and atelectasis and peri-bronchial inflammatory response
heart		normal
adrenal		normal
liver	+	central lobular granular degeneration
kidney	±	interstitial nephritis
spleen		normal
pancreas		normal
testes		normal

rat 2965

lung	±	interstitial pneumonitis with peri-bronchial inflammatory response
heart		normal
adrenal		normal
liver	+	central lobular granular degeneration
kidney	±	interstitial nephritis together with petechial hemorrhages in the cortex
spleen		normal
pancreas		normal
testes		normal

400 cps - 5% - Males

rat 2966

lung	±	interstitial pneumonitis with peri-bronchial inflammatory response
heart		normal
adrenal		normal
liver	+	central lobular granular degeneratio n with an occasional large fat vacuole in the mid-zonal area
kidney	+	slight hydronephrosis with interstitial nephritis and several petechial hemorrhages in the cortex
spleen		normal
pancreas		normal
testes		normal

rat 2967

lung	+	interstitial pneumonitis and emphysema with marked peri-bronchial inflammatory response
heart		normal
adrenal		normal: cortex; medulla not seen
liver	±	slight central lobular granular degeneration
kidney	∓	normal
spleen		normal
pancreas		normal
testes		normal

rat 2968

lung	+	interstitial pneumonitis and emphysema with peri-bronchial inflammatoy response
heart		normal
adrenal	++	hemorrhagic necrosis in the cortex
liver	±	central lobular granular degeneration
kidney	∓	interstitial nephritis with two petechial hemorrhages in the cortex
spleen		normal
pancreas		normal
testes		normal

rat 2969

lung	±	very slight peri-bronchial inflammatory response
heart		normal
adrenal		normal
liver	±	central lobular granular degeneration
kidney	+	interstitial and tubular nephritis
spleen		normal
pancreas		normal
testes		normal

rat 2970

lung	+	interstitial pneumonitis with peri-bronchial inflammatory response
heart		normal
adrenal		normal
liver	±	central lobular granular degeneration
kidney	++	interstitial and tubular nephritis with cast formation
spleen		normal
pancreas		normal
testes		normal

rat 2971

lung	+	interstitial pneumonitis with peri-bronchial inflammatory response
heart		normal
adrenal		normal
liver	±	central lobular granular degeneration
kidney	++	interstitial and tubular nephritis
testes		normal
pancreas		normal
spleen		normal

rat 2972

pancreas		normal
testes		normal
spleen		normal
kidney	++	interstitial and tubular nephritis
liver	±	central lobular granular degeneration
adrenal		normal
heart		normal
lung	±	interstitial pneumonitis and congestion

rat 2973

lung	+	moderate interstitial pneumonitis with emphysema and atelectasis
heart		normal
adrenal		normal
liver	±	central lobular granular degeneration
kidney	++	interstitial and tubular nephritis
spleen		normal
pancreas		normal
testes		normal

4000 cps - 5% - Males

rat 2974

lung		normal
heart		normal
adrenal		normal
liver		normal
kidney	±	interstitial nephritis
testes	±	degeneration of 20% of the tubules
pancreas		normal
spleen		normal

rat 2975

lung	+	interstitial pneumonitis with emphysema
heart		normal
adrenal		normal
liver	±	central lobular granular degeneration
kidney	±	interstitial nephritis with cloudy swelling of renal tubular epithelium
spleen		normal
pancreas		normal
testes		normal

rat 2976

lung	+	interstitial pneumonitis
heart		normal
adrenal		normal
liver	±	central lobular granular degeneration
kidney	±	interstitial nephritis
spleen		normal
pancreas		normal
testes		normal

rat 2977

lung	±	interstitial pneumonitis with peri-bronchial inflammatory response
heart		normal
Adrenal	+	hemorrhagic necrosis in the cortex
liver	±	central lobular granular degeneration
spleen		normal
kidney	+	interstitial and tubular nephritis
testes		normal
pancreas		normal

rat 2978

lung	±	interstitial pneumonitis with some emphysema
heart		normal
adrenal		normal
liver	±	central lobular granular degeneration
kidney	∓	interstitial and tubular nephritis
testes		normal
pancreas		normal
spleen		normal

rat 2979

lung	+	interstitial pneumonitis and emphysema and atelectasis and peri-bronchial inflammatory response
heart		normal
adrenal		normal
liver	+	central lobular granular degeneration with some slight bile duct proliferation portally
kidney	+	interstitial and tubular nephritis
spleen		normal
pancreas		normal
testes		normal

rat 2980

lung	+	interstitial pneumonitis with marked peri-bronchial inflammatory response
heart		normal
adrenal		normal
liver	±	central lobular granular degeneration
kidney	∓+	interstitial and tubular nephritis with cast formation
spleen		normal
pancreas		normal
testes	+++	degeneration of all the tubules

rat 2981

lung	±	interstitial pneumonitis
heart	normal	
adrenal		normal
liver	±	central lobular granular degeneration
kidney	∓+	interstitial and tubular nephritis with cast formation
spleen		normal
pancreas		normal
testes		normal

Control - Female

rat 2982

pancreas		normal
spleen		normal
kidney	++	glomerulo, tubular and interstitial nephritis with cast formation
liver	+	central lobular granular degeneration with occasional large vacuoles in mid-zonal areas
adrenal	+	slight hemorrhagic necrosis of the cortex
heart		normal
lung	±	interstitial pneumonitis

rat 2983

lung	++	bronchial pneumonia
heart		normal
adrenal	+++	hemorrhagic necrosis of the cortex
liver	+	central lobular granular degeneration with occasional large fat vacuoles in the mid-zonal areas
kidney	+	interstitial and tubular nephritis
spleen		normal
pancreas		normal

tumor is a fibro-sarcoma with hemorrhage

rat 2984

lung	±	slight interstitial pneumonitis with peri-bronchial inflammatory response
heart		normal
adrenal		normal
liver	+	central lobular granular degeneration with occasional large fat vacuoles in mid-zonal areas
kidney	+	interstitial and tubular nephritis
spleen		normal
pancreas		normal

rat 2985

pancreas		normal
spleen		normal
kidney	±	interstitial nephritis
liver	±	central lobular granular degeneration with occasional large fat vacuoles in mid-zonal areas
adrenal	+	hemorrhagic necrosis of the cortex
heart		normal
lung	±	slight interstitial pneumonitis and congestion

rat 2986

lung	++	bronchial pneumonia
heart		normal
adrenal	+++	hemorrhagic necrosis of the cortex
liver	+	central lobular granular degeneration
kidney	++	hydronephrosis with interstitial nephritis
spleen		normal
pancreas		normal

rat 2987

lung	±	interstitial pneumonitis
heart		normal
adrenal		normal
liver	±	central lobular granular degeneration
kidney	±	interstitial nephritis
spleen		normal
pancreas		normal

rat 2988

lung	+	interstitial pneumonitis with atelectasis and peri-bronchial inflammatory response
heart		normal
adrenal		normal: cortex; medulla not seen
liver	±	central lobular granular degeneration
kidney		normal
spleen		normal
pancreas		normal

rat 2989

lung	+	interstitial pneumonitis
heart		normal
adrenal	++	hemorrhagic necrosis of the cortex; medulla not seen
liver	+	central lobular granular degeneration with occasional large fat vacuoles in mid-zonal areas
kidney	++	hydronephrosis with interstitial and tubular nephritis
spleen		normal
pancreas		normal

rat 2990

lung	±	interstitial pneumonitis
heart		normal
adrenal	++	hemorrhagic necrosis of the cortex
liver	±	central lobular granular degeneration
kidney	±	interstitial and tubular nephritis
spleen		normal
pancreas		normal

rat 2991

lung	±	interstitialpneumonitis
adrenal	+++	hemorrhagic necrosis of the cortex; medulla not seen
liver	±	central lobular granular degeneration
heart		no section
kidney	++	interstitial and tubular nephritis with cast formation
spleen		normal
pancreas		normal

rat 2992

lung	+	moderate interstitial pneumonitis with some peri-bronchial inflammatory response
heart		normal
adrenal	+++	hemorrhagic necrosis of the cortex
liver	±	central lobular granular degeneration
kidney	++	interstitial and tubular nephritis
spleen		normal
pancreas		normal

rat 2993

lung	+	interstitial pneumonitis with peri-bronchial inflammatory response
heart		normal
adrenal	++	hemorrhagic necrosis of the cortex
liver	±	central lobular granular degneration
kidney	±	interstitial and tubular nephritis
spleen		normal
pancreas		normal

rat 2994

pancreas		normal
spleen		normal
kidney	+	interstitial and tubular nephritis
liver	+	central lobular granular degeneration with occasional large fat vacuoles in mid-zonal areas
adrenal		+++ hemorrhagic necrosis of the cortex
heart		normal
lung	+	interstitial pneumonitis with some emphysema and peri-bronchial inflammatory response

rat 2995

lung	+	interstitial pneumonitis
heart		normal
adrenal	+	slight hemorrhagic necrosis of the cortex
liver	+	central lobular granular degeneration with an occasional large fat vacuole in the mid-zonal area
kidney	++	hydronephrosis with interstitial and tubular nephritis
spleen		normal
pancreas		normal

rat 2996

lung	+	interstitial pneumonitis with some peri-bronchial inflammatory response
heart		normal
adrenal	+	slight hemorrhagic necrosis of the cortex
liver	∓	central lobular granular degeneration with occasional large fat vacuoles in mid-zonal areas
kidney	+	interstitial and tubular nephritis
spleen		normal
pancreas		normal

rat 2997

lung	+	interstitial pneumonitis
heart		normal
adrenal	+++	hemorrhagic necrosis of the cortex
liver	+	central lobular granular degeneration
kidney	∓	interstitial and tubular nephritis
spleen		normal
pancreas		normal

rat 2998

lung	+	moderate interstitial pneumonitis
heart		normal
adrenal	+++	hemorrhagic necrosis of the cortex
liver	+	central lobular granular degeneration
kidney	±	cloudy swelling of renal tubular epithelium together with some interstitial nephritis
spleen		normal
pancreas		normal

rat 2999

lung	++	bronchial pneumonia
heart		normal
adrenal		normal
liver	+	central lobular granular degeneration with an occasional large fat vacuole in the mid-zonal area
kidney	+	interstitial and tubular nephritis
spleen		normal
pancreas		normal

rat 3000

lung	±	slight interstitial pneumonitis
heart		normal
adrenal	+++	hemorrhagic necrosis of the cortex
liver	+	central lobular granular degeneration
kidney	±	interstitial and tubular nephritis
spleen		normal
pancreas		normal

rat 3001

lung	±	interstitial pneumonitis
heart		normal
adrenal	+++	hemorrhagic necrosis of the cortex; medulla not seen
liver	+	central lobular granular degeneration with an occasional large fat vacuole in mid-zonal area
kidney	+	interstitial and tubular nephritis
spleen		normal
pancreas		normal

rat 3002

lung	+	interstitial pneumonitis with peri-bronchial inflammatory response
heart		normal
adrenal	+	hemorrhagic necrosis of the cortex; medulla not seen
liver	+	central lobular granular degeneration
kidney	∓	interstitial and tubular nephritis
spleen		normal
pancreas		normal

rat 3003

lung	+	interstitial pneumonitis with emphysema and atelectasis
heart		normal
adrenal	+++	hemorrhagic necrosis of the cortex
liver	+	very slight central lobular granular degeneration
kidney	∓	interstitial and tubular nephritis
spleen		normal
pancreas		normal

rat 3004

lung	+	interstitial pneumonitis
heart		normal
adrenal	++	hemorrhagic necrosis of the cortex; medulla not seen
liver	+	central lobular granular degeneration
kidney	∓	interstitial and tubular nephritis
pancreas		normal
spleen		normal

rat 3005

lung	±	interstitial pneumonitis
heart		normal
adrenal	+++	hemorrhagic necrosis of the cortex
liver	+	marked central lobular granular degeneration
kidney	+	interstitial and tubular nephritis
spleen		normal
pancreas		normal

rat 3006

pancreas		normal
kidney	±	interstitial nephritis
liver	∓	central lobular granular degeneration with an occasional large fat vacuole in the mid-zonal area
adrenal	++	hemorrhagic necrosis of the cortex
heart		normal
lung	+	interstitial pneumonitis and congestion

rat 3007

lung	+	interstitial pneumonitis with emphysema and atelectasis
heart		normal
adrenal		normal
liver	+	central lobular granular degeneration
kidney	∓+	interstitial nephritis, tubular nephritis and hydronephrosi
pancreas		normal
spleen		normal

rat 3008

lung	+	interstitial pneumonitis with some emphysema and peri-bronchial inflammatory response
heart		normal
adrenal	+++	hemorrhagic necrosis of the cortex
liver	+	central lobular granular degeneration
kidney	∓+	interstitial and tubular nephritis
spleen		normal
pancreas		normal

rat 3009

lung	+	interstitial pneumonitis
heart		normal
adrenal	++	hemorrhagic necrosis of the cortex; medulla not seen
liver	+	central lobular granular degeneration
kidney	∓	interstitial and tubular nephritis
spleen		normal
pancreas		normal

rat 4780

lung	peri-bronchial cell infiltration also the presence of several areas of pneumonitis with macrophages
adrenal	normal
heart	normal
liver	mostly normal with a few areas showing slight increase of the connective tissue around the bile ducts and also proliferation of the bile duct
kidney	normal
spleen	normal
testes	normal
pancreas	normal

rat 4781

lung	bronchitis, peri-bronchial cell infiltration and several areas of pneumonitis also the alveoli contain macrophages
adrenal	normal
heart	moderate degenerative changes in the myocardial muscle fibers particularly in the capillary muscles
liver	normal
kidney	senile changes as characterized by chronic interstitial nephritis also hyliniaation of several glomeruli and a corressponding death of the tubules attached to them, also the presence of hyaline casts
spleen	normal
testes	normal
pancreas	normal

rat 4782

spleen	normal
heart	moderate degenerative changes in the myocardial fibers and slight increase in the interstitial tissue
adrenal	normal
liver	a few foci of focal necrosis and degenerative changes also moderate proliferation of the bile duct epithelium
kidney	degenerative senile changes characterized by increased amounts of interstitial tissue also the presence of hyalinization in many of the glomeruli and corressponding necrosis of the tubular epithelium also the presence of hyaline casts
testes	normal
pancreas	normal

rat 4783

lung	large areas infiltrated with macrophages also the presence of peri-bronchial cell infiltration and small areas of pneumonitis
heart	normal
adrenal	normal
liver	normal
kidney	moderate degenerative changes of senile origin
spleen	normal
pancreas	normal
testes	normal

rat 4784

lung	peri-bronchial cell infiltration and pneumonitis
adrenal	normal
heart	mostly normal
liver	normal
kidney	normal
testes	normal
spleen	normal
pancreas	normal

rat 4785

lung	peri-bronchial cell infiltration and extensive thickening of the bronchial wall also a few areas of pneumonitis and macrophage infiltration of the alveoli
heart	normal
adrenal	normal
liver	normal
kidney	normal
spleen	normal
testes	normal

rat 4786

lung	peri-bronchial cell infiltration and infection
heart	moderate degenerative changesin the myocardium
liver	normal
kidney	senile degenerative changes also increase of interstitial tissue and the presence of hyaline casts
spleen	normal
testes	normal
pancreas	normal

rat 4787

lung	areas of pneumonia and peri-bronchial cell infiltration
adrenal	normal
heart	normal
liver	normal
kidney	normal
spleen	normal
pancreas	normal
testes	normal

rat 4788

lung	several areas of pneumonitis and infection also the presence of peri-bronchial cell infiltration
adrenal	normal
heart	moderate degenerative changes in the myocardium
liver	normal
kidney	normal
testes	normal
pancreas	normal
spleen	normal

rat 4789

testes	moderate degenerative changes in the seminepherous tubules
kidney	normal
liver	several foci of focal necrosis
spleen	normal
pancreas	normal
lung	several areas of pneumonitis, thickening of the alveolar walls and peri-bronchial cell infiltration

rat 4790

lung	peri-bronchial cell infiltration and thickening of the alveolar walls
heart	moderate degenerative changes in the myocardium
adrenal	normal
liver	diffuse degenerative changes and areas of focal necrosis
kidney	normal
testes	normal
spleen	normal

rat 4791

lung	peri-bronchial cell infiltration
adrenal	normal
heart	normal
liver	normal
kidney	normal
pancreas	normal
testes	normal
spleen	normal

rat 4792

lung	peri-bronchial cell infiltration and mild pneumonitis
heart	normal
adrenal	moderate hemorrhage and necrosis in the cortex
liver	normal
kidney	2 glomeruli in the section show a mass of amorphous material outside of the glomerulo tuft and forming what appears to be a crescent
spleen	normal
testes	normal
pancreas	normal

rat 4793

lung	peri-bronchial cell infiltration and pneumonitis
adrenal	normal
liver	normal
kidney	many glomeruli show hylinization and corressponding death of the nephron attached to them - quite extensive also there is chronic interstitial nephritis and the presence of hyaline casts
testes	normal
pancreas	normal

rat 4795

lung	peri-bronchial cell infiltration and pneumonitis
adrenal	small adenoma on the cortex
heart	normal
liver	normal
kidney	normal
pancreas	normal
testes	normal
spleen	normal

rat 4796

lung	several patches of pneumonitis and peri-bronchial cell infiltration
adrenal	normal
liver	a few areas of focal necrosis and slight proliferation of conncetive tissue in the portal spaces
spleen	hemosiderin deposits
kidney	normal
testes	normal
pancreas	normal

rat 4797

lung	peri-bronchial cell infiltration and areas of pneumonitis
adrenal	normal
heart	normal
liver	diffuse cloudy swelling in several areas showing degenerative changes and necrotic changes
kidney	normal
spleen	normal
pancreas	normal
testes	normal

rat 4798

lung	peri-bronchial cell infiltration
adrenal	normal
heart	areas of of the papillary muscles show necrotic changes
liver	normal
kidney	normal
spleen	normal
testes	normal

rat 4799

lung	a large area of peri-bronchial cell infiltration and pneumonitis
heart	normal
adrenal	normal
kidney	normal
spleen	normal
pancreas	normal
testes	degenerative changes of the seminephrous epithelium also edema of the interstitial tissue - no spermatogenesis present

rat 4800

lung	normal
adrenal	normal
heart	normal
liver	mostly normal - slight increase in the connective tissue around the portal spaces
kidney	normal
spleen	normal
testes	normal
pancreas	normal

rat 4801

lung	normal
adrenal	normal
heart	normal
liver	normal
kidney	degenerative changes in epithelium lining, the convoluted tubules cytoplasmis granular also hylanization of the glomerulo tufts
spleen	normal
testes	necrosis and degeneration of the seminephrous tubules - no spermatogenesis
pancreas	normal

rat 4802

lung	peri-bronchial cell infiltration and pneumonitis
liver	normal
adrenal	normal
kidney	normal
spleen	normal
testes	normal
pancreas	normal

rat 4803

lung	peri-bronchial cell infiltration and pneumonitis
adrenal	normal
heart	degenerative changes in the myocardium fibers and also the presence of fibrosis
liver	normal
kidney	several glomeruli show hyalinization and corressponding death of the nephrons also interstitial nephritis
pancreas	normal
testes	normal
spleen	normal

rat 4804

lung	pneumonitis
adrenal	normal
heart	normal
liver	several foci of focal necrosis, mononuclear cell infiltration
kidney	normal
pancreas	normal
spleen	normal
testes	normal

rat 4805

lung	normal
adrenal	normal
heart	normal
liver	normal
kidney	normal
spleen	normal
testes	normal
pancreas	mostly normal - a few areas have cystic formations in them

rat 4805

lung	normal
heart	normal
adrenal	several areas of hemorrhage in the cortex
liver	normal
kidney	mostly normal, slight increase of the interstitial tissue
spleen	normal
pancreas	normal

rat 4807

lung	slight thickening of the alveolar walls and peri-bronchail cell infiltration
heart	normal
adrenal	several areas of hemorrhage in the cortex
liver	normal
kidney	senile changes characterized by decreased amounts of interstitial tissue and occasionally there is an atrophied glomerulus also the presence of hyaline casts in the tubules - also has a certain amount of pyelonephritis and hydronephrosis
spleen	normal
pancreas	normal

rat 4808

lung	slight increase of interstitial tissue and pneumonitis
heart	normal
adrenal	extensive hemorrhage in the cortex
liver	normal
kidney	hydronephrosis
spleen	normal
pancreas	normal

rat 4809

lung	extensive pneumonia associated with formation and granulome
heart	normal
adrenal	extensive hemorrhage in the cortex
liver	many effects characterized by cloudy swelling in the center of the lobule and occasional fat cell
kidney	several glomeruli show hyalinization and corressponding necrosis of the nephrons also there is slight increase of the interstitial tissue
spleen	normal
pancreas	normal

rat 4810

lung	extensive areas show presence of pneumonia and abscess formation
heart	normal
adrenal	extensive necorosis and hemorrhage in the cortex
liver	normal
kidney	hydronephrosis
spleen	normal
pancreas	normal

rat 4811

lung	peri-bronchial cell infiltration and thickening of the alveolar walls
adrenal	enlarged also extensive necrosis
heart	degenerative changes of the myocardial fivers
liver	diffuse cloudy swelling and extensive proliferation of the bile ducts
kidney	hydronephrosis compiled by senile degenerative changes affecting both the glomeruli and interstitial tissue
spleen	normal
pancreas	normal

rat 4812

lung	slight thickening of the alveoli walls
adrenal	extensive hemorrhage
liver	normal
kidney	normal
pancreas	normal
spleen	normal

rat 4813

lung	peri-bronchial cell infiltration
heart	normal
adrenal	hemorrhage and necrosis of the crotex
liver	diffuse cloudy swelling and possible proliferation of the bile ducts also areas of focal necrosis and mononuclear cell infiltration
kidney	normal
pancreas	normal
spleen	normal

rat 4814

lung	peri-bronchial cell infiltration and an occasional area of pneumonitis
adrenal	necrosis and hemorrhage of the cortex
heart	normal
liver	diffuse cloudy swelling
kidney	hydronephrosis and also interstitial nephritis in the remaining part of the kidney
spleen	normal
pancreas	normal

rat 4815

lung	peri-bronchial cell infiltration and slight thickening of the alveolar walls
heart	normal
adrenal	hemorrhagic necoris of the cortex associated with cyst formations
liver	normal
kidney	hyaline degeneration of many glomerulus also the presence of increased amount of interstitial tissue accompanied with hyaline casts in many tubules
spleen	normal
pancreas	normal

rat 4816

lung	areas of thickening of the alveolar walls also peri-bronchial cell infiltration and moderate blocking of bronchioles also areas showing emphysema
adrenal	enlarged along with hemorrhagic necrosis of the cortex
heart	normal
liver	moderate fatty changes in the liver associated also with mono-nuclear cell infiltration in several areas
kidney	normal
pancreas	normal
spleen	normal

rat 4817

lung	moderate thickening of the alveolar walls and pneumonitis
heart	moderate degenerative changes of the myocardial muscle fiber and slight increase in interstitial tissue

adrenal	hemorrhagic necrosis of the cortex
liver	moderate fatty changes
kidney	hydronephrosis
spleen	normal
pancreas	normal

rat 4818

lung	peri-bronchial cell infiltration
adrenal	enlarged and severe destruction of the cortex hyalinization and hemorrhagic necrosis - medulla does not appear on the section
heart	moderate myocardial degeneration
liver	normal
kidney	extensive degenerative changes, hyalinization of the glomeruli and also interstitial nephritis
pancreas	normal
spleen	normal

rat 4819

lung	moderate thickening of the alveoli walls and peri-bronchial cell infiltration
adrenal	hemorrhagic necrosis of the cortex
heart	normal
liver	normal
kidney	normal
spleen	normal
pancreas	normal

rat 4820

lung	peri-bronchial cell infiltration and pneumonitis
heart	moderate degenerative changes of the myocardial fibers
adrenal	enlarged also hemorrhagic necrosis of the cortex and cystic formation
liver	normal
kidney	normal
spleen	normal
pancreas	normal

rat 4821

lung	peri-bronchial cell infiltration
adrenal	hemorrhagic necrosis of the cortex
heart	moderate myocardial degenerative changes
liver	bile duct proliferative changes
kidney	normal
spleen	normal
pancreas	normal

rat 4822

lung	pneumonitis and peri-bronchial cell infiltration
adrenal	hemorrhagic necrosis of the cortex
heart	moderate myocardial degenerative changes
liver	a few areas showing focal necrosis and replacement fibrosis
kidney	hydronephrosis and the remaining part of the kidney tissue show several glomeruli with hyalinization and corressponding death of the nephrons
spleen	normal
pancreas	normal

rat 4823

lung	several areas of pneumonitis
adrenal	hemorrhagic necrosis of the cortex
heart	normal
liver	nommal
kidney	senile degenerative changes characterized by hyalinization of several glomeruli and corressponding of the nephrons. also increase in interstitial tissue
spleen	normal
pancreas	normal

rat 4824

lung	peri-bronchial cell infiltration and pneumonitis
adrenal	hemorrhagic necrosis of the cortex
heart	normal
liver	normal
kidney	normal
spleen	normal
pancreas	normal

rat 4825

lung	thickening of the alveolar walls
heart	moderate myocardial degenerative changes
adrenal	vacuolation and extensive hemorrhagic necrosis of the cortex
liver	diffuse cloudy swelling and thickening of most of the liver cells
kidney	senile degenerative changes characterized by interstitial nephritis and also many of the glomeruli show hyalinization and corressponding death of the nephrons and al so the presence of hyaline casts
spleen	mostly normal
pancreas	normal

rat 4826

lung	thickening of the alveolar walls
adrenal	extensive hemorrhagic necrosis of the cortex also the formation of large cysts
heart	normal
liver	normal
kidney	normal
pancreas	normal
spleen	normal

rat 4827

lung	peri-bronchial cell infiltration and pneumonitis
adrenal	enlarged also hemorrhagic necrosis and cyst formation in the corte
heart	moderate degenerative changes in the myocardial
liver	normal
kidney	normal
pancreas	normal
spleen	normal

rat 4828

lung	extensive peri-bronchial cell infiltration and pneumonitis
adrenal	enlarged and extensive hemorrhagic necrosis and cyst formation in the cortex
heart	moderate degenerative changes in the myocardium
liver	normal
spleen	normal
kidney	generally normal, a few areas only show localized interstitial nephritis and occasional hyalinization of a glomerulus and corressponding death of the nephron
pancreas	normal

rat 4829

lung	peri-bronchial cell infiltration
adrenal	extensive hemorrhagic necrosis and cyst formation in the cortex
heart	normal
liver	a few vacuoles scattered all over the section also cloudy swelling in a few areas of the central part ef the lobule
kidney	normal
spleen	normal
pancreas	normal

rat 4830

lung	pneumonitis and presence of cells
heart	normal
adrenal	hemorrhagic necrosis of the cortex
liver	moderate cloudy swelling in the central part of the lobule
kidney	a few glomeruli show evidence of hyalinization
pancreas	normal
spleen	normal

rat 4831

lung	peri-bronchial cell infiltration
adrenal	extensive hemorrhagic necrosis in the cortex
heart	normal
liver	normal
kidney	normal
spleen	normal
pancreas	normal

rat 4832

lung	peri-bronchial cell infiltration also pneumonitis
heart	normal
adrenal	extensive hemorrhagic necrosis and cyst formation in the cortex
liver	normal
kidney	chronic interstitial nephritis
spleen	normal
pancreas	normal

rat 4833

lung	peri-bronchial cell infiltration and several areas of pneumonitis cells
adrenal	hemorrhagic necrosis of the cortex
heart	normal
liver	cloudy swelling in the central part of the lobule
kidney	normal
spleen	normal
pancreas	normal

rat 4834

lung	peri-bronchil cell infiltration
adrenal	enlarged and extensive hemorrhagic necrosis and cyst formation in the cortex
heart	normal
liver	normal
adrenal	normal
kidney	normal
spleen	normal
pancreas	normal

rat 5176

lung	peri-bronchial cell infiltration
adrenal	hemorrhagic necrosis of the cortex and also cyst formation
heart	normal
liver	diffuse cloudy swelling and congestion of the sinusoids also a few liver cells show focal necrosis
kidney	normal
spleen	nommal
testes	normal
pancreas	normal

rat 5177

adrenal	hemorrhagic necrosis of the cortex
liver	normal
heart	normal
spleen	normal
kidney	normal
testes	normal
pancreas	normal

rat 5178

lung	pneumonitis with extensive organization and infiltration with cellular reaction
heart	normal
adrenal	normal
liver	normal
kidney	normal
testes	degenerative changes in the seminiferous tubules with edema of the interstitial tissue
spleen	normal
pancreas	normal

rat 5179

lung	peri-bronchial cell infiltration
adrenal	normal
heart	moderate degenerative changes of the myocardium and slight increase in the interstitial tissue
liver	normal
kidney	normal
testes	a greater majority of the seminiferous tubules have dissappeared and only rudiments of tubules completely degenerative are present and there appears to be an interstitial cell tumor

rat 5180

lung	peri-bronchial cell infiltration
adrenal	slightly enlarged and moderate degenerative changes in the cortex
heart	normal
liver	normal
kidney	normal
spleen	normal
pancreas	normal
testes	normal

rat 5181

lung	peri-bronchial cell infiltration and thickening of the alveolar walls
adrenal	moderate vacuolation in the cortex
heart	normal
liver	diffuse degenerativec changes
spleen	normal
kidney	extensive senile changes characterized by the presence of hyaline casts and also proliferation of the interstitial tissue many the glomerulus show atrophy
testes	normal
pancreas	normal

rat 5182

lung	peri-bronchial cell infiltration and areas of pneumonitis
heart	normal
adrenal	hemorrhagic necrosis of the cortex
heart	normal
liver	diffuse degenerative changes particularly in the central part of the lobule with beginning fibrosis
kidney	chronic interstitial nephritis
testes	normal
pancreas	normal
spleen	normal

rat 5183

lung	several areas of pneumonitis, thickening of the alveolar walls and peri-bronchial cell infiltration
adrenal	normal
heart	normal
liver	normal
kidney	normal
spleen	normal
testes	normal

rat 5184

lung	peri-bronchial cell infiltration and pneumonitis
adrenal	normal
heart	normal
liver	normal
testes	normal
kidney	hydronephrosis
pancreas	normal
spleen	normal

rat 5185

lung	peri-bronchial cell infiltration
heart	normal
adrenal	normal
liver	normal
kidney	normal
spleen	normal
pancreas	normal
testes	normal

rat 5186

lung	normal
heart	moderate myocardium degenerative changes and slight increase in the interstitial tissue
adrenal	normal
liver	nommal
spleen	moderate hyperplasia of the white pulp
testes	normal
kidney	normal
pancreas	normal

rat 5187

lung	thickening of the alveolar walls and pneumonitis
heart	normal
adrenal	2 areas of vacuolation in the cortex
liver	several areas of degenerative changes, vacuolation and also evidence of regeneration of liver cells
kidney	several glomeruli and undergone hyalinization with corressponding death of the nephron connected to also the presence of hyaline casts on the tubules
testes	normal
spleen	normal
pancreas	normal

rat 5188

lung	thickening of the alveolar walls and the presence of pneumonitis
heart	several areas of the myocardial show degenerative changes
adrenal	normal
liver	normal
kidney	normal
spleen	normal
pancreas	normal
testes	atrophy and degenerative changes of the seminiferous tubules and edema of the interstitial tissue

rat 5189

lung	extensive pneumonia with consolidation of most of the lung
heart	moderate degenerative changes of the myocardium
adrenal	hemorrhagic necrosis of one area of the cortex
liver	diffuse cloudy selling covering the whole section
kidney	diffuse degenerative changes and necrosis of the tubular epithelium
spleen	presence of numerous giant cells all over the section
pancreas	normal
testes	normal

5190

	thickening of the alveolar walls and peri-bronchial cell infiltration
[illegible]	extensive vacuolation of the cortex
heart	moderate degenerative changes of the myocardial muscle fibers also the presence of many massed cells
liver	excessive cloudy swelling and degenerative changes in all of the liver section also congestion of the sinusoids
kidney	excessive proliferation of the interstitial tissue also the presence of degenerative changes of hypercellularity in the glomeruli and degenerative changes of the tubular epithelium
spleen	normal
testes	normal
pancrease	normal

rat 5190A

lung	peri-bronchial cell infiltration
heart	moderate degenerative changes in the myocardial muscle fibers
adrenal	normal
liver	several areas of focal necrosis and slight congestion also diffuse degenerative changes in the liver cells
kidney	extensive hyalinization of the glomeruli and corresponding death of the attached nephrons also the presence of interstitial nephritis and hyaline casts in the tubules
spleen	normal
pancreas	normal
testes	degenerative changes and atrophy of the seminefhrous tubules

rat 5191

lung	mostly normal with the exception of one small areas where there is peri-bronchial cell infiltration
heart	~~normal~~ myocardial degenerative changes also the presence of a slight increase in connective tissue and also accumulation of massed cells
adrenal	slight degenerative changes in the cortex and an area of excessive vacuolation
liver	cloudy swelling of the liver lobules also the presence of numerous large vacuoles
kidney	chronic interstitial nephritis with hyalinization of many glomerulus also the presence of degenerative changes in the tubular epithelium and the presence of hyaline casts
pancreas	normal
spleen	normal
testes	normal

rat 5192

lung	thickening of the alveolar walls and the presence of cells also peri-bronchial cell infiltration
heart	myocardial degenerative changes and increased cellularity also the presence of cells
adrenal	hemorrhagic degenerative changes in the cortex also the presence of excessive vacuolation in some areas
liver	moderate degenerative changes and cloudy swelling of the liver cells
spleen	enlarge with the presence of multi-nucleated giant cells
kidney	atrophy of a few glomeruli also the presence of interstitial tissue
testes	normal
pancreas	normal

rat 5193

lung	peri-bronchiel cell infiltration and moderate thickening the alveolar walls
heart	moderate degenerative changes in the myocardial
adrenal	small area of vacuolation in the cortex
liver	diffuse focal necrosis also cloudy swelling of liver cells
spleen	normal
testes	normal
kidney	normal
pancreas	normal

rat 5194

lung	peri-bronchial cell infiltration, penumonitis and the presence of cells
adrenal	hemorrhagic necrosis of the cortex
heart	moderate degenerative changes in the mycoardium
liver	diffuse cloudy swelling and areas of focal necrosis
spleen	normal
kidney	normal
testes	degenerative end atrophy of the seminiferous tubules
pancreas	normal

rat 5195

lung	peri-bronchial cell infiltration and areas of pneumonitis
heart	moderate degenerative changes of the myocardium
liver	diffuse cloudy swelling and several areas of large focal necrosis
adrenal	normal
spleen	contains a few multi-nucleated giant cells
kidney	slight increase in interstitial tissue and a few glomeruli show hyalinization and the presence of hyaline casts in the tubules
pancreas	normal
testes	normal

rat 5196

lung	peri-bronchial cell infiltration - extensive, also a few areas of thickening of the alveolar walls
adrenal	small areas of vacuolation in the adrenal cortex
heart	moderate degenerative changes in the myocardial fibers also the presence of cellular infiltration
liver	diffuse cloddy swelling and also the presence of cellular infiltration in certain areas - changes observed are mostly close to the portal spaces
kidney	a few areas show increased cellularity of the interstitial tissue also atrophy of a few glomeruli and the presence of hyaline casts in the tubules.
pancreas	normal
spleen	presence of a few giant dispersed in the section
testes	moderate edema of the interstitial tissue

rat 5197

lung	extensive pneumonia with a large area of organization and also infiltration with cellular reaction also a granulomatosis formation
adrenal	small area of hemorrhagic necrosis in the cortex
heart	moderate degenerative changes of the myocardial muscel fibers also the presence of increased cellularity in between the muscle fibers and also the presence of tissue merged cells
liver	slight engorgement of the sinusoids withlblood
kidney	mostly normal
pancreas	normal
spleen	normal
testes	normal

rat 5198

lung	peri-bronchial cell infiltration and pneumonitis
adrenal	normal
heart	normal
liver	cloudy swelling and degenerative changes in several areas close to the mid-zonal areas of the lobule
kidney	normal
testes	normal
pancreas	normal
spleen	normal

rat 5199

lung	peri-bronchial cell infiltration
heart	degenerative changes of the myocardial fibers, several areas of necrosis and the presence of tissed massed cells
adrenal	excessive vacuolation of one area in the cortex
liver	several areas of focal necrosis and pyknosis of the nucleai also many areas show degenerative changes
kidney	mostly normal, slight hydronephrosis
spleen	slight proliferation of the white pulp with many giant cells
pancreas	normal
testes	normal

rat 5200

lung	peri-bronchial eell infiltration
adrenal	vacuolation in a large area in the cortex
liver	several areas of cloudy swelling and degneration, diffusely spread all over the section
kidney	normal
testes	normal
pancreas	normal
spleen	normal

rat 5201

lung	extensive peri-bronchial cell infiltration
heart	normal
adrenal	hemorrhagic necrosis of the cortex
liver	several areas of focal necrosis
spleen	several giant cells spread all over the section
testes	normal
pancreas	normal

rat 5202

lung	thickening of the alveolar walls
adrenal	a small areas of vacuolation in the cortex
heart	degenerative changes and proliferation of connective tissue
liver	moderate degenerative changes in several areas in the liver section in associationnof the central part of the lobule
kidney	several areas show evidence of connective tissue formation and scarring
testes	normal
pancreas	normal
spleen	normal

RAT 5203

lung	peri-bronchial cell infiltration
heart	myocardial degenerative changes also proliferation of connective tissue
adrenal	a large area of hemorrhagic necrosis in the cortex
liver	mostly normal
kidney	chronic interstitial nephritis
spleen	normal
pancreas	normal
testes	normal

rat 5204

lung	slight thickening of the alveolar walls
heart	moderate myocardial degenerative changes and proliferation of connective tissue
adrenal	normal
liver	extensive necrosis of a part of the liver lobule the rest of the section shows areas of focal necrosis of cells
kidney	mostly normal
spleen	several foci of mononuclear cell infiltration with giant cells
pancreas	normal
testes	normal

rat 5205

lung	peri-bronchial cell infiltration
adrenal	large area of vacuolation in the cortex
heart	moderate degenerative changes and similar proliferation of the myocardium
liver	pyknosis of the nucleai in the central part of the lobule and diffuse cloudy swelling
kidney	scarring on the surface also slight increase in interstitial tissue - a few hyaline casts are present
testes	normal

rat 5206

lung	excessive peri-bronchial cell infiltration and large areas of pneumonitis
heart	moderate degenerative changes of the myocardial muscle fibers and connective tissue proliferation
adrenal	normal
liver	diffuse cloudy swelling
kidney	moderate degenerative changes in the tubular epithelium also many glomeruli show hyalinazation and moderate proliferation of the interstitial connective tissue
spleen	presence of many multinucleated giant cells and also mononuclear cell infiltration
pancreas	normal
testes	normal

rat 5207

lung	extensive pneumonitis and peri-bronchial cell infiltration also presence of many basophils
heart	extensive degenerative changes in the myocardial fibers also the presence of fibrosis
liver	diffuse cloudy swelling
adrenal	normal

rat 5207

lung	peri-bronchial cell infiltration and excessive fibrosis and and infiltration
adrenal	normal
heart	normal
spleen	normal
kidney	normal
liver	diffuse cloudy swelling and areas of cellular infiltration
pancreas	normal
testes	normal

rat 5208

lung-	peri-bronchial cell infiltration and areas of pneumonitis
adrenal	normal
heart	normal
liver	a few areas showing focal necrosis also an occasional vacuole is present with areas also of cloudy swelling
testes	normal
kidney	mostly senile degenerative changes
pancreas	normal
spleen	normal

rat 5209

lung	thickening of the alveolar walls
heart	myocardial degenerative changes
adrenal	excessive vauolation of the cortex
liver	moderate degenerative changes in the central part of the lobule
spleen	normal
kidney	a few glomeruli show hypercellularity and moderate hyalination also slight increase in the interstitial tissue
pancreas	normal
spleen	normal
testes	normal

rat 5210

lung	peri-bronchial cell infiltration and pneumonitis
adrenal	enlarge of the medulla with several areas of hemorrhages
heart	normal
liver	central lobular cloudy swelling and degenerative changes
kidney	moderate increase of the interstitial tissue also glomeruli of hypercellularity
spleen	normal
pancreas	nommal
testes	normal

rat 5211

lung	thickening of the alveolar walls and pneumonitis
adrenal	hemorrhagic necrosis of the cortex
heart	moderate hyocardial degenerative changes and infilitration of fibroblast
liver	central lobular degenerative changes and congestion of sinusoids
kidney	slight increase of the interstitial tissue also the presence of hyaline casts and moderate cellularity of the glomerulo tufts
pancreas	normal
spleen	normal
testes	normal

rat 5212

lung	mild peri-bronchial cell infiltration
adrenal	moderate vacuolation of the adrenal cortex
heart	very mild degenerative changes and connective tissue proliferation
liver	several areas of focal necrosis particularly in the central part
kidney	chronic interstitial nephritis, a few glomeruli shows signs of atrophy and a hyaline material in the capsule
pancreas	normal
testes	normal
spleen	normal

rat 5213

lung	peri-bronchial cell infiltration
heart	moderate myocardial degenerative changes and cellular infiltration
adrenal	moderate degenerative changes in the cortex
liver	diffuse cloudy swelling and early proliferative changes of the connective tissue
spleen	normal
testes	normal
kidney	extensive chronic interstitial nephritis with also atrophy and degenerative changes of the tubules with presence of hyaline casts
testes	normal
pancreas	normal
spleen	normal

rat 5214

extensive pneumonia - lung
adrenal normal
heart myocardial degenerative changes and fibroblastic infiltration
liver diffuse degenerative changes also areas of focal necrosis and congestion of the sinusoids
kidney normal
spleen normal
pancreas normal
testes normal

rat 5215

lung chronic pneumonitis
heart degenerative changes of the myocardium also presence of excessive amount of fibrous tissue
adrenal extensive degenerative changes in the adrenal cortex
liver diffuse cloudy and small area of focal necrosis
kidney chronic intersitial nephritis
spleen infiltration with giant cells
testes normal
pancreas normal

rat 5216

lung	several areas of pneumonitis and peri-bronchial cell infiltration
heart	moderate myocardial degenerative changes also the presence of macrophages
liver	diffuse cloudy swelling
spleen	presence of giant cells multinucleated
kidney	mostly normal one area showing evidence of a scar
testes	normal

rat 5217

lung	peri-bronchial cell infiltration and pneumonitis
adrenal	excessive vacuolation of the cortex
heart	extensive degenerative changes in the myocardial fibers
liver	normal
kidney	moderate cloudy swelling of the tubular cytoplas also one or two glomeruli show hyalinazation
spleen	normal
pancreas	normal
testes	atrophy and degenerative changes of the semineferous tubules

rat 5218

lung	thickening of the alveolar walls and several areas of pneumonitis and also peri-bronchial cell infiltration
adrenal	mostly normal
heart	normal
kidney	normal
testes	normal
pancreas	normal
spleen	several areas showing slight increase in mononuclear cells also giant cells

rat 5219

lung	peri-bronchial cell infiltration and pneumonitis
adrenal	normal
heart	normal
liver	normal
kidney	two areas show evidence of hyalinazation of the glomeruli and increased interstitial tissue also hyaline casts present in the tubules
spleen	normal
testes	the organ is affected with a tumor diagnosed as a seminoma

rat 5220

lung	slight thickening of the alveolar walls
heart	moderate degenerative changes in myocardium
adrenal	normal
liver	normal
kidney	slight increase in the interstitial tissue
spleen	normal
testes	atrophy and degenerative changes of the seminefrous tubules

rat 5221

lung	several areas of pneumonitis
adrenal	normal
heart	normal
liver	normal
kidney	chronic interstitial nephritis
testes	normal
pancreas	normal
spleen	normal

rat 5222

lung	extensive pneumonia with
heart	a area of degenerative changes and myocarditis
adrenal	an area of excessive vacuolation and necrosis in the cortex
liver	several areas of focal necrosis, a few glomeruli show shrinkage and hyaline deposit in the capsule also moderate increase of the interstitial tissue
pancreas	normal
spleen	normal
testes	a small seminoma is present; also because of the presence of the tumor and the pressure, atrophy of the adjacent seminefrous tubules is present

rat 5223

lung	thickening of the alveolar walls and peri-bronchial cell infiltration
heart	degenerative changes also moderate increase of the interstitial tissue
liver	normal
kidney	mostly normal
spleen	normal
pancreas	normal
testes	moderate edema of the interstitial tissue

rat 5224

lung	peri-bronchial cell infiltration and thickening of the alveolar walls
heart	moderate increase of the interstitial tissue
adrenal	normal
liver	moderate congestion of the sinusoids also the presence of several areas of focal necrosis also diffuse cloudy swelling
kidney	mostly normal
spleen	normal
testes	normal
pancreas	normal

rat 5225

lung	peri-bronchial cell infiltration also thickening of the alveolar walls and pneumonitis
adrenal	a small area of hemorrhagic necrosis in the cortex
heart	moderate myocardial degenerative changes also slight increase in the interstitial tissue
liver	moderate cloudy swelling
kidney	normal
apleen	normal
testes	normal
pancreas	normal

rat 5226

lung	peri-bronchial cell infiltration and thickening of the alveolar walls
adrenal	normal
heart	moderate degenerative changes also proliferation of the interstitial tissue
liver	diffuse cloudy swelling and several areas of focal necrosis
kidney	mostly normal
spleen	several multinucleated giant cells of the whole organ
pancreas	normal
testes	normal

rat 5227

lung	thickening of the alveolar wallsand pneumonitis
adrenal	normal
heart	moderate degenerative changes in the myocardium and slight increase in the interstitial tissue
liver	moderate cloudy swelling diffused all over the organ
kidney	chronic interstitial nephritis
testes	normal
pancreas	normal
spleen	normal

rat 5228

heart has	a tumor affecting the base of it with or polyheydrant cells rich in cholingin and mytotic figures . The tumor is judged as fast growing and probably malignant. This tumor is diagnosed as of the base of the heart.
adrenal	vacuolation of the cortex
liver	slight increase of the interstitial tissue in the portal spaces
lung	a highly malignant tumor characterised by mytotic figures and invasion of all the lung tissue diagnosed as a carcinoma of the lung
kidney	normal
spleen	normal
pancreas	normal
testes	normal

rat 5229

lung	peri-bronchial cell infiltration also several areas of pneumonitis, thickening of the alveolar walls with what appears to be hemosiderin deposits in macrophages
adrenal	several areas of vacuolation in the cortex
heart	normal, however one area of the heart shows excessive proliferation of macrophages and connective tissues cell and mononuclear cells
liver	a few fat cells scattered all over the section and also one area of mononuclear cell infiltration
kidney	chronic inderstitial nephritis
spleen	normal
testes	normal
pancreas	normal

rat 5230

lung	thickening of the alveolar walls, areas of pneumonitis and peri-bronchial cell infiltration
adrenal	vacuolation of the cortex and cortex appears to be narrow
heart	several areas of degenerative changes of the myocardium and proliferation of the connective tissue
liver	slight granulation of the cytoplas
kidney	a few glomeruli show hyalinazation also the prexence of hyaline casts in a few tubules and a focus of scarring
spleen	a few fields show 1 or 2 giant cells and the presence of hemosiderine deposits - macrophages and mononuclear cell also appear to be prominent.
testes	normal
pancreas	normal

rat 5231

lung	peri-bronchial cell infiltration
heart	myocardial degenerative changes also the presence of connective tissue fibrosis and macrophages
adrenal	normal
liver	normal
kidney	normal
spleen	normal
testes	normal
pancreas	normal

rat 5232

lung	extensive thickening of the alveolar walls, several areas of pneumonitis and peri-bronchial cell infiltration
adrenal	moderate vacuolation of the cortex
heart	moderate degenerative changes in the myocardial fibers
kidney	a few glomeruli show hyalinazation
liver	normal
testes	atrophy of the semineferous tubules
pancreas	normal
spleen	normal

rat 5233

lung	pneumonitis also thickening of the alveolar walls and peri-bronchial cell infiltration
heart	normal
adrenal	one areas shows extensive degenerative changes and cyst formatic in the cortex
liver	diffuse cloudy swelling of the liver cells
kidney	chronic interstitial nephritis with many tubules having hyaline casts
spleen	slight increase in the lymphoid cells also the presence of a fe giant cells
pancreas	normal
testes	normal

rat 5234

lung	peri-bronchial cell infiltration and pneumonitis
adrenal	vacuolation of the cortex
heart	slight increase in the interstitial tissue
liver	diffuse cloudy swelling in the central area of the lobule
kidney	one area of scarring
pancreas	normal
testes	normal

rat 5235

lung	peri-bronchial cell infiltration
heart	myocardial degenerative changes and slight increase in interstitial tissue
adrenal	degenerative changes and vacuolation of the cortex
liver	proliferation of the bile ducts also infiltration of the portal areas with mononuclear cells and proliferation of the connective tissue cells. Mononuclear cell infiltration also is present in the central parts of the lobule with diffuse degenerative changes in the whole liver section
kidney	several areas of infiltration with mononuclear cells similar to the ones observed in the liver, appears to be lymphocytone
spleen	excessive proliferation of the mononuclear lymphoid and the presence of occasional giant cells - same as in liver and kidney
testes	normal
pancreas	normal

rat 5236

lung	extensive consolidation and organization of the globule also had a tumor diagnosed as cronco genic carcinoma
adrenal	hemorrhage necrosis and extensive vacuolation
heart	normal
adrenal	diffuse cloudy swelling particularly in the central areas of the lobule
kidney	normal
testes	normal
pancreas	normal
spleen	normal

rat 5237

lung	peri-bronchial cell infiltration
adrenal	normal
heart	normal
liver	diffuse cloudy swelling
kidney	normal
spleen	normal
pancreas	normal
testes	normal

rat 5238

lung	peri-bronchial cell infiltration and moderate thickening of the alveolar walls
adrenal	hemorrhagic necrosis of the cortex with cyst formation and vacuolation
heart	slight increase of the interstitial tissue
liver	diffuse cloudy swelling and several areas of focal necrosis
kidney	normal
testes	normal
pancreas	normal
spleen	very much enlarged with proliferation of the lymphoid tissue also the presence of endotheial cells and many giant cells

rat 5240

lung	several areas of pneumonitis
heart	slight increase in the interstitial tissue
adrenal	normal
liver	normal
spleen	normal
kidney	chronic interstitial nephritis
testes	normal
pancreas	normal

rat 5242

lung	peri-bronchial cell infiltration and pneumonitis
heart	degenerative changes in the myocardium also increased fibrosis
adrenal	normal
liver	normal
kidney	chronic interstitial nephritis
testes	normal
pancreas	normal
spleen	normal

rat 5243

lung	pneumonitis
heart	normal
adrenal	normal
liver	slight proliferation of the bile ducts
kidney	normal
testes	normal
pancreas	normal
spleen	normal

rat 5244

lung	a few areas of pneumonitis and thickening of the alveolar walls
adrenal	normal
heart	slight increase in the interstitial tissue also a few areas show degenerative changes in the myocardial fibers
liver	normal
kidney	normal
spleen	enlarged also the presence of multinucleated giant cells and slight increase in the lymphoid cells
pancreas	normal
testes	normal

rat 5245

lung	a few areas of pneumonitis
adrenal	several areas of hemorrhagic necrosis of the cortex also an areas of excessive vacuolation
heart	slight increase of the interstitial tissue
liver	a few areas show degenerative changes and necrosis of cells
kidney	normal
spleen	several giant cells are present also slight increase in the lymphoid tissue - appears to be enlarged
testes	normal
pancreas	normal

rat 5246

lung	peri-bronchial cell infiltration
adrenal	excessive vacuolation of the cortex
heart	moderate degenerative changes of the myocardium and replacement fibrosis
liver	normal - probably slight cloudy swelling in the central part of the lobule
kidney	normal
spleen	several areas show many multinucleated giant cells and proliferation of lymphoid cells - a few cells have what appears to be a foreign [illegible]ed object darkly stained
testes	atrophy and degeneration of the semineferous tubules
pancreas	normal

rat 5247

lung	several areas of pneumonitis and fibrotic nodules
adrenal	normal
heart	a few areas show degenerative changes of the myocardium and proliferation of the connective tissue
liver	diffuse degenerative changes and several areas of mononuclear cell infiltration
kidney	chronic interstitial nephritis
spleen	normal
testes	atrophy and degenerative changes of the semineferous tubules
pancreas	normal

rat 5248

lung	per-bronchial cell infiltration and moderate pneumonitis
heart	moderate degenerative changes of the myocardium and increased amount of fibro-blasts
adrenal	excessive vacuolation of the cortex
liver	diffusely degenerative changes particularly in the central part of the lobule also the presence of several areas of focal necrosis
kidney	moderate increase of the interstitial tissue in the presence of hyaline casts in the tubules
spleen	normal
testes	atrophy and degeneration of the semineferous tubules
pancreas	normal

rat 5249

lung	several areas of pneumonitis and peri-bronchial cell infiltration
adrenal	hemorrhagic necrosis of the cortex and several areas of vacuolation
heart	normal
liver	cloudy swelling in the central part of the lobule
kidney	one area shows excessive fibrosis and another has scarringon the surface
spleen	normal
testes	edema of the interstitial tissue and about 20% of the tubules show degeneration

rat 5250

lung	peri-bronchial cell infiltration and pneumonitis
adrenal	extensive hemorrhagic necrosis of the cortex
heart	normal
liver	diffuse cloudy swelling and increased in cells also stayes of blood in the sinusoids
kidney	normal
spleen	moderate increase of the lymphoid tissue also has many giant cells dispersed all over the section
pancreas	normal
testes	normal

rat 5251

lung	extensive pneumonia and peri-bronchial cell infiltration
heart	degenerative changes of the myocardium also replacement fibrosis
adrenal	vacuolation of the cortex
liver	normal
kidney	extensive hyalinazation of the glomeruli, chronic interstitial nephritis
spleen	normal
testes	degeneration and atrophy of the semineferous tubules
pancreas	normal

rat 5252

lung	peri-bronchial cell infiltration and slight thickening of the alveolar walls
heart	normal
adrenal	about 1/2 of the cortex is extensively vacuolated
liver	normal
kidney	moderate increase of the interstitial tissue
pancreas	normal
spleen	normal
testes	normal

rat 5253

lung	extensive peri-bronchial cell infiltration
heart	degenerative changes of the myocardium and proliferation of the interstitial tissue
adrenal	moderate vacuolation of the cortex
liver	mostly normal
kidney	normal
spleen	normal
pancreas	several fields in the section show fibrosis also has an adenoma of the eyelet cells of Langerhans
testes	normal

rat 5254

lung	peri-bronchial cell infiltration, chronic murine pneumonia
heart	normal
adrenal	moderate hemorrhage in the adrenal cortex
liver	moderate proliferation of the bile duct
kidney	normal
spleen	presence of moderate amount of brown hemosiderin stain also the presence of giant cells
pancreas	normal

rat 5255

lung	peri-bronchial cell infiltration and pneumonia
heart	normal
adrenal	large of hemorrhage in the adrenal cortex and also excessive vacuolation, several areas of necrosis also is present
liver	normal
adrenal	normal
spleen	normal, presence of an occasional giant cell
pancreas	normal

rat 5256

lung	normal
heart	normal
adrenal	several areas of hemorrhage and a large cyst formation
liver	normal
spleen	normal, presence of quite a few giant cells
pancreas	normal
kidney	hydronephrosis otherwise normal

rat 5257

lung	thickening of the alveolar walls and the presence of pneumonitis
heart	moderate degeneration changes in the myocardial fibers
adrenal	necrosis and hemorrhage of the cortex also the presence of small cyst formations
liver	normal
kidney	normal
spleen	presence of a few giant cells and slight granulomatosis formation
kidney	normal
pancreas	normal

rat 5258

lung	normal
heart	normal
adrenal	excessive necrosis and vacuolation of the adrenal cortex also the presence of cyst formation
liver	normal
kidney	normal
spleen	presence of giant cells
pancreas	normal

rat 5259

lung	thickening of the alveolar walls and pneumonitis
adrenal	hemorrhagic necrosis of the cortex and the presence of a few cyst
heart	normal
liver	moderate cloudy swelling in liver cells and an occasional area of focal necrosis
kidney	one small area of showing hyaline casts in the tubules and moderate degenerative changes
spleen	presence of giant cells
pancreas	normal

rat 5260

lung	extensive areas of infection and pneumonitis also the presence of bronchiolitis and chronic murine pneumonia
heart	normal
adrenal	hemorrhagic necrosis of the cortex and the presence of large cyst formation
liver	an occasional area of focal necrosis
kidney	normal
spleen	several areas of granulomatosis
pancreas	normal

rat 5261

lung	peri-bronchial cell infiltration
adrenal	hemorrhagic necrosis of the cortex with large areas of cyst formation
heart	normal
liver	moderate cloudy swelling and an occasional area of focal necrosis
kidney	moderate tubular degenerative changes also the presence of hyaline casts and
spleen	normal
pancreas	normal

rat 5262

lung	extensive peri-bronchial cell infiltration and thickening of the alveolar walls
heart	moderate degenerative changes of the myocardium
adrenal	hemorrhagic necrosis of the cortex and the presence of cysts
liver	a few areas of focal necrosis
kidney	normal
spleen	normal
pancreas	normal

rat 5263

lung	chronic murine pneumonia
heart	moderate degenerative changes with the myocardium
adrenal	very much enlarged with the presence of hemorrhagic necrosis in the cortex and cyst formation
liver	diffuse cloudy swelling of liver cells
kidney	normal
spleen	presence of giant cells

tumor present on the section is a thymoma

rat 5264

lung	chronic murine pneumonia
heart	nommal
adrenal	several areas of hemorrhagic necrosis with cyst formation in the cortex
liver	a few areas of focal necrosis also the presence of several foci of mononuclear cell infiltration
kidney	several glomeruli are shrunken some also contain a hyaline material otherwise normal
spleen	mostly normal, a little more pigmentation present and a few giant cell present
pancreas	normal

rat 5265

lung	advance chronic murine pneumonia
adrenal	moderate necrosis of the cortex
heart	normal
liver	normal
kidney	normal
spleen	proliferation of the white pulp and the presence of dark staining mononuclear cells
pancreas	normal

rat 5266

lung	peri-bronchial eell infiltration and chronic murine pneumonia
heart	normal
adrenal	extensive hemorrhagic necrosis of the cortex and cyst formation
liver	moderate cloudy swelling in the central part of the lobule and a few areas showing focal necrosis
kidney	hydronephrosis also the presence of degenerative changes in the tubular epithelium
spleen	moderate infiltration of mononuclear cells
pancreas	normal

rat 5267

lung	pneumonia and infection
heart	normal
adrenal	hemorrhagic necrosis of the cortex and cyst formation
liver	an occasional area of focal necrosis also very slight proliferatic of connective tissue in the portal spaces
spleen	normal
kidney	normal
pancreas	normal

rat 5268

lung	peri-bronchial cell infiltration and pneumonitis
heart	normal
adrenal	hemorrhagic cnecrosis of the cortex and cyst formation
liver	diffuse cloudy swelling in certain areas and vacuolation of the cytoplas
kidney	chronic degenerative changes observed characterized by hyalinization of a few glomeruli also of hyaline casts in the corresponding tubules
pancreas	normal
spleen	normal

rat 5269

lung	peri-bronchial cell infiltration and pneumonitis
adrenal	excessive hemorrhagic necrosis of the cortex also the presence of cysts
heart	moderate degenerative changes in a few myocardial fibers
liver	several areas showing focal areas of necrosis also moderate proliferation of the bile ducts and slight increase in the portal connective tissues
kidney	advanced chronic interatitial nephritis also with envolvement of the glomeruli hyalinization and the presence of in tubules
pancreas	normal
spleen	[illegible]

rat 5270

lung	chronic murine pneumonia
adrenal	moderate hemorrhagic necrosis in the cortex
heart	normal
liver	normal
spleen	normal
kidney	normal
pancreas	normal

rat 5271

lung	chronic murine pneumonia
adrenal	hemorrhagic necrosis of the cortex and cyst formation
heart	moderate degenerative changes of the myocardial fibers
liver	central lobular degenerative changes characterised by cloudy swelling and then occasional area of focal necrosis also slight increase in interstitial tissue in the portal spaces also moderate congestion of the sinusoids
spleen	moderate hyperplasia of the white pulp
kidney	extensive hydronephrosis and interstitial nephritis of the remaining tissues
pancreas	normal

rat 5272

lung	chronic murine pneumonia
heart	normal
adrenal	hemorrhagic necrosis of the cortex
liver	several areas of focal necrosis also diffuse cloudy swelling and slight increase of the interstitial tissue in the portal spaces
kidney	several areas showing senile degenerative changes with hyalinization of the glomeruli and hyaline casts in the tubules
pancreas	normal
spleen	normal

rat 5273

lung	chronic murine pneumonia
adrenal	hemorrhagic necrosis of the cortex and several cyst formations
heart	normal
liver	central lobular cloudy swelling also the presence of an occasional vacuole (fat)
kidney	normal
pancreas	normal
spleen	normal

rat 5274

lung	chronic murine pneumonia
adrenal	hemorrhagic necrosis of the cortex with a large cyst formation
heart	moderate degenerative changes of the myocardial fibers
liver	diffuse cloudy swelling also moderate increase of the interstitial tissue in the portal spaces
kidney	chronic senile changes with also increase in the interstitial tissue, hyalinization of the glomeruli and hyaline casts in the tubules
spleen	normal
pancreas	normal

rat 5275

extensive	chronic murine pneumonia in the lung also an abscess formation
heart	moderate degenerative changes in the myocardial fibers
adrenal	hemorrhagic necrosis of the cortex and cyst formation
liver	moderate proliferation of the bile ducts and an occasional area of focal necrosis
spleen	normal
kidney	senile degenerative changes of glomeruli and tubules
pancreas	normal

rat 5276

lung chronic murine pneumonia
heart moderate degenerative changes and diffuse cloudy swelling of the myocardial fibers
adrenal moderate hemorrhagic necrosis of the cortex
liver diffuse cloudy swelling
kidney moderate proliferation of the interstitial tissue and senile changes
spleen normal
pancreas normal

rat 5277

chronic murine pneumonia
heart normal
adrenal hemorrhagic necrosis of the cortex and cyst formation
liver normal
kidney chronic senile degenerative changes of the tubules and glomeruli
pancreas normal
spleen normal
tumor present is mammary adenocarcinoma

rat 5278

lung chronic murine pneumonia
liver central lobular cloudy swelling
heart diffuse degenerative changes of the myocardial fibers
adrenal hemorrhagic necrosis of the cortex and cyst formation
kidney chronic senile degenerative changes of the kidney
spleen normal
pancreas normal

rat 5279
1
lung chronic murine pneumonia
heart moderate degenerative changes of the myocardial fibers
adrenal hemorrhagic necrosis of the cortex and cyst formation
liver diffuse degenerative changes
kidney normal
spleen normal
pancreas normal

rat 5280

lung chronic murine pneumonia
adrenal hemorrhagic necrosis of the cortex and cyst formation
heart moderate degenerative changes of the myocardial fibers and proliferation of the connective tissue.
liver normal
kidney normal
spleen nommal
pancreas normal

lung	chronic muring pneumonia
adrenal	chronic hemorrhagic necrosis of the cortex and syst formation
heart	several areas of scarring
liver	several areas of diffuse degenerative changes of the liver c cells
spleen	normal
kidney	senile degenerative changes
pancreas	normal

rat 5282

lung	chronic murine pneumonia
adrenal	hemorrhagic necrosis of the cortex and cyst formation
heart	diffuse degenerative changes of the myocardium
liver	diffuse cloudy swelling with several areas of mononuclear cell infiltration and areas of focal necrosis
kidney	senile degenerative changes affecting both glomeruli and tubules
pancreas	normal

rat 5283

haert	moderate degenerative changes of the myocardial fibers and the presence of scarring tissue
adrenal	a small area of hemorrhagic necrosis of the cortex
liver	proliferation of the bile ducts and slight increase in the interstitial tissue in the portal spaces in addition to a few scattered areas of focal necrosis
kidney	senile changes mostly of the nature of interstitial nephritis
spleen	normal
pancreas	normal

rat 5284A

lung	chronic murine pneumonia
adrenal	hypetrophy of the gland also the presence of several areas of hemorrhagic necrosis and large cyst formation in the cortex
liver	a few areas of mononuclear cell infiltration
kidney	a few areas show hyalinization of the glomeruli and corresponding death of the nephron also slight increase in interstitial tissue
spleen	normal
pancreas	normal

rat 5284B

lung	chronic muring pneumonia
adrenal	hemorrhagic necrosis of the cortex and cyst formation
heart	moderate degeneration changes of th myocardial fibers and the presence of scar tissue.
liver	diffuse cloudy swelling in the presence of mononuclear cell infiltration
kidney	moderate hydronephrosis and the presence of a few foci of hyalinization of the glomeruli and corresponding death of the nephron and the presence of hyaline casts in the tubules
spleen	normal
pancreas	normal

lung	moderate infiltrations of the peri-bronchial areas
adrenal	moderate hemorrhagic necrosis of the cortex and small areas of cyst formation
heart	moderate degenerative changes of the myocardial fiber and the presence of scar tissue
liver	normal
kidney	moderate senile degenerative changes
spleen	slight increase of the lymphoid tissue
pancreas	normal

rat 5286

lung	moderate thickening of the alveolar-walls also slight mononuclear cell infiltration in the bronchial walls
adrenal	hemorrhagic necrosis of the cortex and the presence of cysts
heart	moderate degeneration changes of the myocardial fibers
liver	a few foci of focal necrosis and mononuclear cell infiltration
kidney	normal
spleen	normal
pancreas	normal

rat 5287

lung	very slight mononuclear cell infiltration in a small vacuole
heart	normal
adrenal	hemorrhagic necrosis of the cortex
liver	a few areas of focal necrosis, slight congestion of the sinusoids and mononuclear cell infiltration
spleen	normal
kidney	diffuse degenerative changes
pancreas	normal

rat 5288

lung	chronic murine pneumonia
adrenal	extensive hemorrhagic necrosis of the cortex and cyst formation
heart	extensive myocardial degenerative changes and proliferation of the connective tissue and saar formation
liver	diffuse cloudy swelling in the central part of the lobule and a few areas of regeneration
spleen	normal
kidney	extensive senile degenerative changes with hyaliniaation of the glomeruli and hyalinization of the tubules
pancreas	normal

rat 5289

lung	chronic murine pneumonia
adrenal	hemorrhagic necrosis of the cortex and cyst formation
liver	moderate cloudy swelling of the central part of the lobule also areas of focal necrosis
heart	normal
kidney	one area showing degenerative changes in the tubular epithelium also hyalinization of glomeruli and corresponding tubules
spleen	normal
pancreas	normal

rat 5290

lung	moderate peri-bronchial cell infiltration and thickening of the alveolar walls
adrenal	hemorrhagic necrosis of the cortex and cyst formation
heart	moderate myocardial degeneration changes and scarring
liver	congestion of the sinusoids and also congestion of the central vein with diffuse cloudy swelling
kidney	chronic interstitial nephritis
pancreas	normal
spleen	normal

rat 5291

lung	normal
adrenal	hemorrhagic necrosis of the cortex and cyst formation
heart	moderate degenerative changes of the myocardium
liver	moderate cloudy swelling in the central areas of the lobule and slight increase in interstitial tissue in the portal spaces
spleen	normal
kidney	chronic senile degenerative changes
pancreas	normal

rat 5292

lung	chronic murine pneumonia
heart	moderate degenerative changes of the myocardium and the presence of scarring tissue
adrenal	degenerative changes in the cortex with cyst formation
liver	normal
kidney s	senile degenerative changes with chronic interstitial nephritis
spleen	normal
pancreas	normal

rat 5293

lung	moderate chronic murine pneumonia
adrenal	hemorrhagic necrosis of the cortex
heart	moderate degenerative changes of the myocardium also the presence of scar tissue
liver	scattered areas of focal necrosis
kidney	chronic interstitial nephritis
spleen	normal
pancreas	normal

rat 5294

lung	chronic murine pneumonia
heart	normal
adrenal	hemorrhagic necrosis of the cortex and cyst formation
liver	moderate degenerative changes diffused all over the section also slight proliferation of the bile ducts
kidney	moderate degenerative changes of the tubular epithelium
spleen	normal
pancreas	normal

rat 5295

lung	peri-bronchial cell infiltration
adrenal	extensive hemorrhagic necrosis of the cortex and areas of cyst formation
heart	normal
liver	normal
kidney	chronic interstitial nephritis
spleen	normal
pancreas	normal

rat 5296

lung	peri-bronchial cell infiltration and moderate thickening of the alveolar walls
adrenal	normal
heart	moderate degenerative changes in the myocardial fibers and the presence of areas of acarring
liver	several areas of focal necrosis and proliferation of the bile duct epithelium
kidney	moderate increase in the interstitial tissue
spleen	normal
pancreas	normal

rat 5297

lung	slight thickening of the alveolar walls
heart	normal
adrenal	hemorrhagic necrosis of the cortex and cyst formation
liver	one area of necrotic and degenerative changes also several foci of mononuclear cell infiltration
kidney	normal
spleen	normal
pancreas	normal

rat 5298

heart	degenerative changes of the myocardium also the presence of scar tissue
adrenal	extensive hemorrhagic necrosis of the cortex and the presence of two large cysts.
liver	presence of several areas of focal necrosis and mononuclear cell infiltration
kidney	there is about 1/3 of the cortex has a ~~light~~ pulma in it rest of the kidney do not show any other compression effects
spleen	normal
pancreas	normal

rat 5299

lung	chronic murine pneumonia with infection
adrenal	extensive hemorrhagic necrosis of the cortex and the presence of large cysts
heart	moderate degenerative changes of the myocardium
liver	several areas of focal necrosis also the presence of cloudy swelling
spleen	normal
kidney	senile degenerative changes accompanied by interstitial nephritis
pancreas	normal

rat 5300

lung	chronic murine pneumonia
adrena	hemorrhagic necrosis of the cortex and cyst formation
heart	moderate degenerative changes of the myocardium
liver	areas of focal necrosis and also the presence of mononuclear cell infiltration also proliferation of the bile ducts in some areas and diffuse cloudy swelling in other areas.
kidney	senile degenerative changess
spleen	normal
pancreas	normal

rat 5301

lung	thickening of the alveolar walls
heart	moderate degenerative changes and increase of connective tissue
adrenal	hemorrhagic necrosis of the cortex with the presence of cysts
liver	diffuse degenerative changes in the liver in focal areas
kidney	senile degenerative changes characterized by increase of the interstitial tissue, hyalinization of the glomeruli and the presence of hyaline casts in the tubules
spleen	normal

tumor present on the slide is an adenoma probably of the mammary gland

rat 5302

lung	thickening of the alveolar walls also peri-bronchial cell infiltration
adrenal	hemorrhagic necrosis of the cortex and the presence of cyst formation
heart	moderate degenerative changes of the myocardium also the presence of scar tissue
liver	normal
adrenal	chronic degenerative changes also the presence of hyaline casts an d increase in the interstitial tissue
spleen	normal
pancreas	normal

rat 5303

lung	chronic murine pneumonia with a large abseess formation
adrenal	hemorrhagic necrosis of the cortex
liver	areas of focal necrosis and slight increase in the interstitial tissue in the portal spaces
kidney	chronic interstitial nephritis

tumor present on the slide is a hymoma

rat 5304

lung	chronic murine pneumonia
adrenal	extensive hemorrhagic necrosis of the cortex and cyst formation
heart	normal
liver	normal
kidney	normal
pancreas	normal
spleen	normal

rat 5305

lung	peri-bronchial cell infiltration and also the presence of many heart failure cells or macrophages in the alveoli
heart	moderate degenerative changes of the myocardium
adrenal	hemorrhagic necrosis of the cortex
liver	diffuse cloudy in the central part of the lobule
kidney	chronic interstitial nephritis and the presence of hyaline casts also hyalinization and shrinkage of the glomeruli in many areas
pancreas	normal
spleen	normal

rat 5306

lung	peri-bronchial cell infiltration
adrenal	hemorrhagic necrosis of the cortex
heart	moderate degenerative changes of the myocardium and the presence of scar tissue
liver	diffuse degenerative changes also the presence of areas of focal necrosis and replacement fibrosis also regeneration in other areas
kidney	mostly normal
spleen	mostly normal - slight increase in pigmentation
pancreas	normal

rat 5307

lung	peri-bronchial cell infiltration and pneumonitis
heart	moderate degenerative changes of the myocardial
adrenal	hemorrhagic necrosis of the cortex and cyst formation
liver	slight increase of the interstitial tissue in the portal spaces and the presence of focal areas of necrosis and mononuclear cell infiltration
kidney	chronic interstitial nephritis and also the presence of hyaline casts
spleen	normal
pancreas	normal

rat 5308

lung	peri-bronchial cell infiltration and the presence of macrophages in the alveoli
heart	moderate degenerative changes of the myocardial
adrenal	hemorrhagic necrosis of the cortex and the presence of large cyst formation
liver	moderate cloudy swelling in the central part of the lobule
kidney	advanced hydronephrosis
pancreas	normal
spleen	normal

rat 5309

lung	chronic murine pneumonia
adrena	ext. hemorrhagic necrosis in the cortex and presence of cysts
heart	degenerative changes of myocardial
liver	areas of focal necrosis and central lobular degenerative changes
kidney	senile degenerative changes as characterized by increase of the interstitial tissue and also hyalinization of glomeruli and tubules
spleen	normal
pancreas	normal

lung	peri-bronchial cell infiltration
liver	normal
kidney	moderate degenerative changes of the tubular epithelium
spleen	normal
pancreas	normal

rat 5311

lung	peri-bronchial cell infiltration
adrenal	hemorrhagic necrosis of the cortex
heart	moderate degenerative changes of the myocardial
liver	normal
kidney	showed evidence of interstitial nephritis
spleen	normal
pancreas	normal

rat 5312

moderate peri-bronchial cell infiltration and thickening of the alveoli walls in the lung

adrenal	hemorrhagic necrosis of the cortex
heart	moderate degenerative changes of the myocardial
liver	slight increase of the interstitial tissue in the portal spaces also diffuse cloudy swelling
kidney	normal
spleen	normal
pancreas	normal

rat 5313

lung	peri-bronchial cell infiltration and thickening of the alveoli walls also the presence of macrophages in the alveoli
adrenal	hemorrhagic necrosis of the cortex
heart	moderate degenerative changes of the myocardial
liver	diffuse cloudy swelling of liver cells
kidney	senile degenerative changes
spleen	normal
pancreas	normal

rat 5314

lung	peri-bronchial cell infiltration
adrenal	hemorrhagic necrosis of the cortex and cyst formation
heart	~~heart~~ degenerative changes of the myocardium and the presence of scar tissue
liver	diffuse cloudy swelling and proliferation of the epithelium binding of the bile ducts
kidney	senile degenerative changes
spleen	normal
pancreas	normal

lung	peri-bronchial cell infiltration
adrenal	hemorrhagic necrosis of the cortex and cyst formation
heart	diffuse degenerative changes of the myocardium and the presence of scar tissue
liver	diffuse degenerative changes
kidney	chronic interstitial nephritis and hyalinization of the glomeruli
pancreas	normal
spleen	normal

rat 5317

lung	chronic pneumonia
adrenal	hemorrhagic necrosis of the cortex and cyst formation
heart	normal
liver	normal
kidney	normal
spleen	normal
pancreas	normal

rat 5318

lung	thickening of the alveolar walls and the presence of peri-bronchial cell infiltration
heart	normal
adrenal	extensive hemorrhagic necrosis in the cortex
liver	normal
kidney	hydronephrosis
spleen	normal
pancreas	normal

rat 5319

lung	chronic murine pneumonia
adrenal	hemorrhagic necrosis with the cortex
heart	advanced degenerative changes of the myocardial
liver	diffuse cloudy swelling of liver cells
kidney	a few areas show evidence of chronic interstitial nephritis
spleen	normal
pancreas	normal

rat 5320

lung	chronic murine pneumonia
heart	moderate degenerative changes of th myocardium
adrenal	hmoerrhagic necrosis of the cortex
liver	diffuse cloudy swelling of liver cells
kidney	chronic senile degenerative changes of the interstitial tissue and the glomeruli
pancreas	normal
spleen	normal

rat 5321

lung	chronic murine pneumonia
adrenal	hemorrhagic necrosis of the cortex and cyst formation
heart	normal
liver	normal
kidney	chronic interstitial nephritis
spleen	normal
pancreas	normal

lung	normal
heart	moderate degenerativ changes of the myocardial
adrenal	hemorrhagic necrosis of the cortex and cyst formation
liver	normal
kidney	hydronephrosis
spleen	normal
pancreas	normal

rat 5323

lung	peri-bronchial cell infiltration
heart	normal
adrenal	hemorrhagic necrosis of the cortex
liver	normal
kidney	hydronephrosis with chronic interstitial nephritis
pancreas	normal
spleen	normal

rat 5324

lung	chronic murine pneumonia
heart	moderate degenerative changes of the myocardial
adrenal	hemorrhagic necrosis of the cortex and cyst formation
liver	mostly normal
kidney	hydronephrosis
spleen	normal
pancreas	normal

rat 5325

lung	peri-bronchial cell infiltration
heart	normal
pancraas	normal
adrenal	hemorrhagic necrosis of the cortex and cyst formation
liver	normal
kidney	hydronephrosis
spleen	normal

rat 5326

lung	peri-bronchial cell infiltration and thickening of the alveolar walls
heart	degenerative changes in the myocardium
liver	normal
kidney	chronic interstitial nephritis
pancreas	normal
adrenal	hemorrhagic necrosis of the cortex and large cyst formation
pancreas	normal

lung	chronic murine pneumonia
adrenal	hemorrhagic necrosis of the cortex and cyst formation
heart	normal
liver	normal
kidney	normal
spleen	normal
tumor present is a fibro-adenoma	
pancreas	normal

rat 5328

lung	normal - small area of peri-bronchial cell infiltration present
heart	normal
adrenal	hemorrhagic necrosis of the cortex
liver	normal
spleen	normal
kidney	mostly normal
pancreas	normal

rat 5329

lung	thickening of the alveolar walls
adrenal	hemorrhagic necrosis of the cortex
heart	moderate degenerative changes in the myocardium and the presenc of scar tissue
liver	normal
kidney	normal
spleen	normal
pancreas	normal

rat 5330

lung	thickening of the alveolar walls
adrenal	hemorrhagic necrosis of the cortex and cyst formation
heart	moderate degenerative changes of the myocardium
liver	normal
kidney	normal
spleen	normal
pancreas	normal

rat 5331

lung	mostly normal
heart	moderate degenerative changes of the myocardium
adrenal	hemorrhagic necrosis of the cortex and cyst formation
liver	normal
spleen	normal
pancreas	normal

rat 5334

lung	normal
adrenal	hemorrhagic necrosis of the cortex and cyst formation
heart	moderate degenerative changes through the myocardium and the presence of scar tissue
liver	normal
kidney	normal
spleen	normal
pancreas	normal

lung	chronic murine pneumonia
adrenal	normal
heart	moderate degenerative changes of the myocardium
liver	normal
kidney	hydronephrosis
spleen	normal
pancreas	normal

rat 5336

lung	thickening of the alveolar walls
adrenal	a small area ofdegenerative change in the cortex
liver	large area of extensive degenerative changes also moderate proliferation of the bile ducts
kidney	mostly normal
pancreas	normal
spleen	normal

rat 5337

lung	peri-bronchial cell infiltration
adrenal	extensive hemorrhagic necrosis of the cortex
heart	moderate degenerative changes of the myocardial
liver	normal
kidney	senile degenerative changes mostly in the nature of hyalinization of the glomeruli and slight increase in the inserstitial tissue
spleen	normal
pancreas	normal

rat 5339

lung	thickening of the alveolar walls
adrenal	hemorrhagic necrosis of the cortex
heart	normal
liver	normal
kidney	senile degenerativ changes
pancreas	normal
spleen	normal

rat 5340

lung	peri-bronchial cell infiltration
adrenal	normal
heart	moderate dggenerative changes of the myocardial
liver	normal
kidney	chronic interstitial nephritis
spleen	normal
pancreas	normal

rat 5341

lung	chronic murine pneumonia and abscess formation
heart	myocardial degenerative changes
adrenal	hemorrhagic necrosis of the cortex
liver	diffuse cloudy swelling
kidney	chronic interstitial nephritis also hyalinization of the glomeruli
spleen	normal
pancreas	normal

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CONCERNING THE PHYSICAL & CHEMICAL CHARACTERIZATION OF CELLULOSE ETHERS USED AS FOOD ADDITIVES.

4th Communication

A process for proof and determination of cellulose ethers in foods.

CROESSMANN, KUPFER, SOUCI & MERGENTHALER

Communication from the applied section and analytical laboratory of Kalle, Inc., Wiesbaden/Biebrich and from the German Research Institute for Foods, Munich

The purpose of this report is to provide the means for unambiguous determination of the kind and amounts of methyl-cellulose and of sodium-carboxy-methylcellulose in foods. Such a method assumes significance in view of the statuory regulations concerning additives.

Cellulose ethers -- particularly methylcelluloses and sodium carboxy-methylcelluloses -- are valuable emulsifiers, stabilizers and gelling means in the food industry. The advantages from this kind of compounds as regards naturally occurring gelling means and emulsifiers such as tragacanth, guar flour, alginate among others, consist in higher evenness, purity, in higher resistance to bacterial effects and higher constancy with respect to viscous properties from external influences and in the vast technical efficacy of these products. Cellulose ethers have been listed as admissible food additives on the EEC roster. Considering the trend toward unifying food statutes, West Germany too may expect permission for such in the near future.

Earlier communications reported on the manufacture, properties and applications of cellulose ethers in the food industry and proposals for identity and purity requirements were submitted.[1] Furthermore, suitable analytical methods for the testing for cellulose ethers have been worked out with respect to the specifications proposed in Communication I,[2] and the results from such tests on commercial products were submitted[3]. A suitable process is still required, which will allow qualitative detection and quantitative measurement of methyl celluloses and of sodium carboxy-methyl-celluloses when they are also together with permissible gelling means and emulsifiers of different natures.

EXPERIMENTAL PART

Seven whipped cream/horse-radish sauces were used as test materials, since their multifold contents and hence the likely interferences as test substances seemed particularly appropriate for checking the practical suitability of the analysis process.

The whipped cream/horse-radish sauces were of the composition shown in table 1:

Table 1: Composition of the whipped cream/horse-radish sauces

INGREDIENTS	%	INGREDIENTS	%
BINDER	1.60	salt	2.50
ground horse-radish	4.00	white wine	2.00
wine vinegar	14.00	starch syrup	3.50
milk	4.00	spices (R)	0.17
sweet whipped cream	6.00	Nipagin	0.10
Egg yolk & egg white	5.00	Potassium sorbate	0.25
Food oil	12.00	water	39.88
sugar	5.00		

Besides methyl cellulose and sodium carboxy-methyl-cellulose, tragacanth, pectin, alginate and guar flour in various mixtures and quantities were used as a binder. The commercially available methyl celluloses and sodium carboxy-methyl-celluloses held a water content of about 7%. Table 2 lists the proportion of binders in the various samples.

Table 2. Binder content in substances tested

Substance number	% Binders added to					
	methyl cellul.	Na-carb. methyl-cellul.	alginate	pectin	guar	tragacanth
I	0.8	-	0.2	0.6	-	-
II	-	-	0.4	0.4	0.8	-
III	-	0.6	0.7	-	0.4	-
IV	0.6	0.6	-	-	0.4	-
V	-	0.9	-	0.4	-	0.3
VI	0.8	0.8	-	-	-	-
VII(dummy)	-	-	-	-	-	-

Analytical research was divided into 3 sections.

I. DETERMINATION OF THE DRY SUBSTANCE FREE FROM SALTS AND FATS

PROCEDURE

10-20 gm of the samples were precipitated with 15-20 fold amounts of methanol saturated with ammonium carbonate (about 7 gm of $(NH_4)_2CO_3$/100 ml of methanol) while stirring vigorously. After letting rest overnight, the mixture was centrifuged and the precipitate was washed and centrifuged with 80% methand by volume until the residual solution no longer showed any reaction to Cl ions. Then the precipitate and 100 ml of pure methanol were

transferred to a glass filter crucible (1G2) and washed with a total of 100 ml of ether in small quantities. Following intensive evacuation, drying was performed at 105° until weight constancy had been obtained and following cooling, weighing was again performed in the dessicator. As an alternative to centrifuging, the precipitated binders were filtered immediately through a glass filter crucible 1G2 and processed as described above.

RESULT

The results are shown in table 3.

Table 3. Dry-substance content of the test substances.

Substance number	Dry substance (A) free from salts and fats	
	centrifuged,%	filtered,%
I	3.2	3.5
II	3.2	3.6
III	3.3	3.2
IV	3.3	3.2
V	3.4	3.5
VI	3.4	3.3
VII	1.6	1.5

Sometimes direct filtration proved very laborious and time-consuming, whereas centrifuging always could be carried out swiftly and simply. Taking into account the dummy values from sample VII, the total precipitation theoretically must amount to about 3.2%. The values obtained from centrifuging in most cases are more favorable. Therefore centrifuging may be recommended as a practical measure. The values obtained by this procedure (A) were made use of for later computations.

II. QUALITATIVE TESTING FOR THE PRESENCE OF METHYL CELLULOSE (MC) AND SODIUM CARBOXY METHYL CELLULOSE (NaCMC)

a) Color and precipitation reactions.

PROCEDURE

The following eight reactions were carried out, which are characteristic for mc or for Nacmc. An approximately 1% aqueous solution, filtered where necessary from the precipitates obtained as in section I, was used as testing solution .

1. Foaming power: 0.5 ml of the testing solution were diluted with 9.5 ml of solution and shaken vigorously. The solution will foam in the presence of mc, but not in that of Nacmc.

2. Heat coagulation: The testing solution is slowly heated in a test tube. If mc is present, there will be reversible clouding at 65-70°, but the solution will remain clear in the presence of Nacmc.

3. Precipitation with iodine/potassium iodide: 1 ml of a 10% potassium iodide solution is added to 1 ml of testing solution, where the former contains 1 gm of iodine per 100 ml. Mc may be recognized by a brownish flocculation, whereas the solution will remain clear for Nacmc.

4. Precipitation with tannin : 5 ml of the testing solution are reacted with 5 drops of a 10% acetic acid and then with 5 ml of a 10% aqueous tannin solution. If mc is present, there will be a yellow precipitation, whereas the solution will remain clear in the presence of Nacmc.

5. Precipitation with Zephirol^{R}: 3-4 drops of 6% soda lye are added to 0.5 ml of testing solution, and then 4.5 ml of water. Then 5 ml of Zephirol solution will be added dropwise. Nacmc may be recognized by a white flocculation whereas no clouding or flocculation will be observed for mc.

6. Color reaction with chromotropic acid: 20 ml of chromotropic acid solution (100 mg of chromotropic acid in 1,000 ml of concentrated sulfuric acid) are added to 1 ml of testing solution. This is followed by heating for 30 minutes in boiling water bath. If Nacmc is present, there will be a reddish-violet coloring, while mc will merely provide a slight brown coloring.

7. Precipitation with copper sulfate: 2.5 ml of testing solution are diluted with the same amount of water. Then 1 ml of a 5% copper sulfate solution is added dropwise. There will be clouding or flocculation in the presence of Nacmc, but the solution will remain clear in the presence of mc.

8. Precipitation with lead acetate. 2.5 ml of the test solution are dilutedwith the same amount of water. 1 ml of a 5% lead acetate solution ($Pb(CH_3COO)_2.3H_2O$) are added dropwise. There will be clouding or flocculation in the presence of Nacmc, but the solution will remain clear in the presence of mc.

RESULT

The results of these tests are listed in table 4.

Table 4. Color and precipitation reactions.

REACTION	SUBSTANCE # I	II	III	IV	V	VI	VII
Foaming	+	-	-	(+)	-	+	-
heat coagulation	+	-	-	+	-	+	-
Iodine/potassium iodide precipitation	+	-	-	+	-	+	-
tannin precipitation	+	-	-	+	-	+	-
Zephirol precipitation	-	-	+	(±)	+	+	-
color reaction with chromotropic acid	-	-	+	+	+	+	-
copper sulfate precip.	-	-	+	+	+	+	-
lead acetate precipitation	-	-	+	+	+	+	-

The foaming capacity of substance #IV may only be faintly observed, and the Zephir precipitation with this substance also lacks distinctiveness. Obviously the simultaneous presence of methyl cellulose and of sodium carboxy methyl cellulose unfavorably affects the clarity of the particular identification reactions. The distinguishability of both reactions is not too good for substance VI either. From the overview of the reactions, however, the presence of mc or of Nacmc may be determined every time without difficulty.

b) THIN LAYER CHROMATOGRAPHIC SEPARATION OF THE HYDROLYSIS PRODUCTS.

PROCEDURE

Approximately 1 gm of the dry substance that is free from salts and fats and obtained according to section 1 was boiled in a 100 ml Erlenmeyer flask with 15 ml of 5% by volume sulfuric acid and with 5 ml of water for 8 hours at the return condenser. The cooled hydrolysate was neutralized to a pH of 7 with a barium hydroxide suspension

in water at the glass electrode. Following boiling, the separated barium sulfate was filtered off and the clear filtrate was extensively cconcentrated. Following another filtration, there was evaporation in the rotation evaporator for 5-7 minutes and the solution so obtained was used for the thin-layer chromatogram.

CHROMATOGRAPHY CONDITIONS:

Plates: glass plates, 20 x 20 cm, coated with MN cellulose powder 300 G (with plaster addition); plate coating: 15 gm of MN 300 cellulose powder (Macherey, Nagel & Co, Dueren) homogenized with 90 ml of water in an electrical mixer and then coating in usual manner; dynamic phase: butanol/pyridin/water (3+2+1.5); developing: double transit with drying each time at 105°, chamber saturation 20-22°C; time of runs: about 2½ hours; spraying means: aniline phthalate (0.93 gm of aniline and 1.66 gm of o-phthalic acid dissolved in 100 ml of water saturated butanol); post-treatment: heat to 105°C for 10 minutes after spraying.

Galactose, glucose, mannose, arabinose and xylose were deposited as a 1% solution in pyridin as comparison substances. Following development, the hexoses provide brown spots and the pentoses violet ones.

The following derivatives are obtained from the gels used in hydrolysis, and the corresponding R_t values for the conditions being discussed are always shown in brackets:

methyl cellulose	glucose (0.74); 2-3 methyl sugar (0.94-1,00)
sodium carboxy methyl cellulose	glucose (0174); uronic acids (0,15-0.37)
alginate	uronic acids (0.09-0.27)
pectin	uronic acids (0.09-0.18); galactose (0.67); arabinose (0.83); rhamnose
guar flour	galactose (0.67); mannose (0.77)
tragacanth	uronic acids (0.10- 0.20); galactose (o.67); arabinose (0.83); xylose (0.91); fucose.

RESULT

Figures 1 and 2 show the results from the thin-layer chromatographic separation of the hydrolysis products. As shown by fig. 1, substances I and IV provide distinct spots for R_t values between 0.94 and 1.0; these substances evidenced methyl cellulose qualitatively. This proves the presence of methyl sugars. Further, a glucose spot may be noted at R_t= 0.74.The spots from substance VII do not interfere with recognizing the methyl sugars because only a weak spot occurs at that location, probably due to rhamnose. Xylose (R_t=0.91), which frequently occurs in gels and food ingredients, does not interfere either with the proof for methyl sugars. Methyl cellulose is recognized even more distinctly when the hydrolysate is obtained not from the entire precipitation according to section 1, but rather when only an aqueous extract (W) from the entire precipitation is used for hydrolysis. As shown by fig. 2, substance VII then allows recognizing only a glucose spot, because the otherwise interfering derivatives of the structural substance of the horse-radish have been removed. The methyl sugars then may be

distinguished even more reliably. In certain cases such pre-treatment may therefore be appropriate, though in general it is not required. Sodium carboxy methyl cellulose is rendered by a glucose spot at R_t = 0.74 and by several uronic acid spots at R_t values between 0.15 and 0.37, fig. 1. Other gels and food ingredients also frequently causing uronic acid spots, the uronic acid spot with the highest value of R_t= 0.37 is particularly suitable for the identication of sodium carboxy methyl cellulose. The other uronic acids always show lower values of R_t.

Evaluation of the chromatogram showed that samples I and IV contained methyl cellulose and samples III, IV and V contained sodium carboxy methyl cellulose. These findings agree with the results from the color and precipitation reactions.

Fig. 1: DC chromatogram of the hydrolysates of the dry substances (I- V, VII)
METHYLZUCKER: methyl sugar

Fig. 2: DC chromatogram of the hydrolysates of the dry substances (I,IV,VII) or of their aqueous extracts (IW, IV W, VII W).
URONSAEUREN: uronic acids

III. QUANTITATIVE DETERMINATIONS OF THE IDENTIFIED CELLULOSE ETHERS

After the presence of methyl cellulose had been proved in substances I IV and VI and that of sodium carboxy methyl cellulose in substance III,IV and V,VI, according to qualitative analysis, the cellulose ethers were determined quantitatively in those substances.

METHYL CELLULOSE

Procedure

In order to determine methyl cellulose, the OCH_3 content of 200 mg of the precipitations obtained in accordance with section 1 and according to ZEISEL in the VIEBOECK-SCHWAPPACH modification was determined. A more precise analytical procedure will be found in CROESSMANN et al[2].

An average etherification degree of 1.5 (=25% OCH_3) was assumed for the computation of methyl cellulose. The content of water-free methyl cellulose in the substances may be obtained from equation (1):

$$\% \text{ mc} = \%OCH_3.A.0.04 \qquad (1)$$

The equation is based on the following considerations:

One determines the percentage of salt-and-fat-free dry substance of the sauces and the percentage of OCH_3 thereof.

100 gm of sauce contain A gm of salt-and-fat-free dry substance; 100 gm of salt-and-fat-free dry substance contain B gm of OCH_3, or 100 gm of sauce contain BA/100 gm of OCH_3.

An average content of 25% of OCH_3 is assumed for methyl cellulose, hence:

100 gm of sauce contain BA 4/100 = BA.0.04 of methyl cellulose.

RESULT

The results for the quantitative determination of methyl cellulose are rendered in table 5.

Table 5: Methyl cellulose content

Substance	OCH_3	A	water-free methyl cellulose present	found	yield (%)
I	5.2	3.2	0.74	0.67	91
IV	3.9	3.3	0.56	0.51	91
VI	5.4	3.4	0.73	0.73	100

b) SODIUM CARBOXY METHYL CELLULOSE

Procedure

Amounts between 50 and 80 mg of the precipitates obtained according to section 1 were hydrolyzed with 50% sulfuric acid in order to determine the sodium carboxy methyl cellulose. One aliquot part made use of the method of EYLER, KLUG & DIEPHUIS (4) for determining the glycolic acid colorimetrically with 2,7 dihydroxynaphtaline. The following procedure was used in particular.

50-80 mg of the particular precipitations were precisely measured and inserted into a 300 ml iodine number flask by means of a weighing glass. Then 25 ml of 6% soda lye are pipetted to the substance in the iodine number flask and shaking proceeds until the sample has been entirely dissolved. 25 ml of H_2O are added and then carefully 36 ml of concentrated H_2SO_4 and the sdution will be boiled for 3½ hours at the return condenser. Then it will be cooled to room temperature and rinsed with 50% sulfuric acid in a 100 ml flask which is filled to the mark.

A solution of the following composition is used for the DETERMINATION OF THE DUMMY VALUE: 25 ml of 6% soda lye, 25 ml of water, 36 ml of concentrated sulfuric acid. Treatment is the same as for the samples.

1 ml of the solution to be tested then is pipetted from the 100 ml flask into a test tube and 20 ml of 2,7-dihydroxynaphthaline solution (0.100 gm of 2,7-dihydroxynaphthaline are dissolved in concnetrated sulfuric acid; this solution will be let to stand several hours prior to use until it is entirely colorless.) This test tube is brought into a glass of boiling water. After 30 minutes, it will be cooled in ice water and the content will be rinsed with water in a 50 ml little flask and again it will be cooled. Then the little flask will be filled up to the mark with water. Trays of 30 mm layer thickness are filled and Lange-Colorimeter measurements are performed with respect to the dummy value.

Pure, crystallized Glycolic Acid dried for 24 hours under vaccum at room temperature is used for drawing up THE CALIBRATION CURVE. Exactly 193.9 mg (=250.0 mg of sodium glycolate) are weighed and dissolved in water and the vessel is filled to 250 ml. This solution may be controlled by alkametrically titrating 100 ml. On the other hand, one may also weigh the amount of a more or less pure glycolate of known content corresponding to 250.0 mg of pure sodium glycolate. 10 ml of this substance again is diluted to 100 ml and each time one withdraws 1,00; 0.75; 0.50; 0.25 ml for the

calibration curve (corresponding to 0.100; 0.075; 0.050; 0.025 mg of sodium glycolate). Adding water, one fills up each time to 1 ml and further d etermination is carried out as described above following mixing with 2,7-dihydroxynapthaline solution. Here too one must take into account the dummy value with 1 ml of water. The glycolic acid from the calibration curve will be converted on the basis of an average etherification degree of 0.7 to Nacmc.

Eq. 2 then will yield the substances' contents of waterfree Nacmc:

$$\%\text{Nacmc} = \%(\text{glycolic acid})A.0.04 \qquad (2)$$

which is obtained as follows:

The percentage of salt-and-fat-free dry substances in the sauces is determined and also their percentage of glycolic acid.

100 gm of sauce contain A gm of salt-and-fat-free dry substance

100 gm of salt-and-fat-free dry substance contain C gm of glycolic acid. Hence:

A gm of salt-and-fat-free dry substance correspond to CA/100 glycolic acid or 100 gm of sauce correspond to CA/100 gm of glycolic acid.

An average etherification degree of 0.7 is assumed for sodium carboxy methyl cellulose. The molecular weight of a st_ructural element of such a Nacmc is 218 (molecular weight of glucose unit = 162, less 0.7 hydrogen = 161.3, add 0.7 CH_2COONa = 218). Thus,

0.7 glycolic acid = 0.7x76= 53.2 gm of glycolic acid correspond to the amount of 218 gm of Nacmc. Thus the amount of Nacmc is 218/53.2 = 4.1 as large as the amount of glycolic acid found. There results: 100 gm of sauce correspond to 4.1CA/100 = 0.041CA gm of sodium carboxy methyl cellulose.

Table 6: sodium carboxy methyl cellulose content.

Substance	glycolic acid,%	A,%	water-free sodium carboxy methyl cellulose,%		
			present	found	yield
III	4.7	3.3	0.56	0.63	112
IV	4.1	3.3	0.56	0.55	98
V	6.4	3.4	0.84	0.98	106
VI	5.1	3.4	0.73	0.71	97

RESULT

The proportions of sodium carboxy methyl cellulose are summarized in table 6.

As shown from the results listed in tables 5 and 6, the largest deviation from the actual value of mc amounts to -0.07% and to +0.07% for Nacmc (corresponding resp. to -9% and + 12% absolute).

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ON THE PHARMACOLOGICAL EFFECTS OF METHYLCELLULOSE *

F. Cugurra

Institute of Pharmacology -- University of Genoa

The use of methylcellulose (Tylos) as an emulsifying substance, used externally or administered orally, is well known. Here we propose to establish whether or not it is possible to use such a compound as a stabilizing factor in a colloid solution by introducing it parenterally.

This possibility is linked essentially to three facts: the absorption of the substance, its elimination, and the lack of toxification during its presence in the organism. The experiments were therefore programmed as follows: to see, by completing direct observation with the evidence of an autopsy, if the substance introduced subcutaneously in animals (guinea pigs, rats) was absorbed or not. The minimal lethal dosage on dogs and rabbits was ascertained through the introduction of an intraveneously administered solution of methylcellulose. Research on the eventual toxification of the substance was conducted on the basis of symptomatology preceding the death of the animals and the anatomological-pathological and histological-pathological reports concerning the same animals. Finally, to ascertain the elimination of the substance, we examined the urine of the animals we treated, either as they were or as they eventually decomposed (for this purpose we used specific reagents; e.g., the reagent of Radkofer and the liquid of Fehling).

The experiments and their results follow.

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* Methylcellulose Kalle (Tylos S.L. 5).

Absorption

Having stated the problem as to whether the methylcellulose solutions, introduced subcutaneously, would be absorbed, we found that the chemical and physico-chemical characteristics of the substance indicated that absorption was not going to take place. In fact we found that with an increased molecular size the liquid solution has a very high grade of viscosity.

The results of the experiments confirm these observations. Five rats weighing approximately 150 gr. were used, as were five guinea pigs weighing approximately 500 gr. Each animal was injected subcutaneously with a few cubic centimeters of a solution of methylcellulose at 2%. The result: at the point of injection an inflammation appeared; it remained unchanged for some days, and then tended to decrease very slowly. After the animals had been killed, the autopsies showed that the subcutaneously introduced substance remained unchanged, without any concomitant inflammatory effects.

By way of conclusion: methylcellulose, subcutaneously introduced in solution into animals, is not absorbed and shows little or no tendency to spread.

Toxification

A solution of 1% of methylcellulose in a physiological solution was introduced, at a velocity of 1 cc. per minute, into the jugular vein of five rabbits, weighing approximately 1.600 kilograms; the animals died after receiving 14 cc. per kilogram; the minimal immediately lethal dose of methylcellulose was 0.14 gr. per kilogram.

The experiment was repeated with five dogs of varying weight. The solution, at 1%, was introduced at a rate of 2 cc. per minute. The animals were dead after the

introduction of 20 cc. per kilogram. The minimal immediately lethal dose of the substance in the majority of cases was 0.20 gr. per kilogram.

Before dying, both dogs and rabbits exhibited the following symptomatology: respiration difficulties, then always but irregularly strange, deep and interrupted short periods of apnea.

We treated other dogs with a minor dose of the solution of methylcellulose--1%--always given intravenously; every day the animals showed a lack of appetite and an increasing loss of weight; the lethal result was not constant (especially in regard to the smaller dosage).

Properly to interpret these facts, we addressed ourselves to an anatomical-pathological and histological-pathological examination of the animals that were treated.

In the animals injected with an immediately lethal dosage, we have the following report: macroscopically, an increase in the size and weight of the lungs, which became edematous; microscopically, we noticed in the capillaries of the parenchymatose organs (especially the lungs and kidneys) the presence of a large quantity of methylcellulose, ascertained by means of a specific histological reagent, hydriotic acid--iodine (ac. hydriotic, 25 gr, iodine, 0.5 gr.).

In the animals treated with a non-immediately lethal dosage, whose death occurred after a few days, the macroscopic report is slight (some pulmonary hemorrhaging particularly in the lower lobe and heightened renal paleness). On the other hand, the microscopic examination of the internal organs was quite demonstrative: a great number of vessels clotted, especially in the lungs and kidneys, with conspicuous

interstitial reactions (we noticed especially in the kidneys a notable lymphocytaric infiltration and numerous cystiform dilations of the tubes accompanied by regressive facts of the epithelium).

Nothing of importance, either macroscopically or microscopically, was found in the dogs treated with a non-lethal dosage, and whose death did not occur spontaneously, even after some interval.

The conclusion to be drawn from this evidence is obvious: in dogs injected with an immediately lethal dosage, death occurred with acute edema of the lungs, provoked by occlusion of pulminary capillaries on the part of the large molecules of methylcellulose. In the dogs treated with a lesser dosage the same cause acts upon a proportionately lesser vascular area, but the exposure of cellular nutrition is the same in the internal organs, with a lethal result at intervals, if the number of exposed vessels exceeds the organic possibility of compensation.

The toxicity of methylcellulose, therefore, is not a specific toxicity: in fact, its solutions do not spread through the walls of the vessels, with the result that the large molecules of the substance occlude the light of the small vessels with the consequences already described.

Elimination

Is methylcellulose eliminated in the urine? The evidence reported above seems to exclude this possibility.

To ascertain this last fact, a small amount of a solution at 1% of methylcellulose was injected into the jugular vein of some dogs; urine was collected at

regular intervals of a few hours and was treated with the reagent of Radkofer; no trace of the substance appeared. The reagent of Fehling gave negative results, with which we wanted to find the eventual presence in the urine of lessened sugar derived from the decomposition of the methylcellulose. Hence the substance in the experiment was not eliminated by the kidneys.

Conclusions

Summarizing the data explained above, we can conclude briefly that subcutaneously introduced methylcellulose does not spread through the system and is not absorbed; when intravenously introduced, it does not spread through the walls of the vessels; it occludes and hence causes the lesions recorded above, which are especially visible in the lungs and the kidneys, thus demonstrating a non-specific toxicity, found at a high level of viscosity in the solutions and in the size of its molecules; and that it is not eliminated through the kidneys. Therefore it cannot be used as a stabilizing factor in colloids by introducing it parenterally.

Summary

The solution of methylcellulose at 2%, subcutaneously introduced into dogs and rats, does not spread and is not absorbed. The solution at 1%, intravenously introduced in rabbits and dogs, causes the death of the animal through acute edema of the lungs; minimal immediately lethal dosage: 14 cc. per kg. in rabbits, 20 cc. per kg. in dogs. Sub-lethal dosages cause anatomological-pathological results of edema by vascular occlusion in the parenchymatose organs. Methylcellulose, intravenously introduced, is not eliminated in the urine.

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OBSERVATIONS ON THE INGESTION OF METHYL CELLULOSE AND ETHYL CELLULOSE BY RATS*

WILHELM DEICHMANN, PH.D., AND SYLVAN WITHERUP, B.S., CINCINNATI, OHIO

BECAUSE of the potential usefulness of methyl and ethyl cellulose as thickening and binding agents in the pharmaceutic and food industries, the possibility that they might have deleterious effects was investigated by feeding them to rats over a considerable period of time.

Methyl cellulose[1] is a white, fibrous, cellulose ether, soluble in cold water but insoluble in hot water and in most organic solvents. Heating causes most aqueous solutions of methyl cellulose to coagulate, but cooling and stirring restores them to their original smoothness and fluidity. Solutions of the compound are colorless, odorless, and tasteless. Ethyl cellulose,[2] also a cellulose ether, is insoluble in water but soluble in most organic solvents. Its solutions are colorless and odorless.

Acknowledgment is made to the Dow Chemical Company for the financial support of the investigation and for the supply of the materials in the form of Methocel (Lot M-368, viscosity 1500 centipoise) and Ethocel (Lot 2467, viscosity 20 centipoise).

GENERAL EXPERIMENTAL PROCEDURES

Young albino rats, male nad female, bred in this laboratory and all of approximately the same age and weight, were divided into 3 groups of 80, to be fed the two cellulose ethers and the control diet, respectively. They were caged in groups of twenty. The food was supplied in baskets of wire screening, and the drinking water, in bottles fitted with glass tubes, the quantities consumed being recorded weekly. Experiments were started in July and terminated in the following March, at which time a representative number of animals from each group was killed and examined for gross and microscopic tissue changes.

PREPARATION OF FOOD AND WATER

Eighty grams of methyl cellulose were wet with hot water, cooled to room temperature, and stirred until nearly smooth; then one liter of cold water was added, and after the mixture had been kept about twenty-four hours in the refrigerator, the whole was diluted to 1500 c.c. with water, mixed, and added to 10 kg. of Purina Fox Chow pellets. Thus 10 Gm. of the dried food contained 80 mg. of methyl cellulose (8,000 parts per million). The drinking water was prepared so as to contain 10 Gm. of methyl cellulose in one liter of water (10,000 parts per million).

Ethyl cellulose (120 Gm.) was dissolved in 3 liters of 95 per cent ethyl alcohol and added to 10 kg. of Purina Fox Chow. After the alcoholic solution had

*From the Kettering Laboratory of Applied Physiology, College of Medicine, University of Cincinnati.

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AVERAGE WEIGHTS OF RATS FED
METHYL CELLULOSE OR ETHYL CELLULOSE

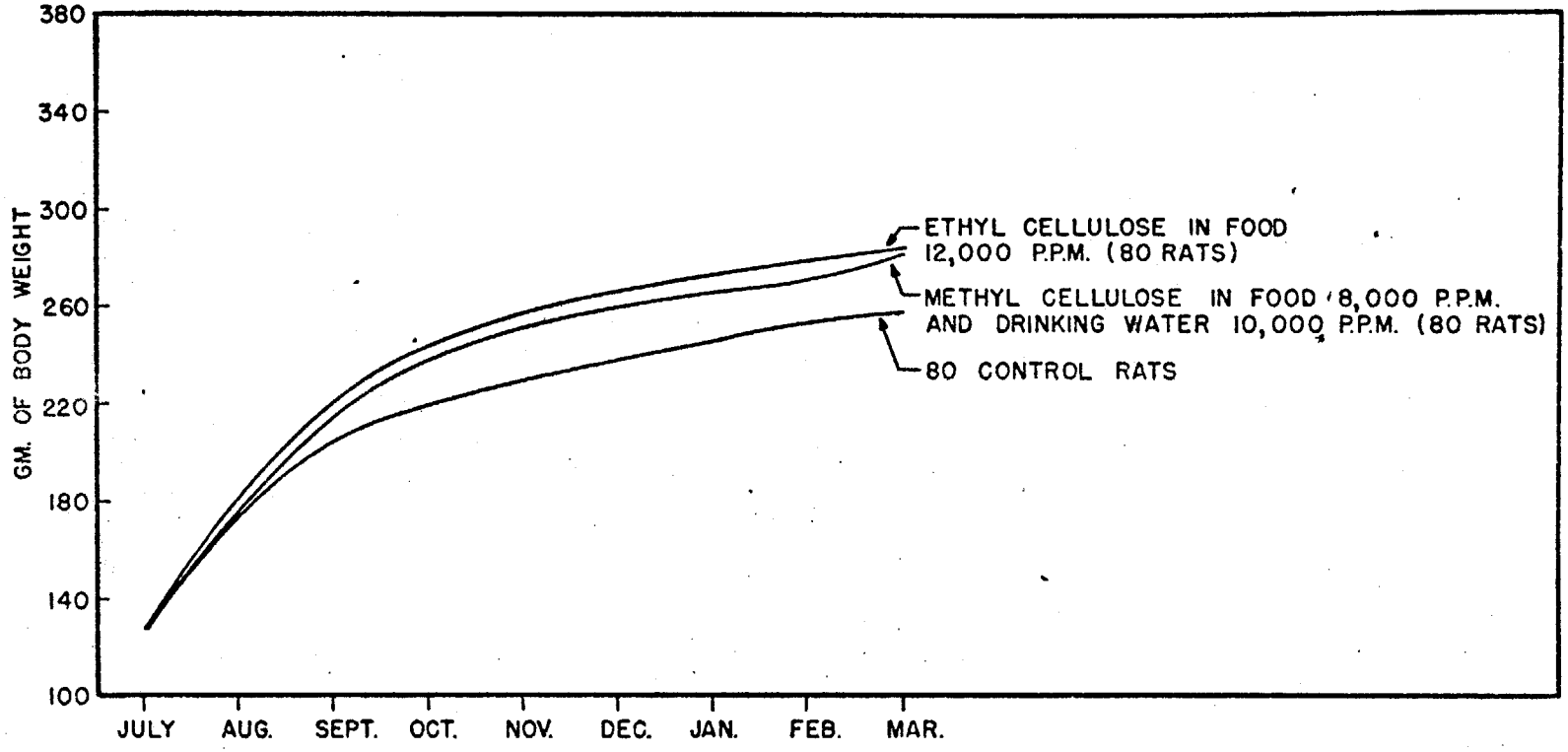

Fig. 1.

soaked in, the pellets were heated for two days at 70° C. in order to expel the solvent. (Analyses proved that the alcohol had been expelled except for insignificant traces.) Ten grams of this food contained 120 mg. of ethyl cellulose (12,000 parts per million).

RESULTS

Group 1.—The 80 rats in this group were given both food and water containing methyl cellulose. The consumption of food varied from 13 to 19 Gm. per day, and the daily intake of water ranged from 25 to 34 c.c. so that the average total daily intake of methyl cellulose was about 436 mg. per rat, 125 mg. being ingested with the food and 311 mg. in the drinking water.

Group 2.—The 80 rats in this group ingested from 14 to 18 Gm. of food per day, the daily intake of ethyl cellulose being about 182 mg. per rat. These animals had free access to uncontaminated drinking water.

Group 3.—The consumption of food and water by the 80 control animals varied within the limits recorded in the case of Groups 1 and 2 above.

Fig. 1 portrays the weight changes that occurred in each group as a whole. The differences among the three groups in this respect were manifestly insignificant. No other evidences of differences in the behavior or well-being of the animals in the several groups were noted.

SUMMARY AND CONCLUSIONS

Eighty rats were given methyl cellulose in their food and drinking water over a period of eight months, and the same number were given ethyl cellulose in their food for a corresponding period. The daily ingestion of quantities of methyl cellulose amounting to about 0.44 Gm. per rat or of ethyl cellulose amounting to about 0.18 Gm. per rat produced no signs of illness. The consumption of food and water as well as the gain in weight by these animals was normal. No evidence was found of significant gross or microscopic abnormalities in the tissues of the animals. It is concluded that the feeding of methyl and ethyl cellulose in these concentrations is harmless to rats.

REFERENCES

1. "Methocel," a brochure published by the Dow Chemical Company, Midland, Michigan, 1942.
2. "Ethocel," a brochure published by the Dow Chemical Company, Midland, Michigan, 1940.

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Effect of Methylcellulose on Growth Response of Rats to Low Vitamin Intakes. (19283)

R. C. ELLINGSON AND O. N. MASSENGALE. (Introduced by Warren M. Cox, Jr.)
From the Research Laboratories, Mead Johnson and Co. Evansville, Ind.

With increasing use of methylcellulose as a laxative in the treatment of chronic constipation(1-4), the question naturally arises—will continued ingestion significantly decrease the intestinal absorption of certain essential nutrients? Perhaps the question has already been answered in the negative by the fact that no clinical symptoms of vitamin deficiencies have been reported in patients who have ingested methylcellulose for long periods of time. Musick(1) cites the unpublished work of Shapiro, who showed that daily ingestion of methylcellulose did not interfere with the absorption of vit. K. However, for more

direct evidence we have studied the effect of ingestion of methylcellulose on the growth response of normal and depleted animals to minimal dosages of thiamine or vit. A. Thiamine and vit. A were chosen as representative of the water-soluble and oil-soluble vitamins, respectively.

Experimental. The given vitamin was dissolved or dispersed in a solution of methylcellulose of such concentration that daily administration by stomach tube of 2.0 cc supplied to each rat the desired quantities of the vitamin and methylcellulose. For the groups receiving no methylcellulose the daily dose of the vitamin was dissolved or suspended in 2.0 cc of water, if water-soluble, or in 0.5 cc of cottonseed oil, if oil-soluble. All dilutions were freshly prepared each day from assayed stock preparations of the vitamins. The experiments were of 28-day duration, during which time the weight responses of the experimental animals were observed. Each group consisted of 5 male and 5 female rats. In all instances, when methylcellulose was administered, a dosage of 50 mg per rat was employed.

Thiamine. Both normal stock rats and thiamine-depleted animals were used. The depletion period was 18 days. The rats were on a thiamine-free diet* and were fed 6 μg of thiamine hydrochloride daily by stomach tube, with or without simultaneous administration of methylcellulose.† The thiamine was supplied as either crystalline vit. B_1 or thiamine in admixture with other vitamins.‡

Vit. A. Stock rats were depleted of vit. A according to the procedure in the U. S. Pharmacopoeia XIV, page 789. Each deficient rat was given 3 units of vit. A daily as either an aqueous vitamin preparation‡ or a fish liver oil concentrate,§ with or without simultaneous administration of methylcellulose.

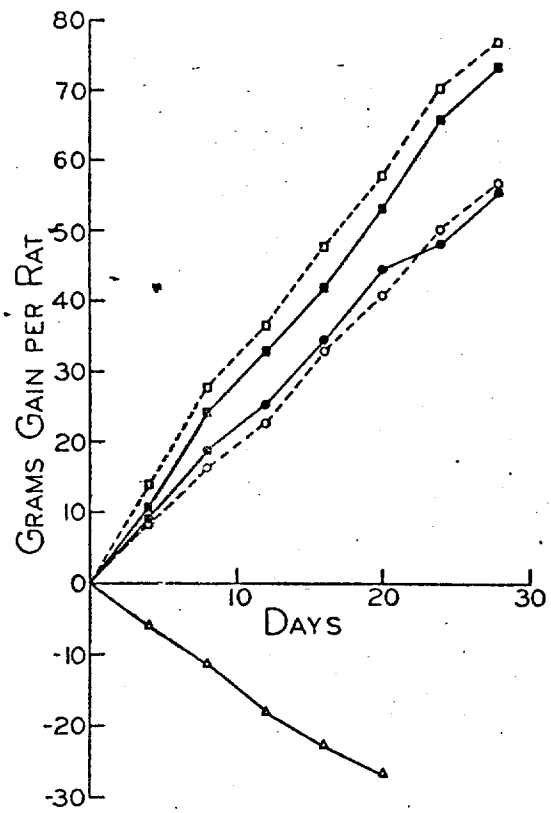

FIG. 1. Weight response curves of B_1-depleted rats given 6 μg of thiamine daily for 28 days with and without 50 mg of methylcellulose. Legend: △——△ control, ●——● cryst. B_1 and methylcellulose, ○---○ cryst. B_1, ■——■ B_1 as aq. vit. preparation and methylcellulose, and □---□ B_1 as aq. vit. preparation.

Results. In Fig. 1 and 2 are plotted the growth response curves of the various groups of animals. Since the response of the groups of normal rats was almost identical with that of the groups of thiamine-depleted rats, curves for the latter animals only are plotted in Fig. 1. Examination of the figures reveals that daily ingestion of methylcellulose by the rat does not significantly change the growth response to minimal doses of vit. A or B_1.

Discussion. Dutcher and coworkers(5) demonstrated, in the rat, that administration of mineral oil with varying amounts of carotene decreases the intestinal absorption of

* Thiamine-free diet: 18% casein (vit.-free), 4% salt mixture, 8% cottonseed oil, 5% autoclaved yeast, 2.5% Ruffex, 60.5% starch, 1% wheat germ oil and 1% cod liver oil.

† Dow Chemical Co. Methocel, 400 cps., Pharmaceutical grade.

‡ Known under the trade name of Poly-Vi-Sol. It contains, in addition to vit. A palmitate, irradiated ergosterol, ascorbic acid, thiamine hydrochloride, riboflavin, niacinamide and small amounts of inert ingredients.

§ Known under the trade name of Mead's Oleum Percomorphum. Consists of liver oils of percomorph fishes, Viosterol and other fish liver oils.

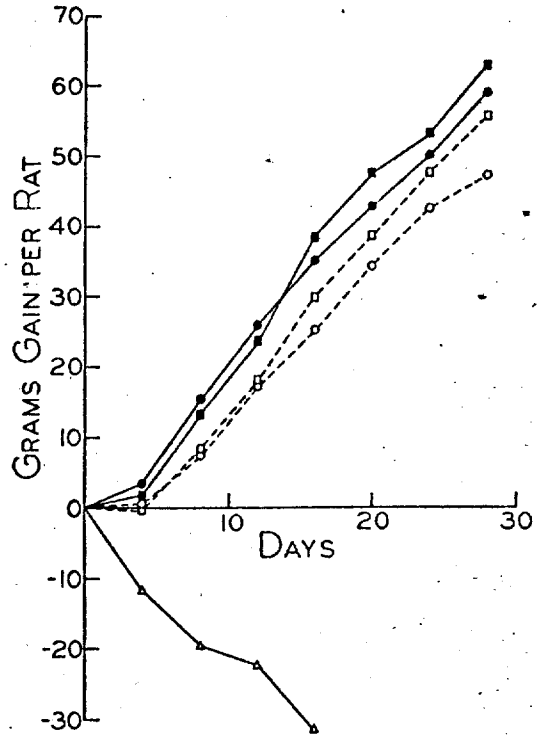

FIG. 2. Weight response curves of A-depleted rats given 3 units of vit. A daily for 28 days with and without 50 mg of methylcellulose. Legend: △——△ control, ●——● A as aq. vit. preparation and methylcellulose, ○---○ A as aq. vit. preparation, ■——■ A as fish oil and methylcellulose, and □---□ A as fish oil.

carotene, but that mineral oil has little effect on the absorption of vit. A administered in cod liver oil or as a vit. A concentrate. The explanation proposed for the different effects of mineral oil was the greater solubility of carotene in mineral oil than in the intestinal fluids and the greater solubility of vit. A in the intestinal secretions than in mineral oil. More recently, however, Paul, Ellis, and Paul (6) propose that the decreased effectiveness of β-carotene administered in mineral oil may be the result of partial isomerization of active β-carotene to stereoisomers of lower biological activity and that stereoisomerization of vit. A in mineral oil does not result in decreased biological activity. Whatever the true explanation may be for the effect of mineral oil on the utilization of carotene and of vit. A by the rat, these studies emphasize the possibility that ingestion of methylcellulose might decrease the effectiveness of the oil-soluble vitamins in the animal.

In order to learn whether ingestion of methylcellulose might decrease absorption of water-soluble or oil-soluble vitamins, low dosages of vit B_1 or A were fed to normal stock animals or depleted rats, and the growth responses were observed. Earlier work with vit. A(7) indicates that if decreased absorption were significant it would be reflected in decreased growth of the rats. The dosage of methylcellulose employed was 0.7 to 1.0 g per kg body weight which is approximately equivalent to 10 times the dosage ordinarily given to man.

From the weight gain of the thiamine-depleted and normal animals given 6 μg of thiamine daily for 28 days with and without simultaneous administration of methylcellulose, it is evident that the latter agent does not reduce the effectiveness of the low thiamine dosage and, therefore, does not decrease the intestinal absorption of the water-soluble vitamin. Similarly, the weight gains of the A-depleted rats show that the simultaneous administration of methylcellulose with 3 units of vit. A does not decrease absorption of the oil-soluble vitamin.

Conclusion. Daily ingestion of methylcellulose does not significantly decrease the effectiveness of (1) thiamine hydrochloride in the depleted or normal rat, or (2) vit. A in the A-depleted rat. These data are interpreted to mean that daily ingestion by the rat of large amounts of methylcellulose does not decrease the absorption of water-soluble or oil-soluble vitamins, and therefore does not increase the daily requirement of the rat for these factors.

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Identification of Stabilizing Agents

M. H. EWART AND R. A. CHAPMAN
Food and Drug Laboratories, Department of National Health and Welfare, Ottawa, Can.

EXTENSIVE use of a variety of polysaccharides as stabilizing or thickening agents in foods has created a need for analytical methods for the identification of commercially available materials and for their detection and quantitative estimation in food products. A number of stabilizers, which are used or have been suggested for use in foods, are listed and their chemical nature is indicated in Table I. Throughout this report the term "gums" is used in referring to all of these materials. Gelatin is included because it has many uses similar to those of the polysaccharides.

As the initial step in a project concerned with the development of the required analytical procedures, methods for the qualitative identification of a number of gums have been studied. Some procedures for the identification of gums are found in the literature. A method described by Jacobs and Jaffe (*15*) classifies the gums on the basis of physical characteristics or appearance of precipitates and thus requires considerable experience on the part of the analyst. Their outline does not include pectic substances, alginates, methylcellulose, carboxymethylcellulose, gum ghatti, starch, or gelatin. It also has the disadvantage of using an unstable reagent (Millon's) which must be freshly prepared each day. A method developed by Cannon and ad-

The present work was undertaken to develop a method for the identification of stabilizing and thickening agents used in food products. The materials studied were pectin, de-esterified pectin, algin, Irish moss, gum tragacanth, gum karaya, locust bean gum, starch, agar, gum arabic, gum ghatti, carboxymethylcellulose, methylcellulose, and gelatin. A proposed identification scheme is based on precipitation reactions with calcium chloride, sodium hydroxide, barium hydroxide, and lead acetate. In addition, reactions of the stabilizing agents with a cationic soap, ammonium sulfate, mercuric nitrate, papain, and gelatin are listed. The proposed scheme should be useful for identification of unknown stabilizing agents. A number of the reactions reported might be employed for the identification of individual stabilizing agents in mixtures of these materials or isolated from foods.

the Association of Official Agricultural Chemists (*3, 5*) does not provide for the identification of pectic substances, alginates, methylcellulose, carboxymethylcellulose, locust bean (carob) gum, or gum ghatti. Bryant (*4*) has described a procedure for distinguishing between pectin and certain gums, but it does not provide for positive identification of the gums. A number of other publications dealing with characteristic properties of these polysaccharides have been summarized by Mantell (*16*), but a systematic procedure is still needed for their identification. Such a procedure would be useful for the identification of products used as thickeners or stabilizers in foods, drugs, and cosmetics and ultimately for the identification of polysaccharides isolated from these materials.

In the present investigation, the manner in which the gums disperse in water after being wetted with alcohol has been a valuable index to the identity of unknown samples. Their solubility properties are summarized in Table II. Use has also been made of the fact that many of the polysaccharides occur as salts of complex organic acids (Table I). The acidic properties may be due to the presence of uronic acid groups, as in gum arabic, or to the unesterified portion of sulfuric acid molecules esterified with the polysaccharide. When mineral acids are added to aqueous solutions or dispersions of these salts, the effective concentrations of the polysaccharide anions are decreased. Thus, although the complex anions may yield insoluble salts with heavy metal cations, most are not precipitated from acid solutions. The amount the pH must be raised in order to precipitate the heavy metal salts—e.g., barium, mercury, or lead—is frequently characteristic of the individual polysaccharide.

Table I. Source and Chemical Nature of Materials Commonly Used as Thickening Agents in Foods

Material	Source	Principal Components	References
Pectic substances	Fruits	Galacturonic acid (occurs as methyl ester)	(*18*)
Algin (sodium alginate)	Seaweeds	Mannuronic acid (Na salt)	(*16*)
Irish moss	Seaweeds	Galactose, galactose 4-sulfate (K and Ca salts)	(*18*)
Agar	Seaweeds	Galactose (D- and L-), galactose 6-sulfate (Ca and Mg salts)	(*18*)
Tragacanth	Plant gum	L-Fucose, D-xylose, galacturonic acid, L-arabinose, D-galactose	(*18*)
Methylcellulose	Modified cellulose	Methyl-D-glucose	(*18*)
Starch	Plants	D-Glucose	(*18*)
Carboxymethylcellulose	Modified cellulose	Carboxymethyl-D-glucose	(*18*)
Locust bean gum (carob gum)	Seed endosperm	Mannose and galactose	(*19*)
Guar gum	Seed endosperm	Mannose and galactose	(*20*)
Karaya	Plant gum	Galactose, acetic acid, galacturonic acid, rhamnose, tagatose	(*14*)
Arabic (acacia)	Plant gum	D-Glucuronic acid, D-galactose, L-arabinose, rhamnose (mixed Ca, Mg, and K salts)	(*18*)
Ghatti	Plant gum	L-Arabinose, galactose, galacturonic acid (Ca salt)	(*10*)
Gelatin	Modified protein	Amino acids	

EXPERIMENTAL

During the present investigation 0.5 to 1.0% aqueous dispersions of the polysaccharides were used for the tests. Aliquots of from 3 to 5 ml. were treated with varying concentrations of the reagents which it was hoped would give characteristic precipitation reactions. Initially the reagents used were those for which Jacobs and Jaffe (*15*) have described reactions with several polysaccharides. Subsequently a number of other reagents were used.

Table II. Dispersion in Water of Gums, Wetted with Alcohol

Gum	Manner of Dispersal in Water
Pectic acid	Insoluble
Pectate (Na, K, or NH_4 salts)	Forms either clear or turbid solution on heating
Pectate (Ca salts)	Insoluble
Pectin	Swells in cold water and dissolves on heating
Alginate	Dissolves slowly in cold water or quickly on heating to form viscous solution
Irish moss	Dissolves slowly in cold water, rapidly on heating to form viscous solution
Agar	Swells in cold water, dissolves on heating, gels on cooling
Tragacanth	Swells to form viscous dispersion in cold or hot water, but does not form true solution
Methylcellulose	Dissolves slowly in cold water but becomes cloudy or gels on heating
Starch	Disperses on heating
Carboxymethylcellulose	Dissolves slowly in cold water, rapidly on heating, giving clear viscous solution with some fine fibrous suspended material
Locust (carob)	Forms viscous suspension but not a true solution
Karaya	Forms viscous suspension. Insoluble particles settle on standing
Arabic (acacia)	Dissolves in cold water to form a clear only slightly viscous solution
Ghatti	Dissolves to form almost clear solution but some insoluble material may remain as fine suspension
Gelatin	Swells in cold water and dissolves on heating

Reactions which were found useful for characterizing the gums are summarized in Tables III and IV. Only those materials having anionic components, such as alginates, or potential anionic components, such as pectin, give pronounced reactions with cationic soap (Table III). As in the case of precipitates with heavy metals, the precipitates with the cationic soap quickly disperse on acidification of the medium. Ammonium sulfate is of interest, in that it gives pronounced precipitation tests with several of the gums but not with alginates, pectin, tragacanth, karaya, arabic, or ghatti, each of which probably contains uronic acid components. The reactions with Stokes's acid mercuric nitrate illustrate the effects of low pH on precipitation of heavy metal salts of the polysaccharide acids. An excess of the reagent makes the solution strongly acidic and thus the weakly dissociated acids redisperse. Alginic acid and pectic acid are insoluble and thus are not dissolved by excess Stokes reagent. Papain and gelatin give pronounced precipitation reactions only with those gums having

Table III. Precipitation Reactions of Polysaccharide Gums and Gelatin

Gum	1 Vol. 1% Solution of Cationic Soap[a]	0.5 Vol. Saturated Ammonium Sulfate	Diluted[b] Stokes's Acid Mercuric Nitrate Added Dropwise	1 Vol. 2% Papain (6)[c]	1 Vol. 2% Gelatin[c]	4 Vol. 95% CH$_3$OH + 2 – 3 Drops Saturated NaCl
De-esterified pectin	Fine opaque precipitate	Gelatinous translucent precipitate	Gels (almost opaque). Insoluble in excess reagent	Precipitate	Precipitate	Gelatinous precipitate, gels (1 vol.)
Alginate	Fine opaque precipitate	Nil	Gels (almost opaque). Insoluble in excess reagent	Precipitate	Precipitate	Gelatinous precipitate (1 vol.) becomes stringy with 4 vol. alcohol
Pectin	Flocculent precipitate	Nil	Forms almost opaque gel which dissolves in excess reagent	Cloudy	No definite effect	Transparent gelatinous precipitate. Gels (1 vol.)
Irish moss	Stringy or flocculent precipitate	Gelatinous precipitate or gel	Transparent gel. Redispersed by excess reagent	Precipitate	Precipitate	Stringy precipitate
Agar	Gelatinous precipitate	Flocculent precipitate	Turbid or cloudy	Cloudy	Precipitate	Fine flocculent precipitate
Tragacanth	Fine opaque precipitate	Nil	Flocculent precipitate. Dissolves in excess reagent	Precipitate	Precipitate	Voluminous precipitate, jellylike
Methylcellulose	Nil	Precipitate	Nil	Nil	Nil	Nil
Starch	Nil	Precipitate	Nil	Nil	Nil	Opaque flocculent precipitate
Carboxyethylcellulose	Gelatinous clotted precipitate	Gelatinous precipitate	Precipitate dissolves in excess reagent	Precipitate	Precipitate	Voluminous clotted precipitate
Locust	Nil	Precipitate (voluminous)	No pronounced effect	No pronounced effect	Nil	Voluminous opaque stringy precipitate, forms clot
Karaya	Flocculent precipitate	Nil	Flocculent precipitate dissolves in excess reagent	Precipitate	Precipitate	Flocculent precipitate, discrete particles
Arabic (acacia)	Precipitate (very fine)	Nil	Flocculent precipitate dissolves in excess reagent	Precipitate	Precipitate	Fine opaque nonsettling precipitate
Ghatti	Fine precipitate	Nil	Nil	Nil	Precipitate	Fine precipitate, nonsettling (2–3 vol.)
Gelatin	Precipitate in alkaline medium (22)	Precipitate	Nil	Nil	Nil	Finely flocculent precipitate, coagulates

[a] Rodalon (alkyl dimethyl benzyl ammonium chloride), Fairfield Laboratories, Plainfield, N. J.
[b] Mercury dissolved in twice its weight of concentrated nitric acid and diluted to 100 times its volume with distilled water.
[c] Precipitates with papain and gelatin are observed only in weakly acidic medium and most exhibit properties of coacervates rather than true precipitates.

pH of the mixture is below the isoelectric point of the protein and it is possible that they would be more correctly called coacervates. They are usually dispersed by a few drops of mineral acid or of dilute ammonium hydroxide. The characteristic manner in which some of the gums are precipitated by alcohol may also be of value in their identification.

The reactions described in Table IV form the basis of a proposed procedure for the systematic identification of the gums.

REAGENTS

Calcium chloride ($CaCl_2$), 3% solution (weight/volume).
Ammonium hydroxide, 3.0 *N* solution.
Sodium hydroxide, 3.0 *N* solution.
Barium hydroxide, saturated solution stored in a bottle equipped with a siphon and a soda-lime tube.
Basic lead acetate, 20% suspension (weight/volume). Heat to boiling, cool, and use supernatant solution.
Hydrochloric acid (or other mineral acid), 3.0 *N* solution.
Methylene blue, 0.1% aqueous solution.
Tincture of iodine (U.S.P., *14*).
Iodine–potassium iodide stock solution, containing 0.5% iodine and 1.0% potassium iodide. Iodine–potassium iodide test solution, consisting of stock solution diluted 1 to 5.
Cupric sulfate ($CuSO_4.5H_2O$), 15% solution (weight/volume).
Borax ($Na_2B_4O_7.10H_2O$), 4% solution.
Ruthenium red test solution (8).
Picric acid, saturated aqueous solution.

IDENTIFICATION PROCEDURE

Wet a 0.25- to 0.5-gram sample of the material to be identified with 1 to 2 ml. of 95% alcohol and add 50 ml. of distilled water. Suspend the solid material in the water by shaking or stirring. Heat the suspension, with frequent shaking, on a hot plate or over a burner. If the sample dissolves, discontinue heating immediately; otherwise hold at 85° to 95° C. for 15 minutes.

Group A. I. Treat a 3- to 5-ml. aliquot of the solution with 0.2 volume of 0.25 *M* calcium chloride. A gelatinous precipitate or gel indicates alginates or de-esterified pectin.

If no reaction is apparent with calcium chloride alone, add 1 volume of 3 *N* ammonium hydroxide to the calcium chloride treated sample. Slow formation of a gel or gelatinous precipitate indicates pectin.

II. If either test in A I was positive, mix a fresh 3- to 5-ml. aliquot of sample with 1 volume of 3.0 *N* sodium hydroxide. Observe the reaction and then heat the mixture in a boiling water bath for 10 minutes.

Immediate formation, in the cold, of a gelatinous or flocculent precipitate indicates either pectin or de-esterified pectin. No precipitate indicates alginates. All three mixtures become yellow on heating, but the precipitates with pectic substances do not dissolve.

Group B. If the material is not an alginate or a pectic substance, carry out the following tests.

I. Mix a 3- to 5- ml. aliquot of the sample with 0.1 volume of saturated barium hydroxide. Observe in the cold and heat in boiling water bath for 10 minutes.

Formation in the cold of a nonsettling, almost opaque, gelatinous precipitate indicates Irish moss. Carry out confirmatory test for Irish moss.

A small amount of flocculent precipitate or cloudiness in the cold and a definite lemon yellow color on heating identify gum tragacanth.

Color changing during heating to yellow, then to green, and finally to gray indicates agar. Carry out confirmatory test for agar.

If the mixture becomes cloudy or forms a gel on heating, but becomes clear on cooling, methylcellulose is indicated. Carry out confirmatory test for methylcellulose.

An opaque flocculent precipitate which may tend to redisperse on heating and reprecipitate on cooling indicates starch. Carry out confirmatory test for starch.

Precipitates which disappear when the barium hydroxide is thoroughly mixed with the sample may be disregarded at this point.

II. If the material has not been identified, mix a fresh 3- to 5-ml. aliquot of sample with 1 volume of saturated barium hydroxide. Observe whether there is an immediate precipitation and examine again after standing 5 minutes at room temperature.

Table IV. Precipitation Reactions Used as Basis of Proposed Procedure for Identification of Gums

(Reactions used as identification tests within heavy lines)

Gum	1/5 Vol. 2.5% $CaCl_2$	1/5 Vol. 2.5% $CaCl_2$ + 1/5 Vol. 3 N NH_4OH	1 Vol. 3 N NaOH	1/10 Vol. Saturated $Ba(OH)_2$ Cold	1/10 Vol. Saturated $Ba(OH)_2$ Heated	1 Vol. Saturated $Ba(OH)_2$	1/5 Vol. Basic Lead Acetate	Basic Lead Acetate + 1/5 Vol. 3 N NH_4OH	Confirmatory Tests
De-esterified pectin	Gelatinous ppt. or gel		Gelatinous or flocculent ppt., yellow color on heating	Gel or gelatinous ppt.	Ppt. and soln. turn yellow	As for 1/10 vol.	Gels	As for lead acetate alone	Gelatinous ppt. when acidified with mineral acids
Alginate	Gelatinous ppt. or gel		Clear yellow soln. on heating	Gel or gelatinous ppt.	Ppt. and soln. turn yellow	As for 1/10 vol.	Gels	As for lead acetate alone	Gelatinous ppt. when acidified with mineral acids
Pectin		Gelatinous ppt. forms slowly	Gelatinous or flocculent ppt., yellow color on heating	Gel or gelatinous ppt.	Ppt. and soln. turn yellow	As for 1/10 vol.	Translucent gel	As for lead acetate alone	
Irish moss	Some samples may give flocculent ppt.			Gelatinous ppt. or gel, almost opaque	Ppt. may flocculate with prolonged heating	Gelatinous ppt. or gel	Gelatinous ppt. or gel	As for lead acetate alone	Forms blue fibrous ppt. with aqueous methylene blue
Tragacanth				May be small amount of ppt.	Lemon yellow color	Flocculent ppt.	Flocculent ppt.	As for lead acetate alone	
Agar				Nil	Becomes yellow then green and gray	Nil	Flocculent ppt.	Gels	Gives blue or black stain with tincture of iodine
Methylcellulose				Nil	Becomes turbid or forms gel; becomes clear on cooling	Nil	Nil	Gels	Aqueous dispersions are not precipitated by alcohol, but form gel or become cloudy when heated
Starch				Opaque flocculent ppt.	May redisperse	Flocculent ppt.	Flocculent ppt.	Very heavy flocculent or gelatinous ppt.	Blue stain with I-KI reagent
Carboxymethylcellulose				Ppt. dissolves on shaking	Nil	Flocculent ppt.	Gels	As for lead acetate alone	Forms clotted ppt. with $CuSO_4$
Locust*						Clotted opaque ppt.	Opaque gel	As for lead acetate alone	Gels with 1/5 vol. 4% borax
Karaya						Flocculent ppt., forms slowly	Flocculent ppt.	As for lead acetate alone	Swells and stains pink with ruthenium red test solution
Arabic (acacia)							Voluminous opaque ppt.	As for lead acetate alone	Readily soluble in water at room temperature
Ghatti							May be small amount of flocculent ppt.	Voluminous opaque ppt.	Fine ppt. with 4 vol. of alcohol. Arabic also gives fine ppt.
Gelatin									Gives fine yellow ppt. when added to saturated picric acid

* Locust bean gum and guar gum give identical reactions and cannot be distinguished on basis of these tests.

Table V. Samples Examined by Proposed Identification Procedure

Gum	Type	No. of Samples
Sodium pectate	Powdered	1
Pectic acid	Granular	1
Pectin	Powdered	2
Sodium alginate	Powdered	5
Irish moss	Powdered	2
Tragacanth	Powdered	1
	Shredded	1
	Granular	3
Methylcellulose	Fibrous	2
Starch	Whole wheat flour	1
	Tapioca flour	1
	Soluble starch	3
	Cornstarch	1
	Amioca (amylopectin)[a]	1
	Clear jel[a]	1
	Clear-Flo-II (sodium salt of starch acid ester)[a]	1
	Dry-Flo (starch ester)[a]	1
	Vulca (starch ether)[a]	1
	Melojel[a]	1
	Nu-film (starch acid ester)[a]	1
Sodium carboxymethylcellulose	Powdered	1
Locust bean (carob)	Powdered	7
Guar	Powdered	1
Karaya	Powdered	1
Arabic (acacia)	Powdered	3
	Lump	1
Ghatti	Powdered	1
	Lump	1
Gelatin	Granular	2

[a] Products of National Starch Products, New York, N. Y.

A voluminous opaque, stringy precipitate which tends to form a clot indicates locust bean gum. This precipitate may appear flocculent if the mixture is shaken vigorously. Carry out confirmatory test for locust bean gum.

A voluminous opaque flocculent precipitate which forms immediately indicates carboxymethylcellulose. Carry out confirmatory test for carboxymethylcellulose.

An opaque flocculent precipitate which forms slowly and is not voluminous indicates gum karaya. Carry out confirmatory test for karaya.

Group C. If the sample has not been identified it may be gum arabic, gum ghatti, or gelatin.

I. Mix a fresh 3- to 5-ml. aliquot of sample with 1 ml. of basic lead acetate solution. Immediate formation of a voluminous opaque precipitate indicates gum arabic.

If there was only a small amount of flocculent precipitate, or no precipitate, with the basic lead acetate add 1 ml. of 3.0 *N* ammonium hydroxide to the lead-containing sample. A voluminous opaque flocculent precipitate indicates gum ghatti. If there is no precipitate, the sample probably is gelatin. Carry out confirmatory test for gelatin.

CONFIRMATORY TESTS

Alginates and De-esterified Pectins. Add 0.2 volume of 3 *N* hydrochloric acid (or other mineral acid) to 3 to 5 ml. of the sample. A gelatinous precipitate confirms alginates or de-esterified pectin.

Irish Moss. Add 2 to 3 drops of 0.5% methylene blue in water to 1 ml. of the sample solution. Precipitation of purple-stained fibers confirms Irish moss.

Methylcellulose. Mix 5 ml. of sample with 25 ml. of 95% alcohol and 2 to 3 drops of saturated sodium chloride. No precipitate confirms methylcellulose.

Agar. Precipitate gum from 5 ml. of sample with alcohol and stain with tincture of iodine (*3*). Starch is also stained blue by this reagent.

Starch. Add 1 to 2 drops of the iodine potassium iodide test solution to 1 ml. of sample. A blue or purple color confirms starch. Some samples of gum tragacanth may give a faint blue test here.

Carboxymethylcellulose. Add 2 ml. of 1.0 *M* cupric sulfate to 3 to 5 ml. of sample solution. An opaque, slightly bluish, clotted precipitate confirms carboxymethylcellulose.

Locust Bean Gum. Add 1 ml. of 4% borax to 3 to 5 ml. of gum solution. If mixture gelatinizes, locust bean gum is confirmed. Guar gum also forms a gel here.

Karaya. Precipitate gum from 5 ml. of solution with alcohol and stain with ruthenium red (*8*). If sample swells considerably and is stained pink, karaya is confirmed.

Gelatin. Add 2 to 3 drops of gum solution to 2 ml. of saturated picric acid. A fine yellow precipitate confirms gelatin.

DISCUSSION

The proposed procedure has been tested with the gums listed in Table V. It was possible to identify the modified starches as starch products, because the solubility of all these materials is decreased by barium hydroxide and all give positive tests with the iodine–potassium iodide reagent. It is possible that not all the thickening agents or gums employed in food products at the present time have been included in this study. Cherry gum and quince seed gum, for example, have been suggested in the literature for use in foods. However, the proposed procedure includes all the gums that were available during the investigation.

The proposed scheme for identification of stabilizing and thickening agents is applicable only when they have not been mixed with other materials. To identify thickening agents in foods by this method it would first be necessary to separate them from the foods, but separation techniques might alter the reaction characteristics of the gums. Much work has been done on methods for the separation and detection of gums in particular foods, such as mayonnaise and French dressing (*2*, *7*, *10*, *11*), soft curd cheese (*1*, *8*, *10*, *11*, *13*, *19*), tomato products (*10*, *15*), starchy foods (*21*), cacao products (*13*, *17*, *23*), ice cream and frozen desserts (*9–11*), canned chicken (*10*), and meat products (*12*). Additional references to methods for separating gums from foods may be found in reviews by Jacobs and Jaffe (*15*) and Mantell (*16*). The emphasis in most procedures has been on detection of the gums without identification. However, Wyler (*24*) has outlined methods for the detection and identification of locust (carob) bean gum, methylcellulose, carboxymethylcellulose, starch, pectin, and alginate in meat products. Thus identification tests proposed in the present paper may be useful for the identification of gums separated from foods by methods already described in the literature. However, it is probable that many special techniques will be required for the analyses of particular combinations of foods and thickeners. A great deal more work must be done before it will be possible to identify all of the gums in the various foods in which they may be used.

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HENRY M. FEINBLATT, M.D., F.A.C.P., THEODORE M. FEINBLATT, M.D., and EDGAR A. FERGUSON, JR., Brooklyn, New York

(From the Medical Service of Kings County Hospital)

SINGLE-DOSE administration of a 1.2-mg. digitalization dose of digitoxin and high maintenance doses of 0.2 mg. have exaggerated the danger of poisoning. An effort has been made to eliminate the danger of too acute and rapid onset of action by modifying digitoxin with synthetic colloids.

Procedure

Two groups of cases were treated. One group was treated with plain digitoxin. The other group was treated with digitoxin plus carboxymethylcellulose. Most of the cases were the same for the first and second groups. The whole procedure was repeated with both types of medication.

Electrocardiograms were taken before medication and at three, six, twelve, twenty, twenty-four, and thirty-six hours. In addition, comparisons were made on a twenty-day basis.

Clinical observations of therapeutic efficiency and toxic side-effects were made in every case.

Method of Analysis of Electrocardiograms

Strict quantitative assay is not possible with the electrocardiogram. However, from a clinical standpoint, the time and acuteness of action may be judged. Robinson and Wilson have shown that an altered T wave occurs with 25 per cent lethal dose, lengthened P-R interval with 50 per cent, idioventricular complexes at 70 per cent, slowing of the heart rate at 70 per cent, and auriculoventricular dissociation at 80 per cent.[1]

These experiments are based on cat studies. The percentages are not exactly applicable to the human but form a convenient basis for comparison.

Slowing of the Heart Rate.—Reduction of heart rate is one of the indications of digitoxin action. Cross-circulation experiments on animals have shown that digitoxin causes characteristic bradycardia when the drug has access to the carotid body. The caroticoaortic vasoreceptor system is stimulated by digitoxin to cause reflex vagal slowing of the heart rate.

PR_2 Interval.—Clinical doses of digitoxin cause slowing of conduction rate between auricle and ventricle. This effect is noted by increased PR interval in the electrocardiograms.

QRS Voltage.—Characteristic voltage changes in the QRS complex are caused by digitoxin. These changes are not quantitative but may form a basis of comparison in the same individual for different types of medication.

T Wave.—T wave alterations in the electrocardiogram showing a change of direction are usually indicative of digitoxin effect.[1]

ST-T Segment.—Characteristic changes in RST and ST-T segments occur after digitoxin therapy. These changes must be carefully distinguished, as they may simulate those resulting from coronary artery disease or coronary occlusion. Changes in the ST-T levels are due to direct action of digitoxin on the heart muscle and are not affected by atropine.

Arrhythmia.—Sinus arrhythmia is an early and minor effect of digitoxin; it may also be abolished by digitoxin. Ventricular tachycardia and alternans, characterized by alternation of direction of QRS complexes, is pathognomonic of digitoxin poisoning. This may occur more readily in injured hearts but is specifically produced by digitoxin.

The following is a typical case illustrating the poisoning effect of plain digitoxin, contrasted with the effect of digitoxin plus carboxymethylcellulose.

Case Report

Case 22.—A thirty-nine-year-old man complained of shortness of breath and anginal pain on exertion. Nitroglycerine was used constantly to relieve pain. He was 67 inches tall and weighed 204 pounds. Symptoms had been becoming worse gradually during the past five years. Palpitation was felt at all times. Shortness of breath was somewhat relieved by digitalis.

On occasion, there were periods of acute shortness of breath, suggestion of gallop rhythm, and poor heart sounds. There were loud diastolic and systolic murmurs. Acute episodes were controlled by full digitalization and bed rest.

There was a history of chorea at the age of six years, followed by confinement to bed for many months. There was rheumatic pain in the joints (especially knees and hips) from the age of eight to thirteen. Since the age of sixteen he had been classified as having a "rheumatic heart."

Physical examination revealed a well-nourished,

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barrel-chested individual. There were increased A_2 sounds with enlargement of the heart to the left and to the right. The veins of the neck were full, and an aortic thrill was present. The heart beat caused a rocking to-and-fro motion of torso and head. There was aortic insufficiency. There were no other physical findings.

Teleoroentgenograms showed enlargement of heart to left and right, the heart occupying 70 per cent of the chest cavity. Chest was clear.

Electrocardiogram revealed an auricular rate of 60 with occasional ventricular extrasystoles. PR_2 interval was 0.24, QRS_2 0.9. There was left axis deviation, and ST-T level was depressed. T_1 was down, T_2 was diphasic, T_3 upright, and T_4 upright.

Digitoxin Plain.—This patient was digitalized with 1.2 mg. of plain digitoxin taken orally.* Four hours after digitalization, the electrocardiogram revealed an auricular and ventricular rate of 71. All other constants were the same except for the more sharply pointed T wave in lead 4 and an increase in the height of T_3.

At eight hours the electrocardiogram revealed a rather sharp depression of ST-T_1 and T_1. The auricular rate was 50, ventricular rate 70, PR_2 interval 0.21, and QRS_2 0.10. All leads showed trigeminal rhythm with numerous ventricular extrasystoles.

The electrocardiogram represented the sharply acute effect of digitoxin. The trigeminal rhythm was pathognomonic.

At the time that this trigeminal rhythm occurred, the patient felt extremely ill at ease, very depressed, and was unable to do any work. There were pains in the extremities, knees, and back. Soon after, he was able to walk about and felt a little better. There was a period, however, during which he felt extremely ill. The acute action of plain digitoxin was demonstrated most clearly at this eight-hour point.

At eleven hours the electrocardiogram revealed an increased ventricular rate (80). PR_2 interval was 0.21, QRS_2 0.10. The first lead showed ventricular extrasystoles, while the second, third, and fourth leads showed many ventricular extrasystoles with a suggestion of trigeminal rhythm. T_1 was depressed, and all other T waves were upright. At this point, the patient felt fairly well but was still uneasy. Some of the acute effects had been dissipated at the eleven-hour point.

At the twenty-three-hour point the ventricular rate was 73. PR_2 interval had increased to 0.23, QRS_2 0.10. Ventricular extrasystoles were present, but trigeminal rhythm was not apparent.

At about thirty hours the trigeminal rhythm returned together with acute clinical manifestations. The patient felt depressed, nervous, and sick. The trigeminal rhythm was not as constant as at eight hours. Forty-eight hours later the trigeminal rhythm had disappeared, but numerous ventricular extrasystoles occurred.

The patient continued taking 0.15 mg. plain digitoxin daily for one week, but medication was discontinued because no improvement was evident, the constant result of plain digitoxin being a condition of uneasiness and subjective instability.

Digitoxin plus Carboxymethylcellulose.—After a period of three weeks during which no medication was given, this same patient was digitalized with 1.2 mg. digitoxin plus carboxymethylcellulose.

The electrocardiogram, taken four hours after administration of 1.2 mg. digitoxin plus carboxymethylcellulose, showed an auricular rate of 77 and a ventricular rate of 77. PR_2 was 0.21, QRS_2 0.10. Axis was left and rate regular. ST-T_1 was depressed slightly. T_1 and T_4 were down. In leads 2 and 3, T was upright. The over-all number of premature ventricular beats was less at the four-hour point.

The electrocardiogram taken after eight hours showed auricular rate of 60 and a ventricular rate of 74. PR_2 was 0.23, QRS_2 0.10. T_1 was more depressed than before medication. All leads showed extrasystoles, and T was upright in all except the first lead.

The electrocardiogram taken at twenty-four hours showed an auricular rate of 60 and a ventricular rate of 80. PR_2 was 0.21 and QRS_2 0.10. Axis was left, and rate (exclusive of premature ventricular beats) was regular. ST-T and T in the first lead were depressed. There was one premature ventricular extrasystole present in lead 1. There were no others, and T was upright in all other leads.

This electrocardiogram demonstrates the myocardial action of digitoxin in that the T waves were made more sharply pointed, and T_4 was slightly depressed. In addition, the number of premature ventricular beats was markedly reduced.

The electrocardiogram taken at thirty-five hours showed a ventricular rate of 80. PR_2 was 0.21, QRS_2 0.10. There was left axis deviation, and the rate (exclusive of ventricular extrasystoles) was regular. T_1 and ST-T_1 were depressed, and T was more sharply pointed downward than it was without medication. T_3 was sharply pointed downward, and T_4 became diphasic.

This series of electrocardiograms demonstrates the action of digitoxin plus carboxymethylcellulose. This action was exhibited continuously all during the period of thirty-five hours.

The patient continued taking 0.15 mg. digitoxin plus carboxymethylcellulose daily for a week. At the end of a week no symptoms of toxic effect had developed.

Tabulation of Cases

Out of the series of 50 cases observed carefully for poisoning effects, 11 showed unmistakable evidence of digitoxin intoxication. Clinical manifestations ranged from green vision with nausea to a subjective sense of instability. While clinical findings were of interest in this group, it is more important to note the mechanism of action as demonstrated by the various electrocardiographic elements.

The following review takes up the elements one by one with reference to a comparison of the

* All digitoxin used was U.S.P. XIII.

TABLE I.—Cases of Myocardial Impairment Demonstrating Poisoning Effect of Plain Digitoxin Contrasted with Effect of Digitoxin plus Carboxymethylcellulose

Case Number	Rate	T Wave	PR_2 Interval	Rhythm
Peak of Activity (Hours) of Plain Digitoxin				
22*	6	3	8	6-8
30	3	3	8	None
28	24	12	6	None
16	12-24	6	8	None
14	3	3	3-6	None
3	6	6	6	None
9†	6	6	12	None
27	6	24	12-24	6-12
5	6	3	6	8
26	8	8	12	6
27	6	8	6	None
Average	8.4	7.5	8.6	4 Cases
Peak of Activity (Hours) of Digitoxin Plus Carboxymethylcellulose‡				
22	12	6	12-24	None
20†	6	12	3	None
19†	24	12	12	None
16	6	6	12	None
14	6	3	6	None
3	6	6	8	None
7	3	6	12	None
27	6	24	24	None
5	12	3	12	None
26	8	6	12	None
27	6	6	6	None
Average	8.6	8.2	11.4	None

* Fully reported in text.
† Paired with case on same line in other section.
‡ All showed subjective manifestations of nausea and changes in vision on plain digitoxin. This was not shown on digitoxin plus carboxymethylcellulose.

effect of plain digitoxin with that of digitoxin plus carboxymethylcellulose (Table I). Since none of these cases showed any poisoning symptoms when digitoxin plus carboxymethylcellulose was used, it is of interest to compare the time of peak activity of each of the electrocardiographic elements.

Rate.—Reduction of heart rate is one of the pathognomonic indications of digitalis action. The peak activity for plain digitoxin was eight and four-tenths hours, while that of digitoxin plus carboxymethylcellulose was eight and six-tenths hours. This small difference is not considered to be significant, and it is also of interest to note that reduction of heart rate is not one of the acute toxic effects of digitoxin.

In most cases after the peak effect was shown, the effect of slowing continued from thirty-six to forty hours with digitoxin plus carboxymethylcellulose, while this effect was sustained for about thirty hours on plain digitoxin.

When digitoxin plus carboxymethylcellulose was used, the reduction of heart rate usually proceeded more smoothly and was less acute in its initial effect.

T Wave Change.—Within three hours after large oral doses of digitalis, the electrocardiogram often shows definite alterations. It is often true that the T wave shows the first change. Normally, upright T waves may become diminished in amplitude or may become flat or inverted. An alteration in T wave direction is usually indicative of digitoxin effect.

Peak activity of plain digitoxin occurred in seven and one-half hours, while peak activity of digitoxin plus carboxymethylcellulose occurred in eight and two-tenths hours. This difference is slightly more significant than the difference in peak activity time of the rate. It is significant to note further that after twenty days the effect from digitoxin plus carboxymethylcellulose became greater, although this occurred more gradually.

PR_2 Interval.—Digitalis causes slowing of conduction between auricle and ventricle. This effect is demonstrated by an increased PR_2 interval in the electrocardiogram. The PR_2 interval reached its peak effect in eight and six-tenths hours with plain digitoxin. This is contrasted with digitoxin plus carboxymethylcellulose, which reached its peak in eleven and four-tenths hours. This difference is even more significant than T wave change or rate. It is interesting to note that in our series the effect of a single dose of plain digitoxin on the PR_2 interval was lost in thirty-six hours. Plain digitoxin effected a much quicker result on the conduction wave, but this effect was not evident after thirty-six hours, while the effect of digitoxin plus carboxymethylcellulose was smoother and the over-all effect greater. In addition, digitoxin plus carboxymethylcellulose persisted in its effect after the thirty-hour point.

Degree of Arrhythmia.—Although sinus arrhythmia may occur as an early and minor effect of digitalis, this early effect is not significant. Arrhythmia has been noted more particularly with plain digitoxin than with older digitalis tincture.

Ventricular tachycardia from plain digitalis is always serious. A particularly ominous type which is thought to signal the beginning of fibrillation of the ventricles is characterized by alteration in direction of QRS complexes. In our cases none showed disturbance of rhythm when digitoxin plus carboxymethylcellulose was used, while, with the same dose, four cases out of the clinical group showing some poisoning effect had disturbance of rhythm when plain digitoxin was used. The greatest disturbance in rhythm was trigeminal pace demonstrated in the detailed case history.

Results

The study revealed that sharp decreases in ventricular rate as measured on lead 1 were noticed at three, six, or twelve hours and that these decreases did not occur after thirty hours.

In each case compared, digitoxin plus carboxymethylcellulose did not show these acute peaks of activity.

The auriculoventricular conduction rate measured by the PR interval at lead 2 showed an effect within six hours and usually diminished after thirty-six hours. With digitoxin plus carboxymethylcellulose this effect was not pronounced until after twenty-four hours and was still evident at thirty and thirty-six hours.

QRS voltage measured on lead 1 was the same for plain digitoxin and for digitoxin plus carboxymethylcellulose. Increase in voltage became evident at three hours. Somewhat slower onset was noted for digitoxin plus carboxymethylcellulose.

T wave change was evident within three hours for both types of medication. The effect on the ST-T segment was evident at three hours with plain digitoxin and at thirty hours with digitoxin plus carboxymethylcellulose. While changes were similar, a different time of activity was evident for each type of medication. The curve of onset of effect was steeper for T wave changes with plain digitoxin.

Toxic arrhythmia was induced in one case with plain digitoxin. This same case responded without arrhythmia when digitoxin plus carboxymethylcellulose was used.

Two cases showed toxic clinical manifestations with relatively small doses of plain digitoxin. These cases showed evidence of rheumatic heart disease with mitral configuration. Digitoxin plus carboxymethylcellulose did not induce these toxic clinical manifestations until the medication had been given for a much longer period of time (one week later).

Conclusions

The full digitalization dose showed no toxic effect in any case when digitoxin was combined with carboxymethylcellulose. Toxic effects were noted with 1.2 mg. of plain digitoxin. Initial onset of action was more intense when plain digitoxin was used. We concluded on the basis of clinical and electrocardiographic studies that carboxymethylcellulose minimizes the acute toxicity of digitoxin.

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SODIUM CARBOXYMETHYLCELLULOSE; LAXATIVE EFFECTS IN CLINICAL USE

JOHN FITTIPOLDI, M.D., AND PARK LEE DAVIS, M.D., F.A.C.P.

Philadelphia, Pa.

INTRODUCTION

Something like 150 "different" laxatives are offered to the medical profession by ethical pharmaceutical manufacturers. As many, or more, are available to the public as patent medicines. Altogether, it is not impossible that a confirmed bowel-conscious neurasthenic of catholic tastes might take his daily medicine for a year without being forced to open the same bottle twice. Whether this situation is due more to the weakness of the human body or to that of the human mind is a profitless debate. Admittedly, these drugs are used by many who might fare better without them. Yet, every day the physician sees patients whom he can help, whatever the basic cause of their constipation, by prescribing a harmless, effective laxative. They are useful drugs.

Because so much constipation is functional, of nervous or emotional origin, it is bound to be chronic and lead to more or less habitual use of some laxative. Most physicians today are at least resigned to this: as Christian puts it, "The ideal would be to manage constipation without the use of drugs, but human nature being what it is, we cannot hope for this. When drugs are used, they should be as simple as possible."[1]

The trend toward simple "physiologically-acting" laxatives has led physicians to an increasing use of the plant gums. These drugs "lubricate and protect the intestinal mucosa, favor soft consistency of the stool by their water binding power and increase its bulk, which through distention of the intestinal wall stimulates the peristalsis."[2] All these useful effects apparently stem from the hydrophilic properties of the gums.

Since certain synthetic colloids are commonly known to be hydrophilic also, it is not surprising that these chemicals have lately been investigated for laxative use. Such synthetics are said to be superior to the natural agents in several important respects: They are "non-toxic when given orally, are not absorbed by the intestinal mucosa, are not degraded by intestinal enzymes and are non-antigenic. In addition to these obvious advantages over the natural gums, they have a high degree of chemical uniformity."[2] These are important issues in an agent fated for more-or-less habitual use—assuming that the synthetic drug can compare with plant gums in clinical results.

A new colloid of this type*, sodium carboxymethylcellulose, was recently offered us for clinical trial. This substance has a rather interesting history. The acid, carboxymethylcellulose, was produced some 30 years ago, but the soluble sodium salt was not repared until 1937.[3] This salt proved to have exceptional hydrophilic properties which led to its adoption by many industries as a "sizer", emulsifier and stabilizer. It was used in the manufacture of textiles, paper, leather, paint and many other goods. Finally, its possible use as a binder or thickener in pharmaceuticals and foods led to an investigation of its toxicity. It was observed and reported,[4] though only incidentally, that the intestinal contents of the animals fed sodium carboxymethylcellulose were bulky and the feces soft. This laxative effect apparently went unnoticed until recently.

Preliminary laboratory studies[5] on the possible use of sodium carboxymethylcellulose as a laxative showed that it compared favorably in hydrophilic properties with a dozen or so of the most widely used gums and other synthetics. The way sodium carboxymethylcellulose "takes up" water differs completely from that of the gums. They absorb water and swell; the synthetic colloid is merely dissolved, becoming evenly dispersed throughout whatever water is present. In such dilutions as occur in the intestinal tract, it remains a viscid solution rather than a gel - a property claimed to minimize any tendency toward obstructive formation within the gut. All toxicity studies[3, 6, 7, 8, 9] indicate that sodium carboxymethylcellulose is completely harmless, even for long-term use. It is almost completely unabsorbed; acute, chronic and lifetime animal tests produced no gross or microscopic pathology; the colloid is non-antigenic, and non-irritating even to the human vaginal mucosa.[7] In a clinical toxicity study,[8] adminsitration of sodium carboxymethylcellulose for several months produced no change in blood formation, liver and kidney function or blood vitamin C levels; nitrogen balance was not affected.[9] On the basis of this evidence and the promising character of its chemical and physical properties, sodium carboxymethylcellulose seemed to warrant clinical trial. The only question remaining was, "Does it work?"

METHODS

One hundred and twenty-eight patients were used in the test; of these, 94 were bedfast with tuberculosis, hypertension, renal or neurologic disease, while 34 were ambulatory private patients with such conditions as diabetes, anemia or anxiety states and associated constipation. Only 4 patients had any organic disease of the gastrointestinal tract; there was one case each of flatulent dyspepsia, duodenal ulcer, hemorrhoids, and infectious hepatitis.

* Supplied by Smith, Kline and French Laboratories, Philadelphia as 'Eskalose' wafers, each containing 2 gm. sodium carboxymethylcellulose.

Ninety-four of the patients were female, 34 male; ages ranged from 10 to 90 years, with 99 subjects within the 21–60 range.

Sixty of these patients were accustomed to taking a daily laxative; 110 of them took laxatives twice or more times a week. Irritant drugs or quick-acting salines had been regularly used by 77, emollients by 61; only 21 had used bulk drugs previously. (Forty-five of the subjects used a combination of 2 or all 3 types.) The patients were shifted to sodium carboxymethylcellulose in various dosages, depending on the previous laxative habit and history.

RESULTS

Adequate laxation was established in most cases in 1–3 days, usually on 2 wafers daily, each wafer supplying 2 gm. of sodium carboxymethylcellulose. The average daily maintenance dose was 2 wafers, taken morning and night.

	DAILY DOSAGE					
	Number of patients needing					
	1 wafer	2 wafers	3 wafers	4 wafers	5 wafers	6 wafers
To start	5	94	13	4	1	11
For maintenance	23	96	7	1	0	1

Days to Establish Laxation

NO. OF DAYS	1	2	3	4	5	6	7	NOT ESTABLISHED
No. of patients	17	52	27	18	3	7	1	3

The effects of sodium carboxymethylcellulose were regarded as satisfactory in all but 14 cases; 109 patients considered the new drug better than the laxative previously used; 5 described its effectiveness as "about the same." Of the 21 subjects who had used bulk laxatives previously in the form of granules, all preferred the sodium carboxymethylcellulose as supplied, in the form of wafers; and in general, this new form of medication seemed acceptable, since 97 of the group commented favorably on the wafer.

There were no side reactions in 109 patients, but 19 subjects made one or more of the following complaints: cramps, belching, fullness, gas constipation, griping. There were no serious side reactions or evidence of any toxic effect.

SUMMARY AND CONCLUSIONS

1. Sodium carboxymethylcellulose, a new synthetic colloid laxative, has been tested for clinical effectiveness in 128 patients. Results with this "bulk" agent were as good or better than those with formerly used laxatives in 109 of these patients. Most of this group, who were accustomed to irritant or saline

drugs, effected a satisfactory change to the colloid within 1 to 3 days on a dose of two 2 Gm. wafers a day.

2. No side reactions were reported by 109 of the group; in the 19 who had any complaints, the symptoms were of the sort commonly encountered in constipation, and none was of serious import.

3. This synthetic colloid appears to be an effective and safe "bulk" laxative for clinical use.

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DIVISIONE RICERCHE LABORATORI GLAXO S.P.A. - VERONA
Director: *Prof. A. Del Campo*

OBSERVATIONS ON SOME CASES OF ALLERGIC REACTIONS IN ADULT CATTLE FOLLOWING TREATMENT WITH SYNTHETIC CORTICOSTEROIDS

C. FRANCIOSI

(Translation)

It appears that written indications of phenomena of an allergic type, following the therapeutical use of synthetic corticosteroids, are not very frequent. Charmillot (1966), was the first to describe two cases of intense allergic manifestations in two bull-calves treated with prednisolone. Later Signorini (1969), indicated that 16% of 149 bovine cattle, had reacted to inoculation of prednisolone, after treatment with a dose of about 20-40 mg/q, of live weight, showing symptoms of an anaphylactic nature, which became evident within 10-30 minutes after administration of the drug. This author has never found a single case of reaction in calves, and maintains, that at the root of allergic tendencies noted, there is some relation between the particular organic condition of the animals (especially in the form of discorticalism) and the corticosteroid administered, in addition to the not less important factor of the dose injected.

In this respect, after studying the sero-proteinic changes induced in the ox by prednisolone and flumetazone, Ballarini (1969), observed a modification of haemo-proteinic characteristics, more intense and prolonged for prednisolone (48-60 hours), than for flumetazone (24-36 hours), showing an increase of protidemia in the first 36 hours for the former (2nd-12th hour), which is in comparison, during the same period, to a reduction for the latter.

In light of this data and considering the fact that in the blood of bovine cattle, transcortine (α-globulin capable of binding the corticosterone and hydrocortisone) is present in quantity very much inferior, in respect of other animal species; the author has put forward an hypothesis that an overdose, and the haematic modification induced by corticosteroids, may be in some way, the origin of the allergic phenomena reported.

In consideration of the scarsity and infrequent indication of such cases, it is thought that it might be interesting to note the clinical and epidemic characteristics of a series of episodes of an allergic nature, observed in adult bovine cattle, in relation to the administration of corticosteroids. Experimental tests, and carefully planned laboratory research to clarify the aetiological aspects of the phenomena, will also be described.

Description of episodes and hypotheses derived

From the beginning of 1966, a number of cases of allergic reaction in adult bovine cattle, treated with a commercial corticosteroid product containing 10 mg/ml, prednisolone alcohol in suspension, were referred to the Laboratory.

Every case, more or less, showed a constant characteristic of clinical manifestations, consisting of: horripilation, urticaria, sialorrhea, vulvar and palpebral oedema, dyspnoea, tachycardia, and state of excitement. The symptoms usually arose within a period of time varying from about 20 to 90 minutes after administration of the drug. In a number of cases, the animal affected, in a very brief space of time, developed a state of shock, which was fatal for some of them.

However, the majority of the episodes were resolved favourably by the rapid administration of antihistamines, which

was followed in about 20-45 minutes by the almost complete normalization in the condition of the animal.

No cases in calves have been reported.

From research carried out subsequently, it resulted that the manifestations described, accompanied the use of the major part of the corticosteroids available commercially, containing different active constituents, and prepared by different pharmaceutical houses.

An examination of available information has also made it possible to reveal that the episodes presented themselves prevalently in determined areas, whereas, others were completely free from the phenomena. In the zones most hardly hit, the number of cases recorded were in the radius of hundreds (it is not possible to give exact data because of the difficulty in finding precise information), while in the other places they were within numbers, generally considered to be « normal », for phenomena of sensibility to drugs containing antibiotics.

It has also been possible to verify, that in England, where identical pharmaceutical preparation to those which are reputed to be the cause of the episodes in Italy, are used on a large scale, collateral effects in reference to those noted in our cattle, have never been observed.

These effects, however, have already been described, as previously mentioned, by Charmillot in 1966, in Switzerland.

The first comment on these episodes, is the prominence of the strange fact that synthetic corticosteroids, notably endowed with exceptional antiallergic action, are at root of evident phenomena on an allergic nature. Such reactions are also very rare in man, although these preparations are today, used on a very large scale (King, 1960; O'Garra, 1962).

From the beginning, it was also logical to take into consideration, that other ingredients contained in the preparations concerned, might be the origin of the phenomena.

Therefore, attention was given to two principal queries:

— What substance was contained besides the active constituents, in all the preparations used, in the animals attacked by these forms.

— What conditions were common to adult bovine cattle, in the areas of Italy where the episodes had taken place, and Swiss cattle mentioned in written works; but not to cattle in

English breeding establishments, which seemed to be immune, although being treated in the same way.

A systematic analysis of the compositions of the various corticosteroid preparations, made it possible to establish that a large number of them contained a common ingredient, carboxymethylcellulose (CMC), polysaccharide frequently used in pharmaceutical preparations for suspension, as it gives better uniformity and stability to suspension.

In fact, this substance has the capacity to produce immediate phenomena of hypersensitivity. Platt (1966), and others, have, in fact, demonstrated that a glycoprotein containing CMC and bovine sero-albumin (BSA), had a very high antigen power.

In the course of an experiment carried out on mice, the author has observed, that also CMC on its own, has a certain antigen power, such as to be reconciled for structural analogy with polysaccharide pneumococcus type 3, which has a good antigen action. The chief power of immunization of glycoprotein (CMC/BSA) seems to be due to the high molecular weight (670,000), in respect of the relatively low weight of CMC, which is about 40,000. In these conditions, the higher molecular compound weight should be less subject to kidney « clearance ».

With regard to the second query referred to, it seemed essential to discover why cattle in English breeding establishments, were different to those of other European nations, not only for particular genetic characteristics, or for the substantial difference of feeding and environment, but for the well known fact that the cattle are not subjected to vaccination against foot-and-mouth disease, contrary to what is an obbligation in all the other countries.

The various elements recounted up to this point, could also be connected to the fact that CMC is used also for tissue cultivation, for the purpose of obtaining a more homogeneous cellular suspension and a more uniform mono-layer of tissue.

As it is common practice for some institutions which prepare vaccines of an antigen virus base, to cultivate the virus on cell layer; the validity of the hypothesis is evident, that the CMC content in the vaccine against foot-and-mouth disease, which was used to immunize the cattle in the zones where

these episodes took place, may be the allergic element capable of sensitizing the animal to a successive contact with the same substance, this time contained in the corticosteroid product.

Confirmation of the direct responsability of CMC, as the cause of a sensitivity was given by Leeman (1969), and others, who have been able to demonstrate that an increased incidence of cases of anaphylaxis and urticaria caused in adult cattle, after injections of products, above all with a penicillin base, was recorded in Switzerland after the vaccination campaign against foot-and-mouth disease, during the winter of 1966-1967.

These authors have been able to clarify that such cases were not to be attributed to the presence of the antibiotic in the vaccine, as was wrongly thought, at first, but to the presence of CMC in the vaccine, of Italian production, as in the major part of antibiotic products, in suspension or in solution.

Experiments

The methods of research used, for verification of the hypotheses of the aforementioned conclusions, were made in two separate phases.

Experimental approach

As a first phase of approach, it was decided to affront the sensitizing action of two corticosteroid preparations, different from each other, not only for the nature of the active constituent but also for the quality of the suspension elements.

The experiment was carried out on eight cows, belonging to the same farm, chosen at random from the total number of animals found there, which had all been repeatedly vaccinated against foot-and-mouth disease.

The animals were divided into two groups: the first inoculated with 20 ml of suspension of prednisolone alcohol 10 mg/ml, containing 5 mg/ml of CMC; the second inoculated with 10 ml of suspension of betamethasone alcohol 2 mg/ml, containing 4 mg/ml of IEC (hydroxyethyl-cellulose).

The trend of morphological composition of the blood of these patients, was also followed, for several hours after treatment; for the purpose of checking eventual alterations, particularly with regard to lymphocytes: in fact, it is well known that corticosteroids have an evident action on lymphocytes, so it could be reasonably suspected that a rapid and consistent lysis of these elements might be one of the components of clinical condition revealed (especially as the ox is an animal of marked lymphatic constitution).

Blood samples were taken before, and at intervals of half, one, two, four, six, and eight hours after the inoculation of the two mentioned corticosteroids.

The results of the experiment are as follows:

In respect of the morphological examination of the blood; from the graphs illustrating the average values recorded (figures 1 and 2), it may be observed that the alterations induced by the two corticosteroids can be more or less superimposed. In fact, it can be noted that the appearance of the initial stages of lymphocytopenia and neutrophilia are evident both for prednisolone and betamethasone, at about an hour after administration of the drugs, and that the curves maintain a falling course for lymphocytes and rising course for neutrophils, still constant eight hours following the injection.

The clinical aspect, however, reveals that all the animals inoculated with prednisolone (and CMC) showed effects of urticaria and horripilation, both starting from the region of the buttocks, with subsequent rapid diffusion in all the body. This first symptom was followed by intense dyspnoea, sialorrhea, palpebral and vulvar oedema, discharge of fluid faeces intermingled with mucus filaments, polyuria, excited state of the animal with periods of decubitus which alternated with stationary periods, anxious attitude, and rapid backward extension of the hind legs.

The time of development of the four animals was: forty, sixty-five, sixty-seven, and ninety-three minutes after the injection of prednisolone (and CMC) respectively. The allergic reactions were resolved by administration of an antihistamine, and after about thirty to forty-five minutes there was a complete abatement of the symptoms.

No reaction was observed in the four patients treated with betamethasone (and IEC).

From the results of the first experiment, it has been possible to deduce, in the first place, that the allergic phenomena observed were not dependent on the administration of a corticosteroid preparation as such; but they were demonstrated to be connected exclusively with a preparation containing prednisolone (and CMC). In addition, no importance can be attributed to the lysis of lymphocytes, inasmuch as the clinical reactions were verified only in one group of animals, while the lymphocytopenia was equal, if not superior (at least within the first ninety minutes) in the animals in which there was no reaction; also, the animals reacted in a period of time in which the lymphocyte values were very near, if not within, the limits of normal values established by Osbaldiston (1968).

Pathogenic experiment

A second experiment was conducted on forty cows, belonging, as in the previous test, to a farm situated in an area where a high number of cases of this kind had been verified in the past.

The animals were divided into five groups; four groups of five animals, and the fifth of twenty animals. The animals of the first three groups were given an intradermic inoculation with a tuberculin syringe, with various combinations of the substances contained in the two corticosteroid preparations mentioned in the previous experiment (prednisolone - CMC - Suspension elements: betamethasone - IEC - Suspension elements) according to the scheme outlined in table 1; thickness of skin in the area of inoculation, was taken previously by means of a cutimeter.

The inoculation area was the sides of the neck and the quantity injected was 0.1 ml. The injections on the side of the neck were spaced at 20 cm from one another.

All the animals treated were observed for the following eight hours and controlled again after twenty-four hours.

None of the animals presented signs of reaction to the intradermic test, contrary to those obtained by Leeman, who, under similar conditions and with doses equal to 0.001 mg of CMC, has obtained, in sensitized animals, lively local reactions.

The negative reaction could be connected, as also referred to by the abovementioned author, to the type of CMC used in this experiment, since it is confirmed that the quality of the substance could be an effective determinant in the results of a skin test. It must also be remembered that the breed of the animals could be a factor capable of influencing the intensity of percutaneous allergic reaction.

To provide a more precise picture of the clinical manifestations under observation, two other groups of animals were taken into consideration (group four and group five), which were administered i.m., with the preparations specified in table, which also shows the results of the experiment.

As it can be seen, following inoculation, one of the two patients treated with 50 mg of CMC, reacted after forty minutes, and the only one treated with 100 mg of CMC, after eighty-five minutes. The symptomatology was identical to that described during the first test (photo 1). Also in this instance, the administration of an antihistamine provoked a resolution after about forty-five minutes.

Not one of the twenty animals inoculated with IEC showed signs of reaction.

Further laboratory verifications

Several checks were made in the laboratory, on sera furnished by veterinary practitioners, taken from animals that had reacted to treatment with various corticosteroid products, apart from these, also on sera (taken before and after the provoked allergic reaction), obtained from the animals which underwent the previously described experiments.

Verifications included the following tests:

Passive cutaneous anaphylaxis upon a guinea-pig: 0.1 ml of serum and its geometrical dilutions in solution buffer phosphate ph 7.2, were administered by intradermic injections. After a latent period of two hours an intravenous injection of 1 ml of solution to 0.5% of CMC and 0.5 ml of solution to 1% Evans Blue, was effected.

Bi-dimensional immune diffusion according to Ouchterlony: carried out on ionagar L 12 with multiple matrices, central wells and fissure. In the fissure solutions to 0.5% and 1% of CMC, in the wells the sera under examination. Diffusion at +4°C for six days.

Passive haemoagglutination with stabilized erythrocytes of sheep: stabilization with $CrCl_3$ and CMC, applying the method described by Baker for pneumococcal polysaccharides. Microscopical reading of reaction.

All the tests had a negative result, with the exception of a sligh agglutination observed in the third, insufficient, however, to be considered specific.

Conclusions

These studies show that clinical phenomena of allergic type may be induced by preparations with a basic combination of corticosteroids, but these phenomena are not connected with the administration of active constituents but rather to a particular suspension element, CMC.

The epidemiologic data recorded give evidence of the close relationship between the allergic phenomena of CMC and previous vaccination against foot-and-mouth disease. Further research has revealed, in fact, that cases of immediate hypersensitiveness to CMC were observed only in regions in which the cattle had been immunized against foot-and-mouth disease with vaccine containing CMC.

It is not possible to produce any pathological phenomenon experimentally, by the use of preparations containing synthetic corticosteroids together with adjuvants different from CMC.

In view of the negative results of laboratory tests carried out to date, a decision has been made to commence a study for the perfection of a technical process, capable of revealing with maximum detail, the presence of anti-CMC antibodies, in order to be able to discover their chemical immunological characteristics.

It is highly probable that the function of CMC in the process of sensitization is of haptenic nature. If the experiments produce satisfactory results, it will be interesting to be able

to specify the protein fraction present in the vaccine against foot-and-mouth disease, which has the quality of combining itself with CMC, transforming it into a complete antigen.

Unfortunately the experiments on adult bovine cattle, animals in which the clinical phenomena reported, were the origin of this article, will not be possible; as noted in data already given, animals not vaccinated against foot-and-mouth disease are not available.

This research, nevertheless, has a certain interest, as the use of CMC and suspension conjugates, and similar tensioactive substances, is becoming more and more diffused, not only in the pharmaceutical but also in the chemical and food industries. It si therefore opportune to study the aetiopathogenetic mechanism by which such substances may induce diseases. In case of specific confirmation it would be desirable that substances like CMC are not used in the preparation of vaccines, as the presence of adjuvants and absorbents in them, aid the easy development of conditions most favourable to a process of sensitization, through local tissue major reaction and an elution probably slowed down and prolonged by substances with allergic powers.

All this is for the purpose of avoiding situations of extreme gravity; today, limited only to animals, but in the future may also involve human beings.

SUMMARY

Cases of immediate hypersensitivity in adult cattle treated with synthetic corticosteroids are reported. From *epidemiologic* observations and experiments it has been possible to exclude the fact that such reactions are caused by constituents. The comparison of two synthetic corticosteroids (prednisolone and betamethasone) enabled it also to be ascertained that slight blood cells changes, following the injection of the drugs, can in no way cause the reactions reported.

Results showed that allergic reactions were due to a *polysaccharide* (Carboxymethylcellulose - CMC) used in most commercial corticosteroid preparations in order to stabilize suspensions. Cattle experimentally injected i.m. with solutions of CMC reacted showing clinical signs comparable to those observed in the field. This substance, since it is present in foot-and-mouth disease vaccines, can be a sensibilizing factor to pharmaceutical products which contain CMC for all vaccinated animals.

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Studies on the Gastro-intestinal Absorption of Purified Sodium Carboxymethylcellulose

J. P. FRAWLEY, A. K. WIEBE* and E. D. KLUG
Hercules Powder Company, Wilmington, Delaware, USA
and
M. V. SHELANSKI
Industrial Biology Laboratories, Inc., Philadelphia, Pennsylvania, USA

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Abstract—Previous reports in the literature have established, within analytical error, that purified sodium carboxymethylcellulose, of a specified viscosity and degree of substitution (commonly called cellulose gum) is recovered quantitatively in the faeces following oral administration. The question remained whether traces of cellulose gum, below the sensitivity of usual chemical procedures, might be absorbed from the gastro-intestinal tract. Radioactive [^{14}C] cellulose gum, containing up to 0·34% radioactive sodium glycollate, was administered orally to rats and the liver, kidney and urine were then examined for radioactivity. No detectable activity (<0·02% of administered dose) was found in the livers and kidneys. An average of 0·14% of the administered radioactivity was found in the urine. Paper chromatography established that this activity was not due to absorption and excretion of cellulose gum. The urinary radioactivity was totally accountable by the free radioactive sodium glycollate present in the samples.

The studies confirm that no systemic effects can result from oral administration of sodium carboxymethylcellulose which is manufactured with specified purity, viscosity, and substitution limitations.

INTRODUCTION

Sodium carboxymethylcellulose is a generic name for a series of ethers obtained by the reaction of cellulose with varying amounts of sodium monochloroacetate. Individual ethers, in this series differ in two respects: (1) the average molecular weight of the cellulose chain and (2) the degree of carboxymethyl substitution. Purity may vary depending on removal of reaction salts (sodium chloride and sodium glycollate), and low molecular weight degradation products.

In some countries, the term "cellulose gum" has been accepted as the usual name for a special food type sodium carboxymethylcellulose, which has a specific range of carboxymethyl substitution and molecular weight. It refers only to a product that has been purified to remove all significant residues of salts and degradation products. Cellulose gum is defined as "the sodium salt of carboxymethylcellulose not less than 99·5 percent on a dry-weight basis, with maximum substitution of 0·95 carboxymethyl groups per anhydroglucose unit, and with a minimum viscosity of 25 centipoises for 2 percent by weight aqueous solution at 25°C." (United States Food and Drug Regulations). The investigations reported herein were conducted on sodium carboxymethylcellulose whose composition meets the above definition of cellulose gum.

Cellulose gum is used widely by the food industry to stabilize the texture of puddings, frozen dairy products, pie fillings, cheese spreads and other food products. It is also used as

*Present Address: River Outarde Road, Ormstown, Quebec, Canada.

a non-irritating bulk laxative by the pharmaceutical industry. Several toxicological investigations have been reported on cellulose gum, including 2-yr chronic and 3-generation reproduction studies, which demonstrate an extremely low order of toxicity and suggest lack of gastro-intestinal absorption. (Rowe, Spencer, Adam & Irish, 1945; Shelanski & Clark, 1948; Brick, 1952). Nevertheless, some doubt has remained whether any cellulose gum can be absorbed during ingestion. The current investigation was designed to resolve this question.

METHODS

Previous attempts have been made to evaluate whether various types of sodium carboxymethylcellulose are absorbed from the intestinal tract. (Shelanski & Clark, 1948; Zeigelmeyer, Columbus, Klausch & Wieske, 1951). These attempts were hampered by the lack of a sensitive analytical method to detect traces of excretion in the urine. Nevertheless, by conventional chemical methods, these investigators recovered from 90–100% of the administered cellulose derivative in the faeces.

The current investigations were based on the incorporation of ^{14}C into the carboxymethyl group of cellulose gum and the examination of urine, kidneys and liver for any traces of the radioactive material after administration to rats. No attempt was made to recover radioactive materials in the faeces, since previous investigators had shown essentially quantitative recovery with conventional chemical analysis.

Preparation of radioactive cellulose gum. Cellulose gum is manufactured by converting cellulose to alkali cellulose and reacting an excess of alkali cellulose with controlled amounts of sodium monochloroacetate according to the following reaction:

$$RONa + 2\,ClCH_2COONa + NaOH \longrightarrow ROCH_2COONa + 2\,NaCl + CH_2OHCOONa$$

where R represents the cellulose structure. Repeated washing of the reaction products reduces the salts content of cellulose gum below 0·5%.

Using sodium monochloroacetate containing ^{14}C in the carbonyl group, two samples of radioactive cellulose gum were manufactured under the same conditions of reaction and washing as the commercial product. The two samples A and B were prepared from two samples of cellulose and were reacted to slightly different degrees of substitution. Table 1 provides a description of the properties of these samples and a non-radioactive control sample (C), including the activity per unit weight.

Table 1. *Characteristics of cellulose gum samples*

Cellulose gum sample	Degree of CM substitution*	Viscosity† (cP)	Radioactivity (μC/g)	Unreacted radioactivity (%)
A	0·80	300	2·0	0·34
B	0·72	450	1·9	0·30
C	0·77	600	0·0	—

*Carboxymethyl groups per anhydroglucose unit.
†Viscosity of a 2% aqueous solution at 25° C.

Determination of unreacted radioactivity. In the reaction described above, some radioactive sodium glycollate is formed. Commercial washing techniques remove all but approximately 0·2% of the free glycollate. Additional purification beyond commercial procedures was not performed on the radioactive sample because it was felt that these techniques might remove fractions of the cellulose gum and leave a radioactive sample which would not be

typical of the commercial product. Therefore, it was necessary to pre-determine the exact amount of readily absorbable radioactive sodium glycollate in these samples.

This was accomplished by dialyzing a 0·2% aqueous solution of the radioactive cellulose gum against a similar solution of non-radioactive material. The cellulose dialysis tubing had an average pore size of 48 Å and the equal concentration of cellulose gum on both sides of the membrane prevented development of an osmotic pressure differential. The rate of diffusion of the glycollate was rapid and equilibrium was reached within 48 hr for both radioactive samples. The free glycollate content of the two samples was determined to be 0·34% and 0·30% as shown in Table 1.

Administration to rats. Three groups of 5 male albino rats weighing 250–275 g each were used. Each animal was administered 0·40 g of cellulose gum contained in 18 ml of aqueous solution. The total volume was delivered to each rat in a series of stomach tube administrations within a 2-hr period. Two groups were administered the radioactive samples of cellulose gum and the third group received non-radioactive cellulose gum. The dose administered corresponded to a dose approximately 100 times the maximum daily dose likely to be ingested by man.

Each animal was restrained on a wire rack to prevent any movement of the posterior portion of the body. A 35 ml, wide-mouthed jar was placed in direct contact with the belly so that urine could be collected without contamination by faeces. Urine receptacles were replaced at the end of 24 hr, except when samples were large, in which case receptacles were replaced at the end of 12 hr. Urine was collected for 48 hr after which the rats were sacrificed for removal of their liver and kidneys.

Radioactive counting. Radioactive measurements were made with a windowless flow counter on infinitely thick samples (>27 mg/cm^2) contained in stainless steel planchets of 4·5 cm^2 cross section. The control urine samples were pooled and evaporated to dryness in an oven at 70°C. The urine samples from rats fed with radioactive cellulose gum were individually dried and the weights recorded prior to radioassay. The kidneys and livers from each group of 5 animals were pooled, ground, and dried for radioassay.

Chemical analysis of urine for cellulose derivatives. Urine solids were analyzed for cellulose materials by acid hydrolysis and paper chromatography. The digestion techniques involved a room temperature hydrolysis for 4 hr with 50% sulphuric acid, followed by 6 hr reflux in 5% sulphuric acid. The solution was then neutralized with $BaCO_3$, filtered, and evaporated to dryness. Paper chromatograms of the residue and pyridine extracts of the residue were developed using the chromatographic procedure of Partridge (1948), the water–acetic acid–ethyl acetate solvent [3:1:3 (v/v)] system of Jermyn & Isherwood (1949), and the aniline hydrogen phthalate spray reagent of Partridge (1949). This procedure will detect 5–10 μg of glucose or other aldosugars.

RESULTS

Liver and kidney. Table 2 gives the radioactivity detected in the pooled liver and kidney samples from the three groups of rats. The table also shows the limit of detection for each of the samples, expressed as a percentage of the administered dose, and as ppm of tissue. The data reveal no indication of any absorption or storage of radioactive cellulose gum.

Urine. The radioactivity found in the urine solids is reported in Table 3. During the collection period for the first 12 hr, no detectable radioactivity (<0·01% of the administered dose) was found. However, during the 12–24 and 24–48 hr collection periods, measurable activity was found in both samples. Considerable variation was noted between animals

Table 2. *Radioactivity in liver and kidney*

Cellulose gum sample	Activity of sample (counts/min)*	Total dose† (g)	Tissue	Dry weight (g)	Activity‡ of tissue (counts/min)	Level (ppm)	Percentage of administered dose
A	38,200	2·0	Liver	4·7	15·6±0·9	<0·05	<0·018
			Kidney	1·7	17·5±1·0	<0·08	<0·007
B	36,500	2·0	Liver	5·4	15·8±0·9	<0·08	<0·021
			Kidney	1·1	16·1±0·9	<0·07	<0·004
C	0	2·0	Liver	—	17·0±1·0	—	—
			Kidney	—	15·9±0·9	—	—

*Counts/min of infinitely thick sample
†Dose given to each group of 5 rats.
‡Mean counts/min±95% confidence limits.

for the rate of excretion, but since many of these levels of activities were only slightly above background (36±5 counts/min), the individual values are subject to considerable error. Nevertheless, the values unquestionably establish that only a fraction of a percentage of the administered radioactivity is excreted in the urine. The mean values for the two samples for the total 48-hr collections are 0·17 and 0·10% of the administered dose.

Table 3. *Excretion of radioactivity in urine*

Cellulose gum sample	Activity of sample (counts/min)*	Dosage (g)	Rat number	Urinary activity† (net counts/min) x g			Percentage of administered radioactivity in urine
				0–12	12–24	24–48	
A	38,200	0·4	1		15·9	<0·1	0·10
		0·4	2		5·4	2·2	0·05
		0·4	3	<0·1	10·6	34·8	0·30
		0·4	4	<0·1			
		0·4	5	<0·1	7·3‡	24·0‡	0·21
							Mean 0·17
B	36,500	0·4	6		13·1	8·7	0·15
		0·4	7		5·7	2·6	0·06
		0·4	8		6·3	6·0	0·08
		0·4	9		2·3	14·0	0·11
	0	0·4	10	<0·1	8·8	3·0	0·08
							Mean 0·10

*Counts/min infinitely thick sample.
†Net counts/min above control urine multiplied by weight of urine solids.
‡Sample contaminated with faeces.

Since Sample A contained 0·34% and Sample B contained 0·30% radioactive sodium glycollate, it was expected that some portion of this amount of radioactivity would be absorbed from the gastro-intestinal tract and excreted in the urine. The levels of radioactivity found in the urine were totally consistent with the amount of free radioactive sodium glycollate, even if no radioactive cellulose gum had been absorbed and excreted.

In order to determine whether any of this urinary radioactivity was due to absorption of low molecular weight cellulose gum, several of the samples containing the highest radioactivity were analyzed for cellulose and cellulose derivatives. The urine solids were digested to hydrolyze the cellulose to glucose and the residues analyzed by paper chromatography. No sugars were detected in any of the samples. The method was sensitive enough to give a positive reaction if 15% or more of the radioactivity had been due to low molecular weight cellulose derivatives.

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Etudes sur l'Absorption Gastro-intestinale de Carboxyméthylcellulose de Sodium

Résumé—De précédentes études dans la littérature ont établi, compte-tenu des erreurs d'analyse possibles, qu'on retrouve quantitativement dans les fèces, après administration orale, le carboxyméthylcellulose de sodium purifié, de viscosité et degré de substitution donnés (généralement appelé gomme de cellulose). La question restait en suspens de savoir s'il se pourrait que des traces de gomme de cellulose, au-dessous de la dose sensible aux procédés de l'analyse chimique courante, fussent absorbées par le tractus gastro-intestinal. De la gomme de cellulose radioactive (^{14}C), contenant jusqu'à 0,34% de glycolate de sodium radioactif, fut administrée oralement à des rats, dont le foie, les reins et l'urine furent examinés au point de vue de la radioactivité. On ne détecta aucune radioactivité (<0,02% de la dose administrée) dans le foie et les reins. On retrouva en moyenne 0,14% de la radioactivité administrée dans l'urine. La chromatographie sur papier établit que cette radioactivité n'était pas due à l'absorption et à l'excrétion de la gomme de cellulose. La radioactivité urinaire devait être mise toute entière sur le compte du glycolate de sodium libre présent dans les échantillons.

Ces études confirment qu'aucun effet nocif sur l'organisme ne peut résulter de l'administration orale de carboxyméthylcellulose de sodium, à condition qu'il soit fabriqué dans des limites données concernant sa pureté, sa viscosité et ses possibilités de substitution.

Untersuchungen über die Gastrointestinale Resorption von Gereinigter Natriumcarboxymethylcellulose vom Magen–Darm-Kanal aus

Zusammenfassung—Es wurde in der Literatur bereits darüber berichtet, dass innerhalb analytischer Fehlergrenzen-gereinigte Natrium-carboxymethylcellulose einer bestimmten Viskosität und Substitutionsgrad (gewöhnlich als Zellulosegummi bezeichnet) nach oraler Eingabe quantitativ wieder in den Faeces gefunden wird. Es bleibt nun die Frage offen, ob Spuren von Zellulosegummi unterhalb der Nachweisgrenze gebräuchlicher chemischer Verfahren doch vom Gastrointestinaltrakt aus resorbiert werden. ^{14}C markierter Zellulosegummi mit einem Gehalt bis zu 0,3% radioaktivem Natrium-glykolat wurde an Ratten per os verabfolgt. In Leber und Nieren wurde keine nachweisbare Radioaktivität festgestellt (< als 0,02% der zugeführten Menge). Durchschnittlich 0,14% der eingegebenen Radioaktivität wurden im Harn wieder gefunden. Durch Papierchromatographie wurde nachgewiesen, dass diese Aktivität nicht eine Folge der Resorption oder Ausscheidung von Zellulosegummi war. Die im Harn festgestellte Radioaktivität kam einzig und allein von dem ungebundenen radioaktiven Natriumglykolat, das sich noch in den Proben befand.

Die Untersuchungen bestätigen, dass die orale Verabreichung von Natrium-Carboxymethylzellulose, bei deren Herstellung auf eine bestimmte Reinheit, Viskosität und Polimerisationsgrad geachtet wurde, keine Wirkungen auf den Organismus haben kann.

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Determination of Carboxymethylcellulose with Chromotropic Acid

HORACE D. GRAHAM[1]
University of Puerto Rico, Chemistry Department, Mayaguez 00708

Abstract

Chromotropic acid (1,8-dihydroxynaphthanene-3,6-disulfonic acid) in concentrated sulfuric acid was used to determine carboxymethylcellulose (CMC) isolated from milk, chocolate milk, chocolate and vanilla ice cream, and other products. For maximum color development, the reagent must be dissolved in concentrated H_2SO_4 and the CMC—reagent mixture must be heated for 60 to 90 min at 99 ± 1 C. The characteristic purple color developed has maximum absorbance at 570 nm. Color density increased as the degree of substitution of the CMC increased. Of 30 different food gums and related substances tested, only pectin and pectic acid gave the characteristic purple color. The reagent is more specific but less sensitive than the 2,7-naphthalenediol reagent. Interference from chocolate ingredients is less than when the 2,7-naphthalenediol and anthrone reagents are used and much less than with the phenol-H_2SO_4 reagent. In addition, determination is more rapid than with 2,7-naphthalenediol. Recoveries from various products were 88% and upwards, based on the addition of CMC added to water and carried through the entire and identical isolation procedure. The method will detect CMC in food products at 0.025% (w/w).

Introduction

Several reagents have been recommended for the colorimetric determination of carboxymethylcellulose (CMC) after its isolation from products of various sorts and complexities. The best known of these reagents are anthrone (1), phenol-H_2SO_4 (12) and 2,7-naphthalenediol (6, 13, 18). Of these, the latter seems to be preferred because of its high sensitivity, good specificity and stability. The anthrone reagent is unstable. The phenol-H_2SO_4 reagent, though very simple, stable and fairly sensitive, is highly nonspecific and is responsive to interference in chocolate products. Other methods of assay of CMC involve its precipitation as the copper (3) or aluminium (2) salts and determination of the metal content of the precipitate. Copper in the precipitate may be determined by iodometric titration (3) or by measuring absorbance of the copper-amine complex at 590 to 600 nm (15). When the hydrocolloid is separated as the uranyl salt, the uranium is determined gravimetrically (13) or by a radiometric finish as mentioned by (15).

Graham (10) developed a clean-up process to enable improved determination of CMC in the presence of other food gums. Here the CMC was determined by the 2,7-naphthalenediol method which requires a rather extended heating of 3 hr for maximum color development. Interference from chocolate products was less than in the phenol-H_2SO_4 method but was bothersome at times.

Carboxymethylcellulose, when heated in concentrated sulfuric acid, will yield formaldehyde. Chromotropic acid, (1,8-dihydroxynaphthalene-3,6-disulfonic acid), has been recognized for a long time as a very specific and sensitive reagent for the detection and determination of formaldehyde in air and in various and sundry places and compounds (5, 16, 17). However, as far as is known, it has never been used to assay CMC. Since preliminary experiments indicated that reagent blanks from chocolate products gave comparatively low absorbance with this reagent, a thorough study was made of its use for the colorimetric determination of CMC in milk and milk products. This report indicates its usefulness for such an assay.

Experimental Procedures

Materials. The hydrocolloids used, their preparation and characterization, as well as the analytical procedures for the characterization of the products have been described (8). Acetate buffer was prepared as described (9) except that the pH was 5.5 instead of 6.5.

The chromotropic acid reagent consisted of 150 mg of chromotropic acid per 100 ml of concentrated H_2SO_4. Chromotropic acid was obtained from K and K Laboratories, New York City.

The anthrone reagent consisted of 0.2 g of anthrone in 100 ml of concentrated H_2SO_4.

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[1]Address until June 30, 1972: Department of Dairy & Food Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

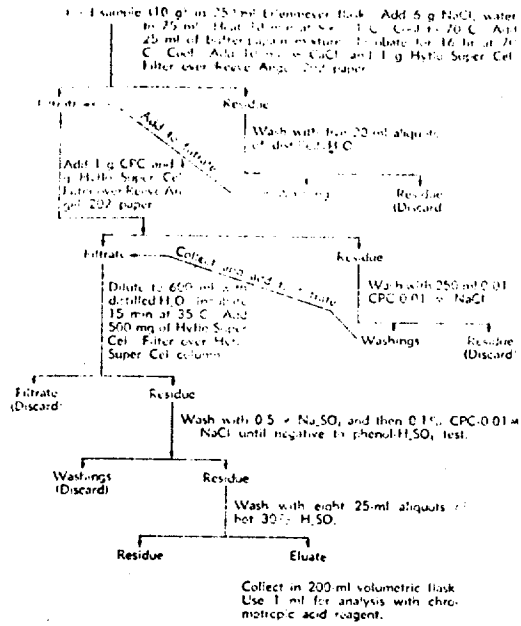

FIG. 1. Flow sheet for the separation and determination of carboxymethylcellulose by chromotropic acid method.

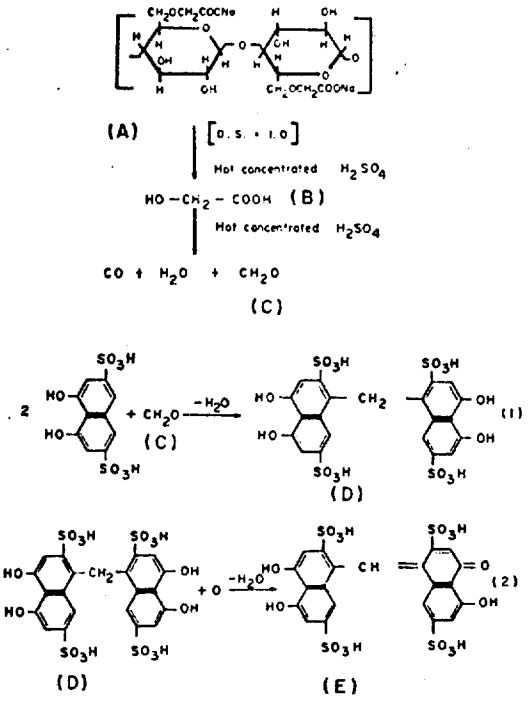

FIG. 2. Mechanism of color production in reaction of carboxymethylcellulose with chromotropic acid—H_2SO_4 (5).

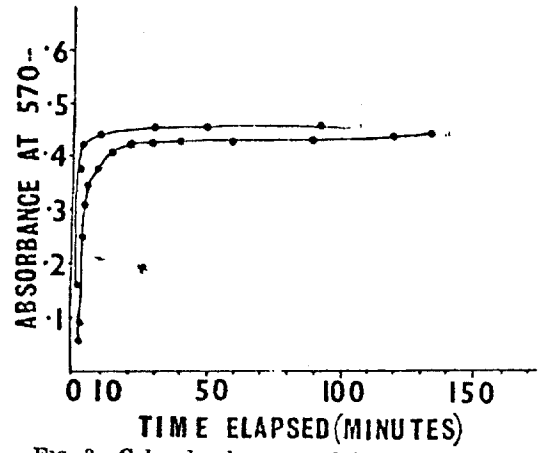

FIG. 3. Color development of formaldehyde and sodium glycolate with chromotropic acid.
I—Formaldehyde (no heat applied).
II—Glycolate (heated for varying time periods).

The anthrone was recrystallized three times from benzene. The reagent was prepared daily and was never used, if older than 24 hr.

The phenol-H_2SO_4 reagent (4) was used as described (7).

Formaldehyde (36.7% formaldehyde) was from Baker Chemical Co. Sodium glycolate was from City Chemical Corporation.

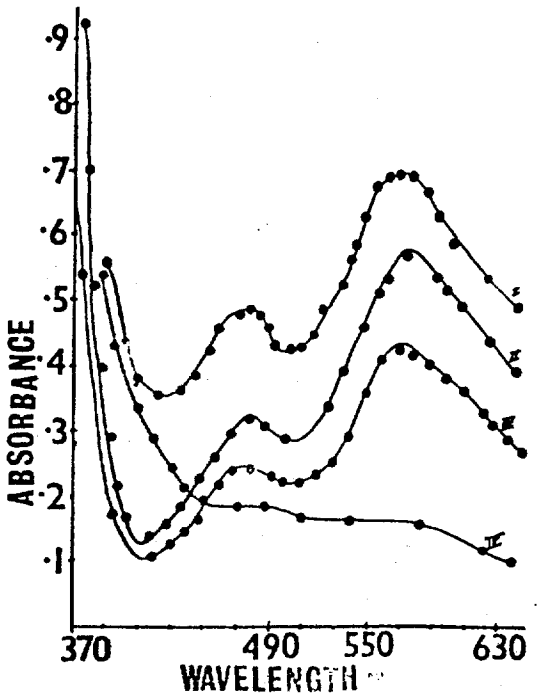

FIG. 4. Absorption spectra of colors produced by carboxymethylcellulose, glycolate, formaldehyde and reagent blank when heated with chromotropic acid—H_2SO_4. I—Carboxymethylcellulose, II—Glycolate, III—Formaldehyde, IV—Reagent blank.

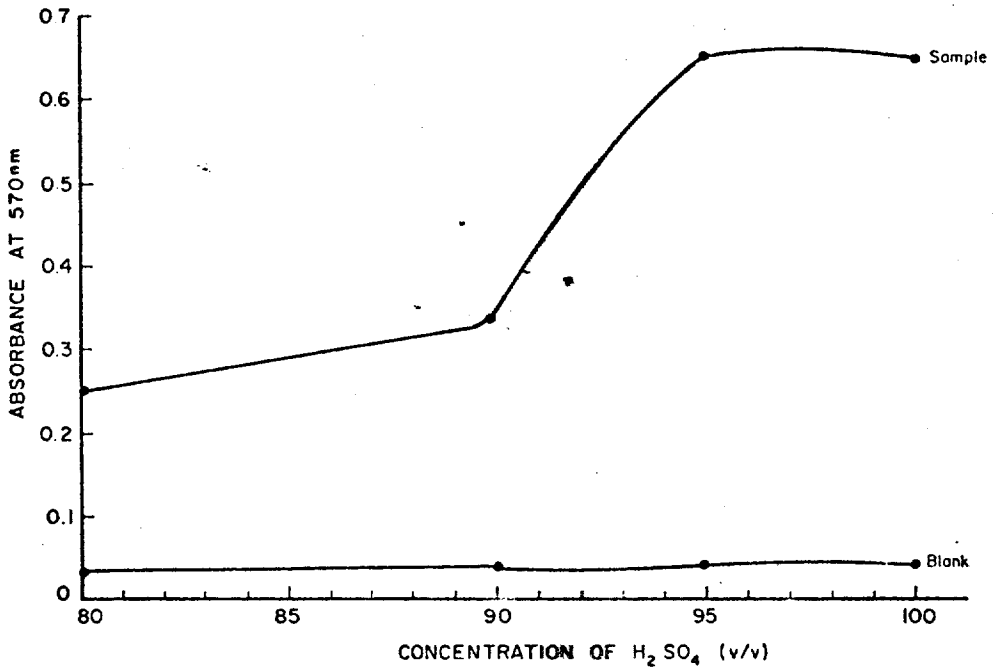

FIG. 5. Influence of concentration of H_2SO_4 (v/v) in the chromotropic acid reagent used for color development.

Cetyl pyridium chloride (CPC) was from Fine Organic Chemicals Co., Philadelphia. Prepared as outlined (7, 10).

Equipment. A Bausch and Lomb Spectronic 20 Colorimeter and 25 × 300 mm test tubes with metal caps, were used.

Procedure. Digest samples of the food products (5 to 25 g) with papain to degrade the proteins and to release any of the CMC bound to proteins. Precipitate alginate and pectic acid as the insoluble calcium salts. Filter these off, along with the protein degradation prod-

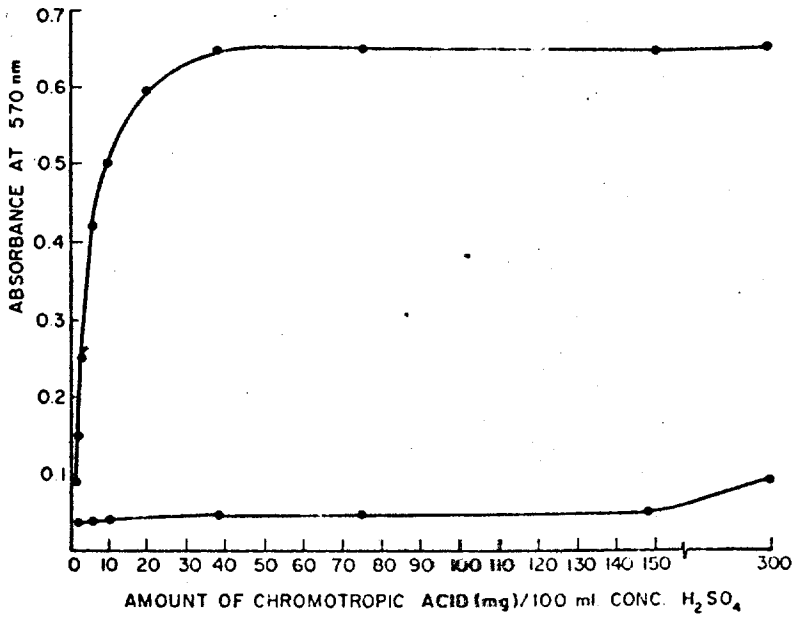

FIG. 6. Influence of amount of chromotropic acid (mg/100 ml of conc H_2SO_4) in the chromotropic acid reagent used for color development.

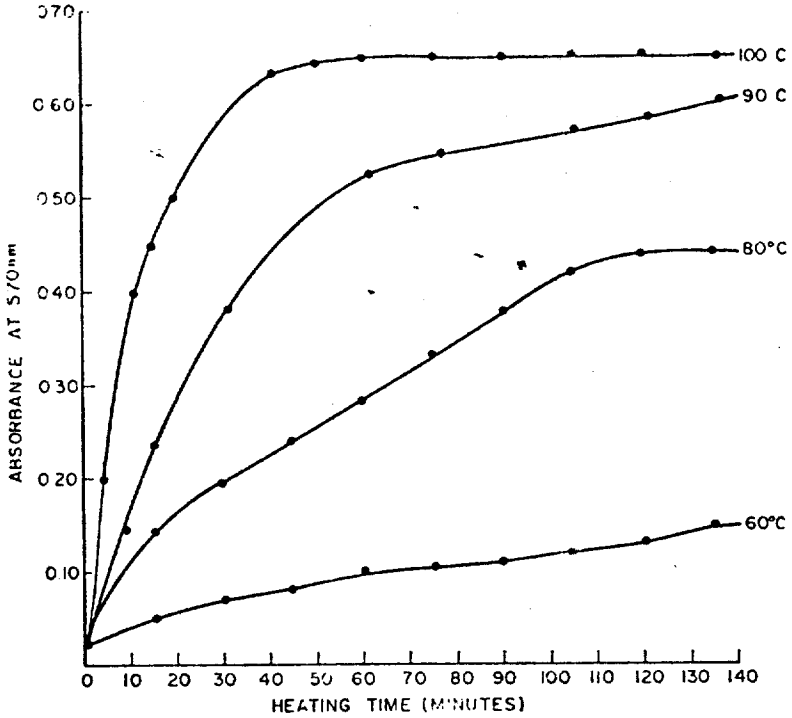

FIG. 7. Influence of time and temperature of heating on the density of the color developed by the carboxymethylcellulose-chromotropic acid mixture.

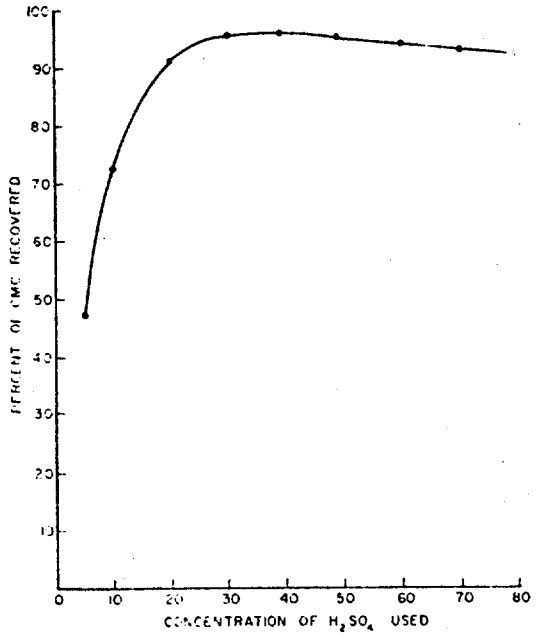

FIG. 8. Influence of strength of H_2SO_4 used to disperse the carboxymethylcellulose-cetylpyridinium complex.

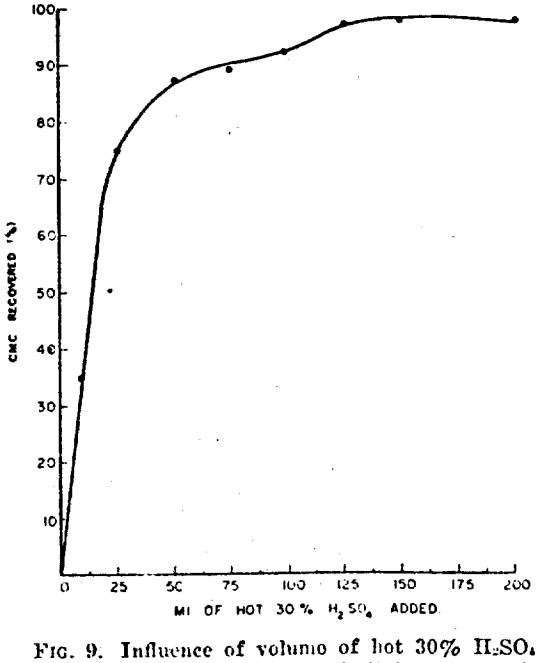

FIG. 9. Influence of volume of hot 30% H_2SO_4 used to disperse carboxymethylcellulose-cetylpyridinium complex.

ucts. Remove carrageenan and other sulfated polysaccharides as the insoluble cetylpyridinium complex in 0.5 M NaCl and filter to remove the precipitate. Then precipitate the CMC by cetylpyridinium chloride (CPC) in 0.20 M NaCl. Collect the CMC-CP complex on a Hyflo Super Cel column, wash it with 0.5 M Na_2SO_4 and then with 0.1% CPC-0.01 M NaCl until the washings are negative to the phenol-H_2SO_4 test. Disperse the complex with hot 30% H_2SO_4 and collect 200 ml of the acidic eluate. Place 1 ml of this eluate in a 25 × 300 ml borosilicate test tube. Add 9 ml of the chromotropic acid reagent and heat the mixture for 90 min in a boiling water bath (99 ± 1 C). Cool mixture for 5 min in tap water and measure the color developed at 570 nm.

Results and Discussion

Details for the isolation and quantitative determination of sodium carboxymethylcellulose are shown in Figure 1.

The mechanism of the reaction is as follows: Carboxymethylcellulose (A) is degraded by hot concentrated sulfuric acid to produce glycolic acid (B). Glycolic acid is degraded further by the hot acid to produce formaldehyde (C) which then condenses with chromotropic acid. The condensation product (D), in the hot acid, is transformed to its *para*-quinoidal form (E), which produces the typical purple color which has maximum absorption at 570 nm (5). The sequence of reactions is in Figure 2.

Formaldehyde develops color with chromotropic acid without heat. Maximum color density was attained within 10 min after the addition of the chromotropic acid (Fig. 3). On the other hand, glycolate had to be heated to produce the maximum color. At 100 C, this was achieved after 20 min. Since CMC, too, needed heating to produce color, this helps to confirm that degradation to formaldehyde is necessary for color development with chromotropic acid. In addition, the similarities in the absorption spectra of these compounds (Fig. 4) substantiates the claim that the production of formaldehyde and its condensation with chromotropic acid is responsible for the purple color formed.

Variations in color intensity with heating time and the temperature of heating, the concentration of chromotropic acid in the mixture, the concentration of acid in which the chromotropic acid was dissolved, the degree of substitution of the carboxymethylcellulose, as well as other variables were studied.

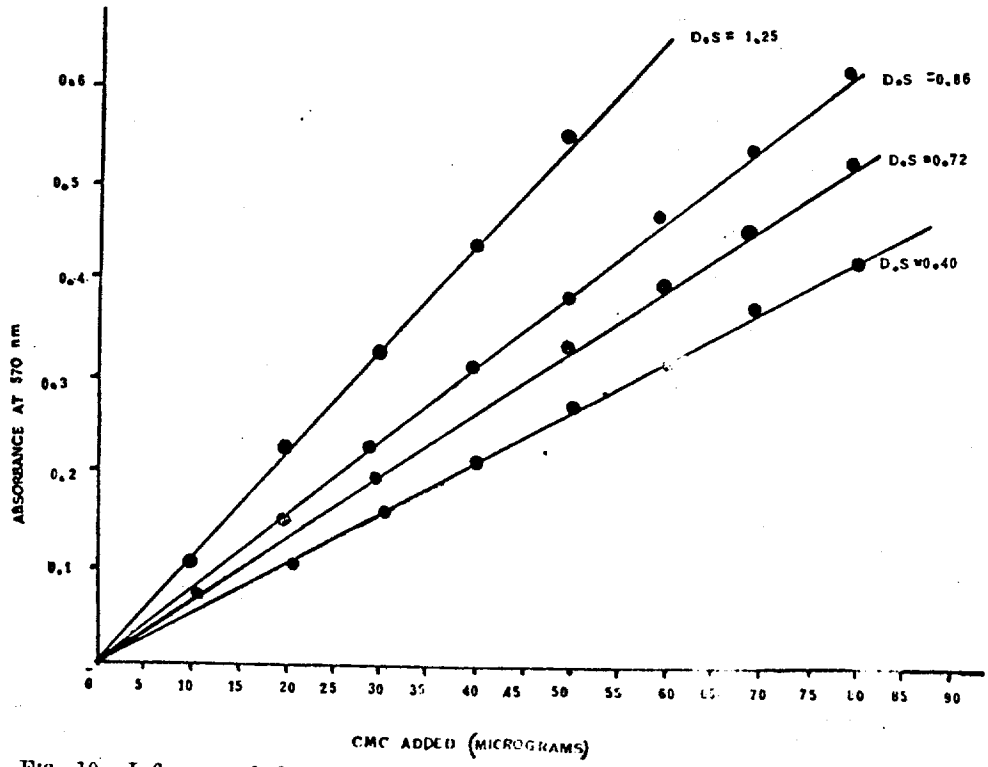

FIG. 10. Influence of degree of substitution (DS) of carboxymethylcellulose on color intensity.

To obtain maximum color, a minimum of 95% (v/v) H_2SO_4 must be used to prepare the chromotropic acid reagent (Fig. 5). Concentrations below 75% (v/v) are not practical since the reagent is not readily soluble at this level of acid. Concentrated H_2SO_4 (sp gr 1.84), 98.8% was always used.

At 50 μg of CMC per reaction tube, the chromotropic acid reagent should contain a minimum of 50 mg of chromotropic acid per 100 ml of concentrated H_2SO_4 to ensure maximum color development (Fig. 6). Beyond 150 mg/100 ml of concentrated H_2SO_4, color of the reagent blank increased. A level of 150 mg/100 ml of concentrated H_2SO_4 was always used.

Color development varied greatly with the temperature of heating and with the duration of heating at any particular temperature (Fig. 7). Below 60 C, hardly any color developed even after heating for 3 hr. At 90 C maximum color was not achieved even after 140 min. However, at 100 C, maximum color occurred after 60 min. To ensure a safety factor, heating 90 min at 99 $\pm$1 C was used throughout these investigations.

The CMC-CPC precipitate, trapped on the Hyflo Super Cel column, could not be eluted by salts such as Na_2SO_4 or $MgCl_2$, although in test tube trials the precipitate dissolved readily in 0.3 M or greater of these salts. Cold 60 to 80% H_2SO_4, when added to the column, will disperse the precipitate and elute the CMC. However, intense heating results when the acid is added to the wet column and the eluate will be colored, from the formation of decomposition products of CMC in the hot acid. Hot acid is beset with this same problem. For best results, 30 to 40% hot H_2SO_4 should be used to disperse the precipitate and elute the CMC (Fig. 8). Higher concentrations may be used but there is danger of using such hot acid, making pipetting difficult. Beyond a concentration of 70% (v/v) recovery decreased, probably from degradation of the CMC with concomitant loss of formaldehyde and probably from errors in pipetting such a viscous mixture. A level of 30% (v/v) was used.

A volume of 125 ml of hot 30% (v/v) H_2SO_4 was sufficient to completely disperse and elute the CMC (Fig. 9). However, to ensure maximum recovery at all levels of CMC, 200 ml were used.

Color density varied with the degree of substitution (DS) of the CMC (Fig. 10). This is readily explicable since degree of substitution is defined as "The average number of carboxymethyl groups that are substituted per anhydroglucose unit". If all three hydroxyl groups are substituted in each anhydroglucose unit, the maximum degree of substitution of 3.0 is obtained. The formaldehyde produced is derived from the carboxymethyl (—— OOC — CH_2 ——) groups.

After establishing the limit of these variables, CMC was isolated from mixtures of gums added to milk. In addition, CMC was recovered from other dairy and nondairy food products. The results are summarized in Tables 1 and 2.

Recoveries were over 80% in the presence of all food gums. With starch, recovery was 78% but this might have been caused from difficulty encountered in filtration. Recovery from the various food products was good also. The lowest recovery was from chocolate ice cream, the digest of which was difficult to filter, also.

For comparison, the 2,7-naphthalenediol, phenol-H_2SO_4 and the anthrone reagents were used to determine the amount of CMC in the eluates. These reagents were used also to determine color development in control blanks obtained from experiments using chocolate milk.

Comparisons showed that the chromotropic acid test was more specific than the 2,7-naphthalenediol test because only pectin and pectic acid gave the characteristic purple color. In the 2,7-naphthalendiol test, carrageenan, furcellaran, and other gums gave slight coloration similar to that produced by CMC; less heat needed with chromotropic acid is probably the reason for this. Interference from chocolate, which is severe in the phenol-H_2SO_4 test, was minimal with the chromotropic acid reagent. Of the four methods, chromotropic acid reagent gave the lowest reagent blank reading. (Table 3).

Although the 2,7-naphthalenediol reagent is the most sensitive of the four reagents (Fig. 11), superior specificity, tolerance to chocolate ingredients and relatively shorter heating time needed in the chromotropic acid tests when compared to the 2,7-naphthalendiol method, make the reagent useful for the assay of CMC isolated from food products. The ready removal of other major food gums by the clean-up process (10) lends assurance to any assay based on the proposed method. Although certain sugars and other substances can produce formaldehyde when heated with hot concentrated H_2SO_4 (15, 16, 18), such substances are all soluble in water and will be removed during isolation. Secondly, they are not precipitated by CPC and will not be trapped on the Hyflo Super Cel column.

TABLE 1. Recovery of carboxymethylcellulose from milk (10 g) in the presence of other gums. (10 mg of CMC added in all cases)

Gum added	CMC recovered		Recovery (Average)	
	Range	Average		
	(mg)	(mg)	A[b] (%)	B[c] (%)
Water	8.3–9.4	8.7	87.0	
None	8.4–9.0	8.5	85.0	97.7
Carrageenan (10)[a]	8.5–8.9	8.6	86.0	98.8
Furcellaran (10)	8.3–8.7	8.5	85.0	97.7
Alginate (10)	8.2–8.7	8.5	85.0	97.7
Pectin (10)	8.5–8.9	8.6	86.0	98.8
Pectic acid (10)	8.2–8.7	8.4	84.0	96.6
Gum arabic (20)	8.3–9.0	8.5	85.0	97.7
Gum karaya (20)	8.1–8.7	8.5	85.0	97.7
Gum tragacanth (10)	7.6–8.2	8.0	80.0	92.0
Quince seed mucilage (10)	8.1–8.4	8.3	83.0	95.4
Gum guar (20)	8.2–8.6	8.3	83.0	95.4
Locust bean gum (20)	8.0–8.5	8.3	83.0	95.4
Gum ghatti (20)	8.0–8.5	8.3	83.0	95.4
Starch (20)	7.5–8.3	7.8	78.0	89.0
Carrageenan + alginate (10 of each)	7.7–8.5	8.0	80.0	92.0
Carrageenan + gum guar (10 of each)	7.4–8.0	7.7	77.0	90.0
Carrageenan + gum arabic + gum guar (10 of each)	7.3–8.0	7.7	77.0	90.0

[a] Numbers in parentheses indicate milligrams of gum added to the milk.
[b,c] See legend at bottom of Table 2.

TABLE 2. Recovery of carboxymethylcellulose from water and various food products.

Product	CMC recovered		Recovery (Average)	
	Range	Average		
	(mg)	(mg)	A[a] (%)	B[b] (%)
Water	8.3–9.4	8.7	87.0	
Milk	8.3–9.2	8.4	84.0	96.6
Chocolate milk (1% commercial chocolate added to commercial milk)	7.9–8.1	8.1	81.0	93.1
Ice cream (vanilla)	8.4–9.1	8.6	86.0	98.0
Ice cream (chocolate)	7.4–8.0	7.6	76.0	87.4
Powdered beverage base (grape)	7.7–8.5	8.2	82.0	94.2
Powdered beverage base (orange)	8.0–8.4	8.3	83.0	95.4
Dietetic food, liquid (strawberry)	7.8–8.4	7.9	79.0	90.8
Dietetic pancake syrup	7.8–8.7	8.3	83.0	95.4
Pancake syrup	7.5–8.6	7.8	78.0	90.0
Cake mix	7.6–8.2	7.9	79.0	90.8
Canned sardines in oil	7.7–8.2	7.9	79.0	90.8
French salad dressing	7.9–8.4	7.6	76.0	87.4
Fruit slaw	7.7–8.4	7.9	79.0	90.8

[a] Recovery based on amount of CMC added to the mixture and carried through the entire process.

[b] Recovery based on CMC recovered from water when 10 mg of the hydrocolloid was carried through the entire process.

Corresponding value in (column A ÷ 87.0) × 100

TABLE 3. Variations in readings of chocolate milk blanks tested with four different reagents.

Reagent	Absorbance of: A Reagent blank	B Milk blank	C Chocolate milk[a] blank	Per cent increase of chocolate milk blank over: Reagent blank $\frac{C-A}{A} \times 100$	Milk blank $\frac{C-B}{B} \times 100$
Chromotropic acid	0.045	0.065	0.075	66.6	15.0
2,7-Naphthalendiol	0.060	0.090	0.110	83.3	22.0
Anthrone	0.035	0.05	0.085	143.0	58.3
Phenol-H_2SO_4	0.045	0.060	0.135	200.0	115.4

[a] Contained 0.08 ± 0.016% true chocolate as determined by the method of Hansen (11). Average of 5 different determinations.

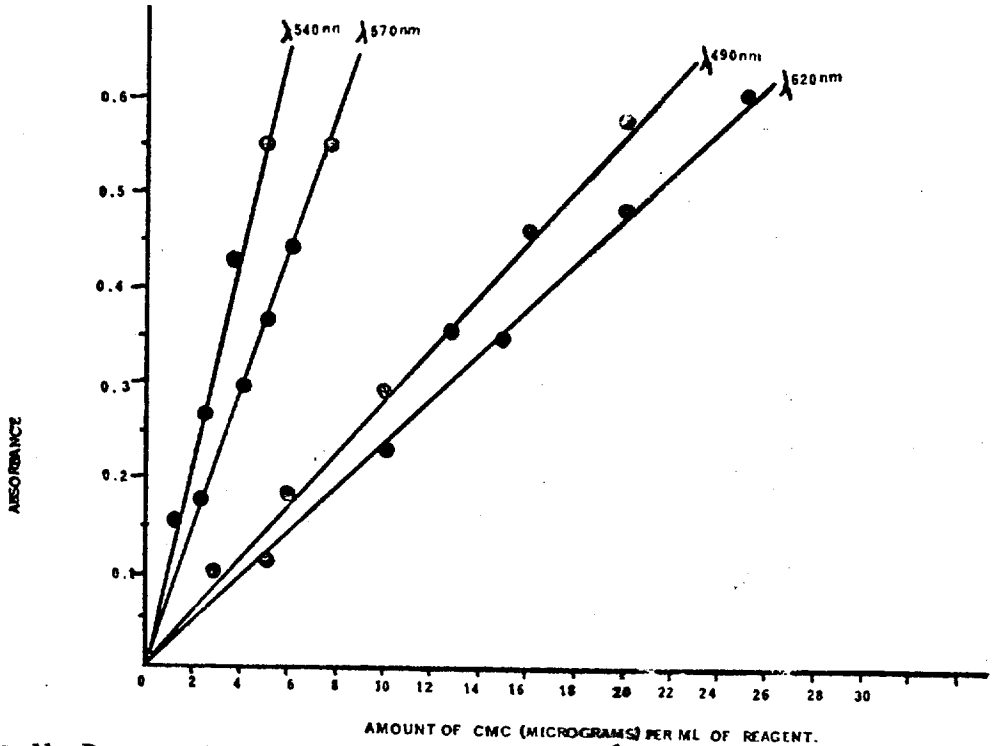

FIG. 11. Response of carboxymethylcellulose to the 2,7-naphthalenediol (540 nm), chromotropic acid (570 nm), phenol—H_2SO_4 (490 nm) and anthrone (620 nm) reagents.

Acknowledgments

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Thanks are due to the Hercules Powder Company, Wilmington, Delaware, for the sodium carboxymethylcellulose.

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KUNSTSTOFFE
58(5): 365-366, 1968

Chromatographic identification of the cellulose esters of volatile aliphatic fatty acids.

By engineer W. Hekeler, Munich.

Report from the Technological Laboratory of the Optical Factories G. Rodenstock.

A simple and tested method is specified for the paper-chromatographic identification of homologous volatile fatty acids, separated from cellulose esters.

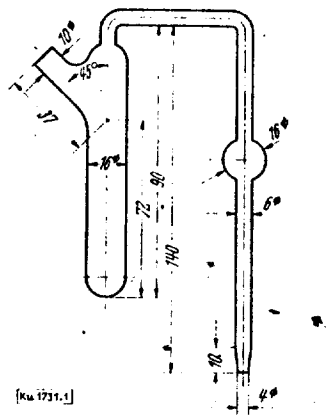
Cellulose acetate,-propionate and -butyrate are used for the preparation of injection molding- and extrusion masses, of foils and varnishes and likewise serve for manifold purposes in electrical engineering, precision mechanics and optics. Mixtures of these cellulose esters are thereby frequently used as well; an analytical method for their identification is therefore of interest. Accordingly, a tested method, simple to apply, will be disclosed concerning paper chromatographic identification and discrimination .

Principle.

After the saponification of the cellulose esters with sulfuric acid which esters were previously, if necessary, freed from softening agents through ether extraction, the released volatile fatty acids are distilled and subjected to chromatography as hydroxamacids according to FINK[1].

To prepare hydroxamecids , mixing with methanol and distillation of the methyl esters is subsequently carried out; after adding a hydroxilamine solution, the methyl esters are briefly boiled. The hydroxamacids obtained as indicated are applied on unwashed filter paper (20-43 mlg, Schleicher & Schüll), and are ascendingy developed with propanol-ammonium carbonate (2 : 1).[2] The coloring is implemented by spraying with an iron (III) chloride solution, whereby purple spots become visible.

1) K. FINK and R.M. FINK, Proc. Soc. exp. Biol. Med., Vol.70(1949), p.654.
2) Chromatography, with specific reference to paper chromatography. Publication of the business firm Merch Ltd., Darmstadt, p.92.



Ill. 1. Distilling tube made of Pyrex or Duran glass, 1 mm thick.

Required equipment

Chromatography installation for ascending paper chromatography;
two distilling tubes, 10 to 12 ml, made of Pyrex or Duran glass, with polyethilene stoppers, Ill. 1;
heater unit (block) with 3 bores according to HOLNESS[3], made of brass, Ill.2;
Bunsen burner and
two test tubes, 100 X 10 mm.

Procedure

The distrilling tube according to Ill. 1 proved adquate for the saponification of the substance and for the distillation of the fatty acids; said tube is fitted into a bore of the heater unit (block). A test tube serves as receiver.

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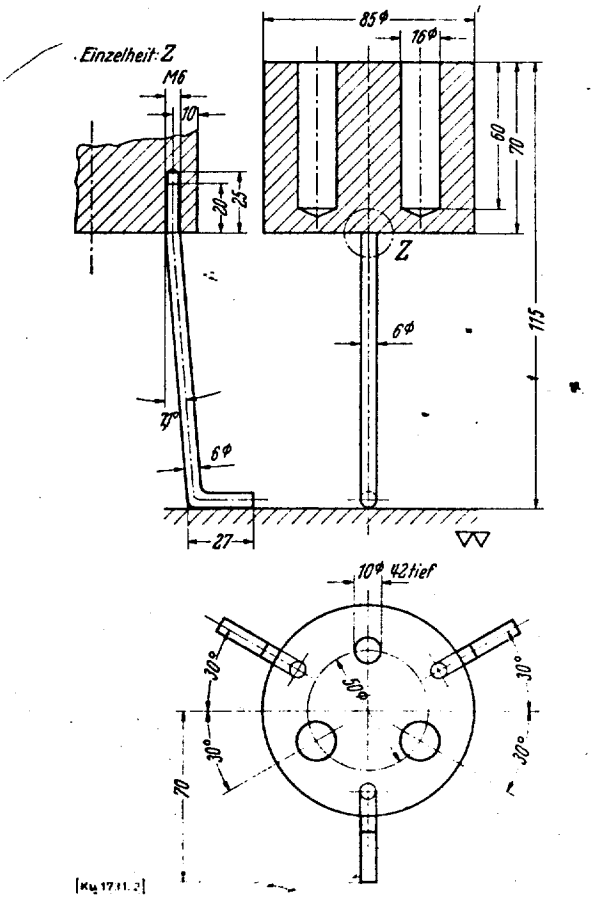
3) H. HOLNESS, Advances inorg. qual. Anal. (1953), p.23.

4) The height of the shown heater unit (block) is adequate for a micro-Bunsen burner. When using a standard Bunsen burner, the height must be correspondingly increased. The polyethylene stoppers needed for sealing the distilling tubes can be prepared according to the following simple method: a polyethilene rod is pressed into the inlet opening which is strongly heated over a Bunsen burner (low-pressure polyethilene is preferable for this purpose); the diameter selected for said rod should be somewhat larger then the inlet opening of the distilling tube. After gradual cooling and solidification of the stoppers obtained as indicated, they are pulled out of the distibbiling tube and cut



Ill.2. Heater unit (block) according to HOLNESS. Material used: brass or copper.

After feeding a total of 1 g substance and 1 ml concentrated sulfuric acid into the distilling tube and after sealing the same with a polyethilene stopper, the heating unit is heated. The substance carbonizes, while the volatile fatty acids are over-distilled and are collected in the supplied test tube. The volume of the distillate is approximately 0,5 ml.

The fatty acids are then placed into a second distilling tube for esterification, mixed with the same volume of methanol; 0,5 ml of concentrated sulfuric acid is added; slow distillation follows. A second test tube serves as receptacle.

After the termination of the distilling process, the distillate within the test tube is mixed with 1 drop (about 1 ml) of 5 n ammonia solution for the purpose of neutralizing the eventually over-distilled sulfuric acid; 1 ml. of the hydroxylamine solution is

added, agitated, then carefully and briefly brought to boiling in a bore of the heater - unit (block).

0,005 to 0,03 of the obtained hydroxamacid solution is placed on unwashed filter paper as a spot which should be as small as possible;(2043 mgl, Schleicher & Schüll).The development occurs ascendingly with propanol-ammonium carbonate (2 : 1).

According to experience, a separation sufficient for the identification of fatty acids takes place after a development period of 3 to 4 hours. After marking the solvent front, the chromatogram can be carefully dried. The chromatogram is then carefully sprayed with a 10% watery solution of an iron (III)- chloride solution, and subjected to final desiccation. The separated fatty acids become visible immediately during the spraying as brown to violet-colored spots.

Evaluation

R_F-values of the hydroxamacids:

formic acid	0,29
acetic acid	0,57
propion acid	0,70
butryc acid	0,79

Preparation of the hydroxylamin solution

7 g of hydroxylaminhydrochloride is dissolved with 100 ml of methanol. After adding 200 ml of 1-standard alcoholic KOH, 10 minutes of vigorous agitation follow; the precipitated KCL is subsequently filtered.

Preparation of the propanol-ammonia carbonate solution (2 : 1)

The ammonium carbonate solution consists of 2 vol.-parts of a 10% watery ammonium carbonate solution and of 1 vol-part of a 5-standard ammonia solution.

1 part of said ammonia carbonate solution yields 2 parts of propanol-1.

(1731)

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Technologists

J. HIDALGO and P. M. T. HANSEN
Department of Dairy Technology, The Ohio State University, Columbus

Moving Boundary Electrophoresis of Food Stabilizers

SUMMARY—The electrophoretic characteristics of locust bean gum, guar gum, arabic gum, carboxymethylcellulose, κ- and λ-carrageenan, alginate and the non-dialyzable fraction of corn syrup solids were studied by moving boundary electrophoresis. The physical properties of these colloids require the use of modified techniques in order to eliminate boundary disturbances which otherwise might be interpreted as heterogeneity of composition.

A high degree of homogeneity was observed for carboxymethylcellulose, arabic gum, alginate and λ-carrageenan. In contrast, κ-carrageenan was composed of a mixture of migrating components. The colloidal fractions of guar gum, locust bean gum, and corn syrup solids did not migrate in the electrical field.

Mixtures of carboxymethylcellulose with guar gum or gum arabic could be separated at pH 7. Mixtures of carboxymethylcellulose with carrageenan could be effectively separated at pH 2. Electrophoretic separation at 23°C of different stabilizer mixtures was unsatisfactory and the patterns showed evidence of interactions.

INTRODUCTION

INTEREST IN THE CHARACTERISTICS and mechanism of stabilizer action prompted an examination of the electrophoretic purity and behavior of a number of hydrocolloids. Published findings indicate that zonal electrophoresis (Lewis *et al.*, 1967) as well as moving boundary electrophoresis (Asano, 1966; Colvin *et al.*, 1952; Northcote, 1954; Smith *et al.*, 1954; Smith *et al.*, 1955; Ward *et al.*, 1947; and Whistler *et al.*, 1965) can be used for this purpose. Lewis *et al.* (1967) employed electrophoresis on glass-fiber paper and reported that gum arabic, carrageenan, and agar are heterogeneous polysaccharides. Colvin *et al.* (1952) examined a number of polysaccharides dissolved in alkali by the moving boundary technique. They found inherent difficulties in the resolution of the patterns, but reported that this technique may be applicable for characterization and fractionation of the class of polysaccharides which are soluble close to neutrality. Whistler *et al.* (1965) suggested moving boundary electrophoresis as useful for the determination of polysaccharide homogeneity and for the determination of polysaccharide mixtures. Northcote (1954) measured the mobility of neutral polysaccharides, including starches and glycogens of different origins, in borate buffer. Apparently these colloids acquire a negative charge by interaction with the borate ion and, consequently, move in the electrical field. Excellent patterns were reported for this buffer system, whereas less satisfactory results were obtained with veronal and glycine buffers.

Smith *et al.* (1954) used free boundary electrophoresis in their study of carrageenan. Abnormalities associated with incipient gelation were encountered with concentrations higher than 0.2% for mixtures of the κ- and λ-fractions, but even at this low concentration they were unable to obtain a definite electrophoretic separation into components. Ward *et al.* (1947) have reported a detailed electrophoretic investigation of pectinates. Recently, Asano (1966) studied the interaction between milk protein and carboxymethylcellulose in fruit-flavored milk by moving boundary electrophoresis.

The present investigation was undertaken in order to study the electrophoretic behavior of common food stabilizers as single entities and as related mixtures.

PROCEDURE

Instruments

Electrophoresis was performed with a Perkin-Elmer Model 38-A electrophoresis apparatus employing a 2 ml

Tiselius cell of open design. The conductivity was measured with an L & N portable electrolytic resistance indicator using the Perkin-Elmer conductivity cell. The viscosity was measured with a Brookfield L.V.F. viscometer.

Sample preparation

The following materials were used: sodium alginate KGHV and KGLV (Kelco Company, San Diego, California); potassium salts of λ- and κ-carrageenan (Marine Colloids, Inc., Springfield, New Jersey); sodium carboxymethylcellulose, 7-HP and 7-LP, with a designated substitution range of 0.65 to 0.85 (Hercules Powder Company, Wilmington, Delaware); and commercial grades of gum arabic, guar gum, locust bean gum, and corn syrup solids type 36 DE (10% nondialyzable matter). The samples were prepared by dissolving at 60°C a weighed amount of dry stabilizer in buffer. The high viscosity of stabilizer solutions limited the sample concentration to approximately 0.5%.

After cooling, the solutions were dialyzed against 20 volumes of buffer for at least 24 hr at 5°C. Insoluble material, if present, was removed by centrifugation at 1000 G for one hour. The amount of insoluble material was estimated by weighing the sediment after drying at 110°C until constant weight (12 hr). Only locust bean gum and guar gum contained significant amounts of insoluble material (7 and 18% respectively).

Preliminary investigations revealed that the use of conditions similar to those commonly employed for the study of protein systems gave rise to considerable boundary disturbances. These consisted mainly in the appearance of a large number of peaks in the sample side of the Schlieren patterns which did not reproducibly reflect a heterogeneity of composition. Inspection of the patterns revealed the presence of both positive and negative refractive index gradients, similar to those observed if the system has not attained temperature equilibrium.

It is questionable, however, that unequal temperature arising from slow heat dissipation was the responsible factor, since the abnormal patterns did not occur when electrophoresis was performed on equally viscous solutions of neutral stabilizers (guar gum and locust bean gum) in which Joule heating would also be generated. The phenomenon appeared to be related to the rate of electrophoretic migration, and was particularly pronounced for stabilizers of high electrophoretic mobility. It was found that a reduction in the field strength to a value below 2 volts/cm effectively prevented these disturbances in most cases. These conditions were achieved by using an auxiliary power supply delivering 2 to 5 ma at 10 volts for a buffer of a specific resistance of 125 ohms.

Electrophoresis at 0–2°C

The electrophoresis cell was filled according to the usual procedures and then mounted in the tank of the instrument. The boundaries were moved into view at room temperature (23°C) at a slow, constant rate by injecting buffer into the descending-side buffer bottle. This was accomplished by means of a capillary tube connected to a closed glass bulb, equipped with platinum electrodes and filled with buffer, in which gas pressure was produced electrolytically.

The time required to move the boundaries into view by this technique could be regulated conveniently by adjusting the flow of current between the electrodes, and a rate of movement of 1 cm/hr was sufficient to prevent initial boundary disturbances. After the boundaries had been shifted into position, the temperature was reduced to 0–2°C by ice water, and approximately 60 min were allowed for the system to reach temperature equilibrium.

The field strength used for electrophoresis was calculated from the relationship $\frac{I}{k \times a}$,

where I = current (amperes)
k = specific conductance of sample ($ohms^{-1} \cdot cm^{-1}$)
a = cross sectional area of cell (cm^2).

This value did not exceed 2 volts/cm.

Electrophoresis at 23°C

For a number of samples, which were either highly viscous or showed tendency towards gelation at 0–2°C, it was possible to perform electrophoresis at room temperature, if the field strength was reduced drastically. A similar procedure has been used successfully by Johnson *et al.* (1948) for analyzing colloids which were only sparingly soluble at lower temperatures. No special thermostat was necessary, since the instrument was located in a constant-temperature room at 23 ± 1°C, and the resulting temperature fluctuations of the water bath during electrophoresis were negligible. The field strength used did not exceed .2 volts/cm. Mobility measurements obtained at this temperature were corrected to 0°C by multiplying the observed values by the ratio of buffer viscosity at 23°C to buffer viscosity at 0°C (Johnson *et al.*, 1948; and Ward *et al.*, 1947).

The expected linear relationship between solute mobility and buffer viscosity was confirmed by the experimental

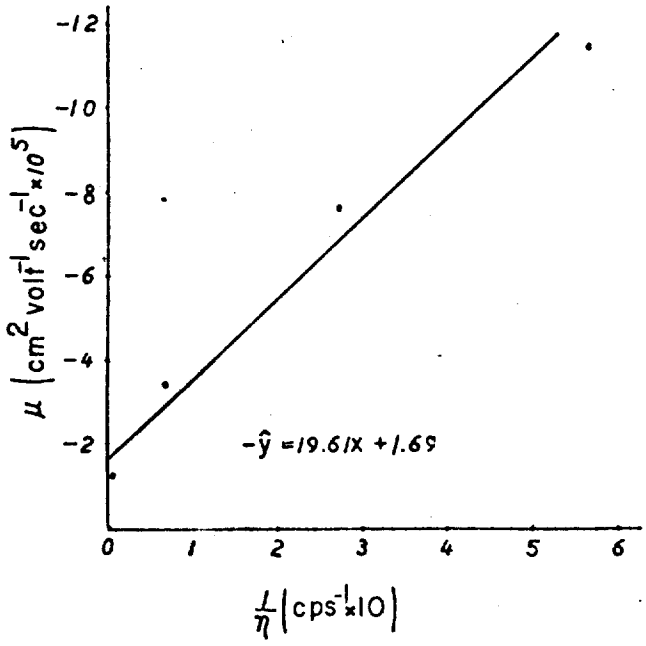

Fig. 1. Relationship between electrophoretic mobility of alginate and buffer viscosity at 0–2°C (sodium phosphate/sucrose buffer pH 7, ionic strength 0.2).

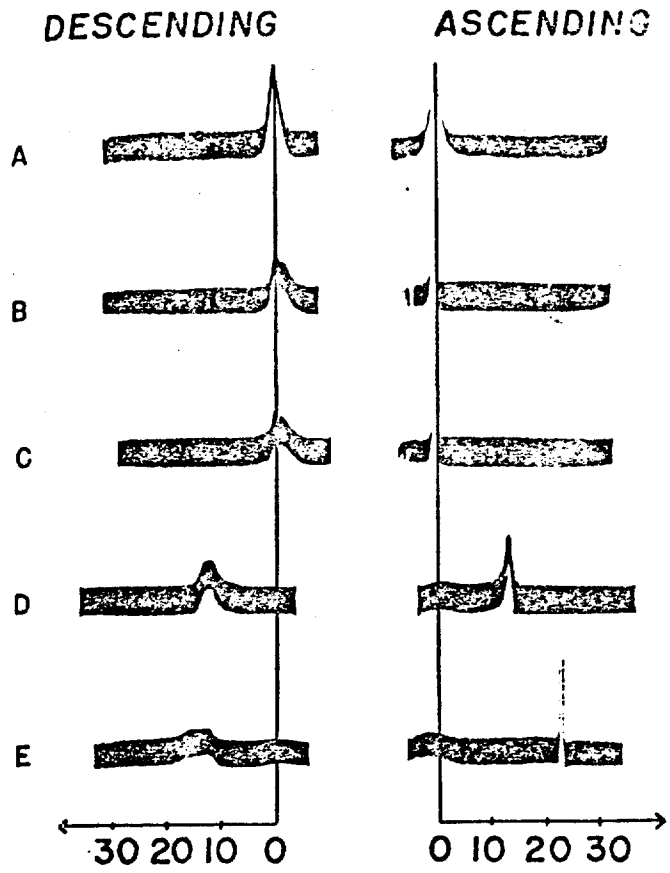

Fig. 2. Electrophoretic patterns of selected food stabilizers (sodium phosphate buffer, pH 7, ionic strenght 0.02. Mobility scale: — $cm^2\ volt^{-1}\ sec^{-1} \times 10^5$).
A. Corn syrup solids (nondialyzable fraction: 10–15%)
B. Locust bean gum (0.3%)
C. Guar gum (0.3%)
D. Arabic gum (0.25%)
E. Carboxymethylcellulose (0.25%)

values in Fig. 1 which were obtained for alginate in sodium phosphate buffer containing different amounts of sucrose.

RESULTS

THE PATTERNS FOR A NUMBER of different stabilizers, including corn syrup solids, are shown in Fig. 2. At pH 7 the colloidal fraction of the corn syrup solids did not possess any net electrical charge since it did not move in the electrical field. Locust bean gum and guar gum did not contain components of appreciable net charge either, and the slight [illegible]ration observed in the descending patterns for these [illegible]al stabilizers was apparently caused by a displacement of the false boundary. No electrophoretic migration could be detected in their ascending patterns, which showed a hypersharp peak at the initial position.

No gross electrophoretic heterogeneity was revealed by the remaining stabilizers, although abnormal spreading of the descending patterns always occurred and, in most cases, precluded accurate mobility measurements. The ascending boundaries remained sharp and appeared to be more suitable for mobility calculations except that, at low ionic strength, the ascending mobilities were considerably higher than those calculated from the descending side and were sensitive to small changes in the ionic strength.

The effect of variations in the ionic strength of the buffer on the electrophoretic patterns of sodium alginate at 0–2°C is illustrated in Fig. 3. At increasing ionic strength, the inequality of the mobilities from the ascending and descending patterns decreased. For both patterns, the higher ionic strength suppressed the electrophoretic mobility, but the effect was most pronounced for the ascending pattern. Apart from the influence of buffer salt upon the electrophoretic mobility, the shape of the peaks remained the same at all salt concentrations.

Electrophoresis of λ-carrageenan presented special problems which were manifested by gross disturbances in the descending pattern arising when the initial boundary was moved into view. Inspection of the cell image revealed a penetration of buffer into the sample solution during boundary displacement, causing a blurred Schlieren pattern with false peaks (Fig. 4A). In contrast, the ascending side displayed a sharp and well-defined initial boundary at all times.

Attempts to perform electrophoresis on λ-carrageenan without shifting the initial boundaries into view did not prevent the gradual deformation of the descending boundary; nor did the addition of a surface active agent (0.01% sodium lauryl sulfate). The disturbances were completely eliminated, however, by performing the analysis at elevated temperature (23°C) or by reducing the pH of the buffer to 2 at 0–2°C. Patterns obtained under these conditions are shown in Fig. 4B and 4C and show that clearly-defined patterns were resolved at sample concentrations as high at 0.5%. In both cases, the stabilizer appeared electrophoretically homogeneous. The mobilities observed at pH 2 were significantly lower than at pH 7.

Electrophoretic patterns of κ-carrageenan are shown in Fig. 5. For this stabilizer, consistent data could not be obtained in neutral buffer at 0–2°C, because at this pH and temperature the mobilities were found to be dependent upon the concentration of the sample. Thus, the κ-carra-

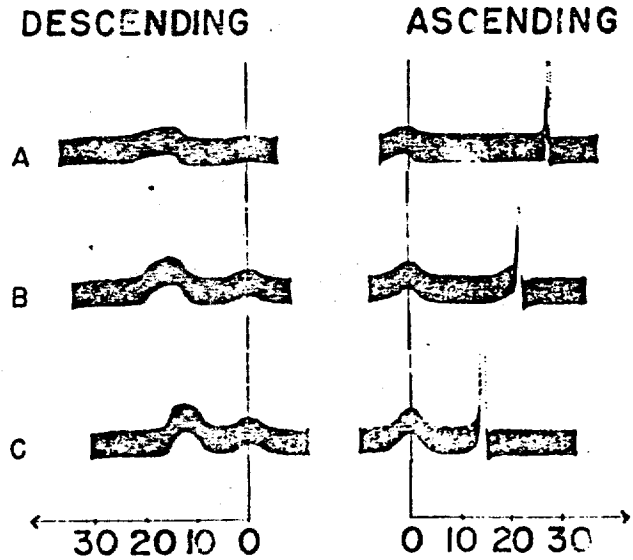

Fig. 3. Effect of buffer ionic strength on the electrophoretic mobility of 0.5% alginate (sodium phosphate buffer, pH 7. Mobility scale: — $cm^2\ volt^{-1}\ sec^{-1}$).
A. Ionic strength 0.02
B. Ionic strength 0.10
C. Ionic strength 0.20

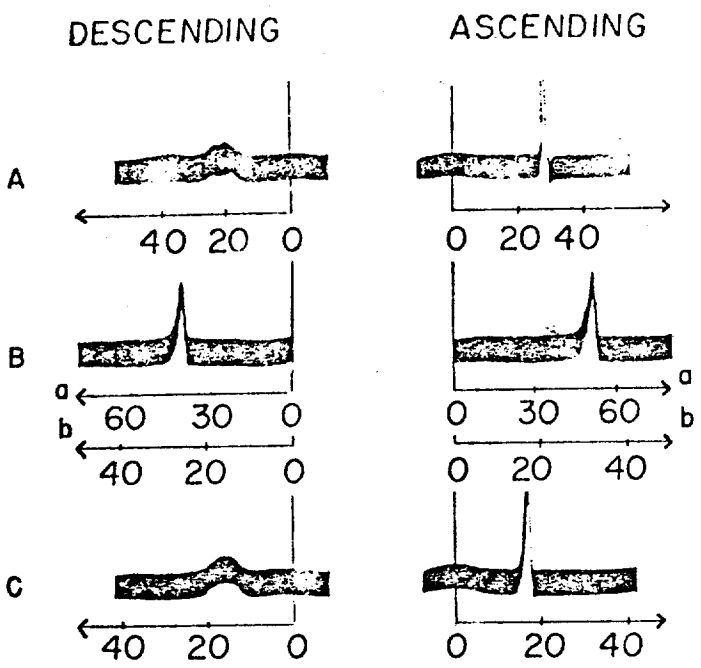

Fig. 4. Electrophoretic patterns of 0.5% λ-carrageenan (buffer ionic strength 0.2. Mobility scale: — $cm^2\ volt^{-1}\ sec^{-1} \times 10^5$).
A. Electrophoresis at 0–2°C. Sodium phosphate buffer, pH 7
B. Electrophoresis at 23°C. Sodium phosphate buffer, pH 7 (a = mobility at 23°C; b = mobility at 23°C, corrected to 0°C for buffer viscosity change)
C. Electrophoresis at 0–2°C. Sodium chloride buffer, pH 2.

geenan exhibited a mobility of close to 15 electrophoretic units at low concentration (0.2%), but only 2 electrophoretic units at the concentration of 0.5% (Fig. 5A). A similar behavior was not found for the other stabilizers investigated and it is possible that incipient gelation at the higher concentration may have retarded the migration of κ-carrageenan.

Well-defined electrophoretic patterns which were not affected by the sample concentration within the range of 0.2–0.5% were obtained for κ-carrageenan in phosphate buffer of pH 7 at 23°C (Fig. 5B), and in sodium chloride buffer of pH 2 at 0–2°C (Fig. 5C). Multiple peaks in both patterns revealed this stabilizer fraction to be a heterogeneous mixture.

Solutions containing mixtures of stabilizers were subjected to electrophoresis to explore the conditions under which the individual components may be identified by this technique. The patterns for a mixture of carboxymethylcellulose and arabic gum in Fig. 6A revealed that a separation of the pair was achieved. A comparison of the mobilities calculated from this pattern with those obtained singly (Fig. 2), show some decrease in the mobility of carboxymethylcellulose with a corresponding increase in that of the arabic gum.

A similar effect was also observed for other pairs of stabilizers. Therefore, effective separation can be achieved only if the individual mobilities are sufficiently different. In the case of carboxymethylcellulose, alginate, and carrageenan, the individual mobilities at pH 7 did not permit a definite separation of their mixtures; however, as shown in Fig 6B and 6C, a complete separation of carrageenan from carboxymethylcellulose was possible at pH 2, because at this pH carboxymethylcellulose did not migrate.

The low concentration of individual components in mixtures of stabilizers made an accurate evaluation of their electrophoretic patterns difficult. An apparent increase in

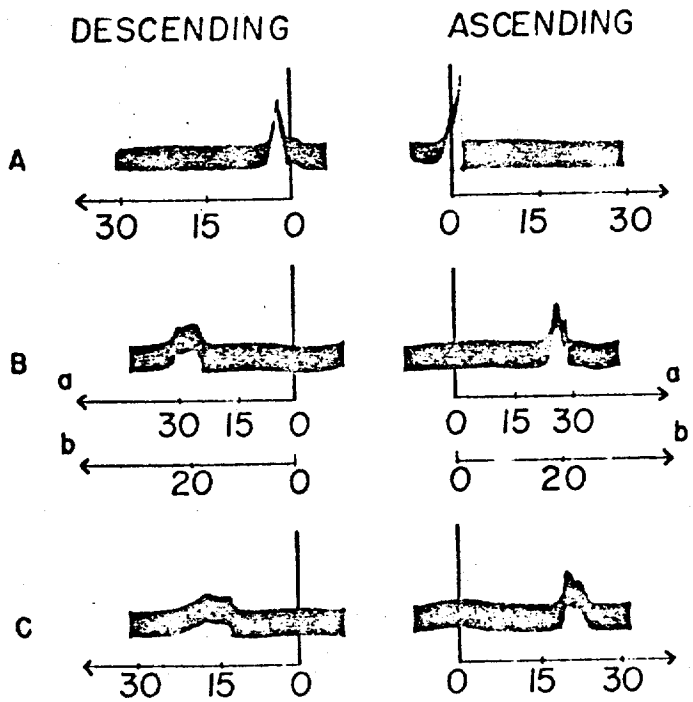

Fig. 5. Electrophoretic patterns of 0.5% κ-carrageenan. (Buffer ionic strength 0.2. Mobility scale: — $cm^2\ volt^{-1}\ sec^{-1} \times 10^5$).
A. Electrophoresis at 0–2°C. Sodium phosphate buffer, pH 7
B. Electrophoresis at 23°C. Sodium phosphate buffer, pH 7 (a = mobility at 23°C; b = mobility at 23°C, corrected to 0°C for buffer viscosity change)
C. Electrophoresis at 0–2°C. Sodium chloride buffer, pH 2.

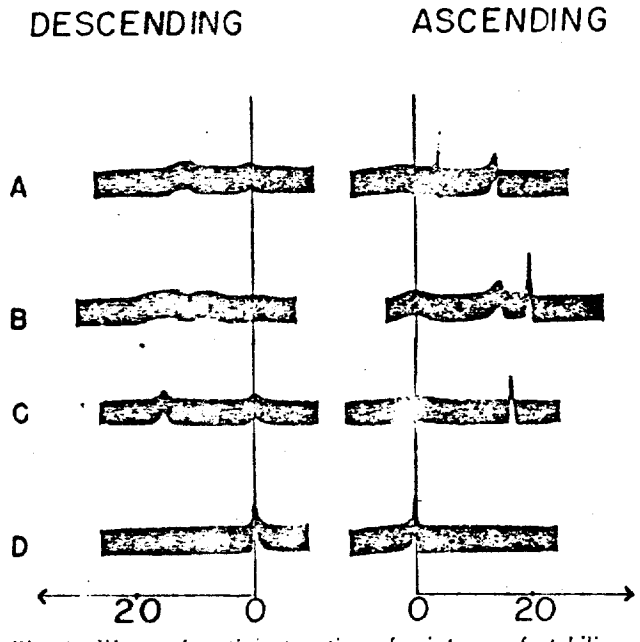

Fig. 6. Electrophoretic separation of mixtures of stabilizers at 0–2°C. (Mobility scale: — $cm^2\ volt^{-1}\ sec^{-1} \times 10^5$).
A. Guar gum and alginate (concentration: 0.5%. Sodium phosphate buffer, pH 7, ionic strength 0.02)
B. Arabic gum and carboxymethylcellulose (concentration: 0.3%. Sodium phosphate buffer pH 7, ionic strength 0.02)
C. Carboxymethylcellulose and κ-carrageenan (concentration: 0.3%. Sodium chloride buffer, pH 2, ionic strength 0.1)
D. Carboxymethylcellulose (concentration: 0.15%. Sodium chloride buffer, pH 2, ionic strength 0.1).

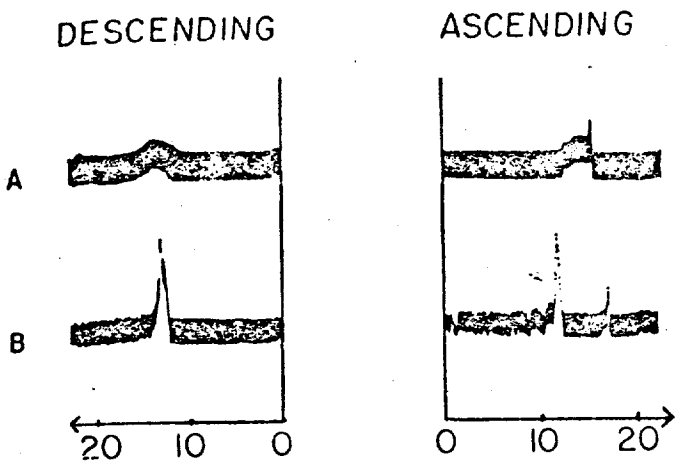

Fig. 7. Electrophoretic separation of mixtures of carboxymethylcellulose, carrageenan and guar gum at 23°C. (Sodium phosphate buffer, pH 7, ionic strength 0.2. Mobility scale: — cm^2 $volt^{-1}$ sec^{-1} × 10^5).
A. κ-carrageenan, carboxymethylcellulose and arabic gum (concentration 0.3%)
B. λ-carrageenan, carboxymethylcellulose and guar gum (concentration 0.6%)

viscosity or tendency towards gelation in some of the combinations imposed a further limitation upon the maximum sample concentration for which electrophoretic patterns could be obtained at 0–2°C. Analysis of such mixtures at elevated temperature (23°C) permitted the use of higher sample concentration but gave evidence of interaction under these conditions.

The ascending patterns for the two mixtures in Fig. 7 show multiple peaks, while only one peak was apparent in the descending side. Such lack of enantiography may possibly be explained by a reversible binding of undissociated buffer acid to the colloids (Cann *et al.*, 1965) or by an interaction between the colloids in the mixture. The irregular patterns obtained in Fig. 7B illustrate the analytical difficulties encountered when gross interaction occurs.

DISCUSSION

THE RESULTS OBTAINED in this study suggest that free boundary electrophoresis may be useful in studies on food stabilizers. However, the method is of limited value for neutral stabilizers, except in analysis of their mixtures with colloids containing ionized groups.

The examination of corn syrup solids was included in order to compare this material with other stabilizers, since Wolfmeyer (1963) has pointed out that the higher polysaccharides present in these products may have attributes similar to some of the vegetable gums. According to our analyses, the particular sample investigated contained approximately 10% of non-dialyzable polysaccharides, which at the levels of corn sweetener used in many foods could exert an important influence on their texture. The electrophoretic neutrality of the material suggests its action would be similar to guar gum or locust bean gum.

The patterns obtained for single entities of stabilizers revealed these were, in general, homogeneous, except for κ-carrageenan. Such homogeneity is surprising, since a considerable range of molecular weights has been demonstrated for a number of stabilizers (Smith *et al.*, 1954; Ward *et al.*, 1947). Therefore, the observed electrophoretic homogeneity suggests that if different molecular sizes occur, these would either possess a uniform electrical charge per unit surface or would be complexed in a manner that would not permit electrophoretic separation. The electrophoretic analysis of mixtures of stabilizers gave evidence of some interaction, particularly at 23°C, but even for these patterns heterogeneity could be demonstrated on the ascending side.

The homogeneous patterns for λ-carrageenan obtained under different conditions (Fig. 4) are of interest because Smith *et al.* (1954) have reported heterogeneity of this fraction and assumed it occurred as the result of components possessing different hexose-sulfate ratios. Although heterogeneity cannot be discounted, the present study does not support that it would be due to components of varying ester sulfate content. There can be little doubt that the electrophoretic technique would detect differences in the degree of sulfation of carrageenan. Other studies showed that the heterogeneous patterns for κ-carrageenan reflected the presence of components of different ester sulfate content.

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CHRONIC ORAL TOXICITY OF A HIGH GEL POINT METHYLCELLULOSE (METHOCEL HG) IN RATS AND DOGS

HAROLD C. HODGE, ELLIOTT A. MAYNARD, WILLIAM G. WILT, JR., HARVEY J. BLANCHET, JR. AND ROBERT E. HYATT

Division of Pharmacology and Toxicology, University of Rochester School of Medicine and Dentistry, Rochester 20, New York

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The demonstration of the low toxicity of Methocel HG is important if this substance is to be added to food or used in pharmaceutical preparations. Methocel HG is a modified Dow methylcellulose, a water-soluble cellulose ether, similar to Methocel (1) in all properties except the gel point. Both of these materials are chemically inert, synthetic gums, yielding in cold water viscous solutions that are colorless, odorless, tasteless, neutral in pH and resistant to fermentation. Methocel HG has a higher gel point by 10° to 15° C. than regular Methocel which improves the compatibility with many pharmaceutical ingredients and gives better storage properties, especially in warm climates.

A number of uses for Methocel or Methocel HG have already been reported. They are suggested for the thickening of sugar-free syrups for diabetic persons (2), for use as a bulk laxative in constipation (3, 4), in a protective mixture for stomach ulcers (5), and in the preparation from flours that do not contain gluten, of breads for patients with wheat allergies (6).

The published data on the oral toxicity of methylcellulose are not extensive and no data were heretofore available on Methocel HG. The urgent need for a blood substitute in the early days of the war and the combination of osmotic activity and isoviscosity of Methocel solutions led to tests of its usefulness intravenously in the treatment of shock (7). Hueper (8) observed in rabbits and dogs given 2 per cent aqueous solutions intravenously that spleens were enlarged, foam cells were found in livers, and the hematological picture was disturbed, but the most striking finding was the development of atheromatous lesions in arterial walls. No acute toxic response was seen. In contrast, little effect except a laxative action was noted even with large doses by mouth. Tainter (4) fed rats 10 per cent of Methocel in their diets and in their drinking water to a total amount of about 400 mgm./day. The results of the various tests reported herewith confirm the previous observations. Methocel HG has a low, but measurable, toxicity when given parenterally. Given orally, even in large amounts over extended periods, Methocel HG is singularly free from toxic properties (9).

Acute Toxicity. The acute toxicity of Methocel HG is low. In a series of experiments on 67 adult (9 months old), male, albino rats (body weight averages—333 to 349 gm.) in which Methocel HG was administered in aqueous solution intraperitoneally, the LD_{50} (10) proved to be of the order of 5 gm./kgm. Statistical treatment of the data according to the method of Litchfield and Wilcoxon (10) gives doses in mgm./kgm. with 19/20 confidence limits as follows: LD_{16}-4200

(4500 to 4700); LD_{50}-5200 (4800 to 5000); LD_{84}-6300 (5700 to 7000). Methocel HG was administered intraperitoneally to 138 adult albino mice; the LD_{50} was found to be of the order of 5 gm./kgm. When an aqueous solution was given orally by stomach tube to eleven fasted adult albino rats, 4 gm./kgm. produced no evidence of injury.

Short-Term Studies. Short-term feeding tests (one to three months) were carried out on rats, rabbits and dogs as shown in table 1. Ten male and ten female weanling (age 28 days) rats per group were fed diets containing 0, 2, 10 and 25 per cent Methocel HG for 30 days. Only on the highest dietary percentages were any evidences found of interference with growth. Severe diarrhea was observed in the 25 per cent group, and a few rats died. No diarrhea was seen in other groups. The results of urine analyses and hematological examinations were normal except for a minor decrease in red blood cell count in the 25 per cent group. Organ weights were, in general, normal; compared to those of the control rats, the weights of the testes in the 25 per cent males were small but of approximately normal weight for rats of their body weights. There was no evidence of any histological injury.

Six rabbits (about 6 months old; body weights 1.8 kgm.) per group were fed for 30 days diets containing 0, 10 and 25 per cent of Methocel HG. Those on the 25 per cent diet maintained body weight; the other rabbits gained a little weight (about 0.5 kgm.). All survived. Urine analyses, hematological findings and organ weights were uniformly normal. There was no evidence histologically of specific tissue effects.

Two dogs (body weights 11 and 13 kgm., respectively) were fed Methocel HG for 30 days; one dog received 25 gm., the other 50 gm./day. The dog receiving the larger dose exhibited some diarrhea, lost a little weight (about 1 kgm.) and showed a slight fall in red blood cell count. The other dog showed no change in body weight or in hematological values. The urine analyses were uniformly negative. The histological examinations showed only normal tissues except for minor abnormalities unrelated to the treatment.

Chronic Oral Toxicity. In a preliminary chronic-feeding test (table 1), ten male and ten female rats per group were fed diets containing 20 and 25 per cent of Methocel HG, respectively, for one year. Some retardation in growth was apparent. Mortalities were those customarily seen in control groups. No abnormalities were found in the urine analyses, hematological values or on histological examination.

Two-Year Feeding Tests: Rats. Six hundred male and female rats at the time of weaning were given the basal ration; individual growth records were matched and, after two weeks, balanced groups having equal average body weights and normal average growth curves were selected. Four groups of 50 male and 50 female rats each were given diets containing 0 (control groups), 1, 5 and 20 per cent Methocel HG, respectively. The Methocel HG was mixed in Purina chow meal with meat and the rats (five per cage) were permitted *ad libitum* access to the diet.

The *growth curves* for the male and female groups are shown in figure 1. The

TABLE 1

Short-term feeding tests to rats, rabbits and dogs with Methocel HG

SPECIES	DURATION	DOSE	BODY WEIGHT	MORTALITY	URINE ANALYSIS	HEMATOLOGY	ORGAN WEIGHTS	PATHOLOGY
	days	*% of diet*	*change*					
Rat-male and female, 10/group	30	0	Gain	None	Negative	Normal	Normal	No regular tissue changes as a result of treatment
		2	Same gain	None	Negative	Normal	Normal	
		10	Small loss	None	Negative	Normal	Normal	
		25	Larger loss	Males 3/10 Females 6/10*	Negative	? RBC	Normal for rat of this bd. wt.	
		% of diet						
Rabbit-6/group	30	0	Small gain	None	Negative	Normal	Normal	No tissue changes from treatment
		10	Small gain	None	Negative	Normal	Normal	
		25	No gain	None	Negative	Normal	Normal	
		gm./day						
Dog-1 at each dose	52	25	No loss	Survived	Negative	Normal	Normal	No tissue changes due to treatment
		50	Small loss	Survived	Negative	Some ↓ RBC	Normal	
Preliminary Chronic Feeding Test								
Rats-male and female, 10/group	1 year	*% of diet* 20	Some growth retardation	M—1/10 F—1/10	Negative	Normal	Normal	No evidence of tissue changes from treatment
		25	More growth retardation	M—3/10 F—2/10	Negative	Normal	Normal	

* 1 rat was missing.

portion of each curve recorded in the first year (weights taken weekly) has been compressed into a small part of the figure on the left. It is apparent that the male rats fed the 1 and 5 per cent diets grew practically identically with the control rats during the first year but that the 20 per cent group exhibited some diminution in growth amounting to approximately 30 gm. No such divergence occurred in the averages for the female rats; the four curves are closely placed. During the second year (weights taken biweekly), the established trends were

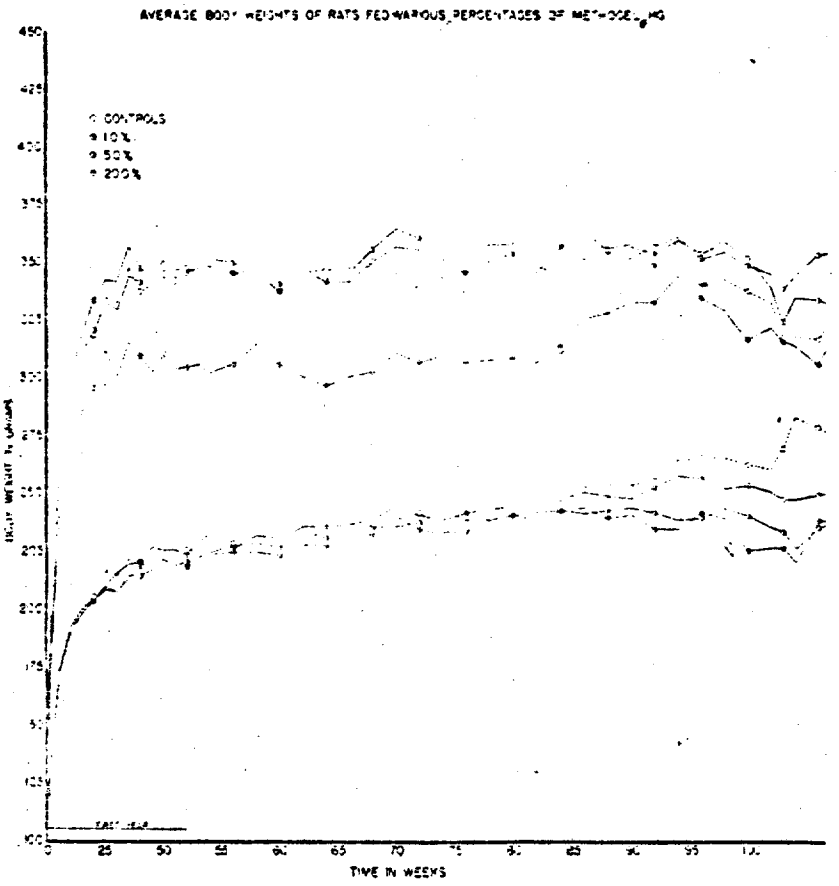

FIG. 1

continued, although, near the end, considerable fluctuations were produced by disease.

The *mortalities* in various groups ranged from 69 to 84 per cent. There was no indication that the ingestion of Methocel HG influenced the life span adversely.

Pooled samples of urine were examined periodically for sugar and protein. There is a trend for normal rat urine samples to contain more sugar and more protein as the rat becomes older. There was no indication that feeding Methocel HG influenced this trend.

Blood samples were taken for the usual hematological observations prior to beginning the study, eight times during the study, and at the termination. In general, only the normal patterns of change with age were observed; a possible exception was the small decrease in red blood cell counts and hemoglobin values, probably of nutritional origin, in the rats given the 20 per cent diets (table 2).

Organ weights were recorded at autopsy. There was a notable lack of variability in organ weights, a finding entirely consonant with the small differences in average body weight.

Histological examinations were made of stomach, small bowel, large bowel, liver, kidneys, lungs, heart, brain, bladder, spleen, testes and bone marrow. Aside from the scattered abnormalities, such as an occasional parasite or the evidence of pulmonary disease frequently found in old rats, there was no evidence of tissue injury. Tumors were found in breast, testes, mesenteric lymph nodes, stomach and liver; sections were examined by Dr. G. Burroughs Mider of the

TABLE 2

Average hematological values in male and female rats after two years on diets containing Methocel HG

METHOCEL IN DIET	SEX	NO. RATS	DAYS ON DIET	TOTAL RED BLOOD CELLS	HEMOGLOBIN	TOTAL LEUCOCYTES
%				*Millions/cmm*	*gm./100 cc.*	*Thous./cmm.*
Control	M	10	714	8.1	14.7	20.1
Control	F	10	714	7.8	14.5	17.0
1	M	11	715	7.9	13.5	23.0
1	F	15	719	7.7	14.0	22.0
5	M	7	720	8.4	13.6	21.2
5	F	15	721	7.8	14.6	13.6
20	M	10	728	7.2	12.4	23.3
20	F	13	721	6.5	11.9	13.0

Department of Cancer Research. This strain of rats has a high incidence of spontaneous tumors; however, as far as can be ascertained, the administration of Methocel HG had nothing to do with the origin of the tumors.

One-Year Feeding Tests: Dogs. Two dogs tolerated without toxic manifestations for a year each of the following doses of Methocel HG; 0, 0.1, 0.3, 1.0 and 3.0 gm./kgm./day. The dogs maintained body weights or gained slightly. None died.

Bimonthly throughout the year, urine analyses were made for sugar and protein. Trace amounts of sugar were found in practically every sample and, occasionally, a small amount of protein was reported. There was no correlation with dosage of Methocel HG.

Blood samples were taken for hematological examination prior to starting the experiment, four times during the experiment, and terminally. All of the observations were within normal limits.

At the time of autopsy, organ weights were recorded. Despite considerable variability, there was no indication of any deleterious effect.

A number of tissue sections were prepared for microscopic examination. These tissues included heart, lung, spleen, stomach, large and small intestine, pancreas, liver, gall bladder, adrenal, kidney, bladder, gonads, thyroid, bone marrow and brain. The histological examinations revealed no specific tissue effect from the ingestion of Methocel HG. The normal appearance of the mucosa of the stomach and of the small and large bowel deserves special mention.

SUMMARY

1. Groups of 50 male and 50 female rats were fed for a period of two years diets containing the following percentages of Methocel HG: 0 (control groups), 1, 5 and 20. Growth retardation was exhibited only by the male rats maintained on the 20 per cent diet. The addition of Methocel HG up to 20 per cent of the diet did not influence the life span adversely. At two years, the males averaged around 82 per cent mortality, the females around 66 per cent, regardless of diet. Urine analyses and hematological examinations gave mostly normal values. No significant variation in organ weights existed from group to group. From histological examinations, no evidence of tissue injury was found.

2. Two dogs each were given for one year the following doses of Methocel HG by mouth: 0 (a control group), 0.1, 0.3, 1.0 and 3.0 gm./kgm./day. The dogs maintained body weights or gained slightly. There were no deaths. Urine analyses for sugar and protein gave uniformly negative results. Periodic hematological examinations gave mostly normal values. Gross autopsy and histological examinations revealed scattered abnormalities but no evidence of any tissue change ascribable to the administration of Methocel HG.

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Methocel is the registered trade mark of the Dow Chemical Company.

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Changes in Serum Proteins during Methylcellulose-Induced Regression of Murphy-Sturm Lymphosarcoma*

Zdenek Hruban, M.D., Allan Lazar, M.D., and Agris Slesers, B.S.

Methylcellulose is a long chain polymer which has recently been shown to produce regression of several transplantable animal tumors.[1-3] The mechanism of regression is not clear. The inhibition of tumor growth may be related to: (1) changes in the host observed following administration of methylcellulose, for example, hypersplenism and hemolytic anemia,[4-6] and leukocytosis and changes in the coagulability of the blood;[5] (2) hyperplasia of the reticuloendothelial system, possibly associated with increased production of tumor growth-regulating macromolecules as previously proposed by Stern;[7] and (3) an enhancement of a homograft reaction possibly due to increased production of antitumor antibodies.

The present experiments were performed to study the behavior of serum proteins in tumor bearing rats during treatment with methylcellulose (MC). The spleens of rats that were given injections of tumor cells killed by freezing were studied to establish how the pyroninophilic response is influenced by administration of MC.

PLAN OF EXPERIMENTS

The effects of continuous injections of MC on the serum proteins of tumor-free rats were studied in the first experiment. The rats received an intraperitoneal injection of MC three times weekly, and samples of serum were obtained on the 11th, 13th, 22nd, 29th, and 38th days after MC treatment was started.

In experiment 2, samples of serum were obtained from untreated rats bearing subcutaneous Murphy-Sturm lymphosarcoma; the serum samples were taken on the 12th and 28th days after transplantation of tumor.

The effect of treatment with MC on rats bearing subcutaneous Murphy-Sturm lymphosarcoma was studied in the third and fourth experiments. In experiment 3 treatment with MC was started at the following times: 10 days before transplantation of tumor (pretreated group); 1 day after transplantation of tumor (simultaneous treatment); and 8 days after transplantation of tumor (delayed treatment). Samples of blood in experiment 3 were obtained on the 12th and 28th days after transplantation of tumor.

Treatment with MC in experiment 4 was started on the fifth day after transplantation of tumor, the rats were killed on the 13th day after transplantation and serum samples were obtained from cardiac blood. Whereas in the first three experiments the rats were fed Purina laboratory chow diet *ad libitum*, the rats in the fourth experiment were force fed a maintenance amino acid diet.[8]

The splenic immune response[9-12] was studied in experiments 5 and 6. In experiment 5, 2 ml. of 2.5 per cent MC were injected intraperitoneally once. Four days later 1 ml. of isotonic homogenate of Murphy-Sturm lymphosarcoma was injected into a vein in the tail. The cells in this homogenate were killed by repeated freezing and thawing. Animals were killed 12, 24, 36, and 48 hours, and 3, 4, 5, and 6 days later. In experiment 6, 5 ml. of 1 per cent MC in saline was injected intravenously. On the fourth day 2.5 ml. of a similar lymphosarcoma homogenate were injected intraperitoneally, and animals

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From the Department of Pathology, University of Chicago, Chicago 37, Illinois. Dr. Hruban is an Advanced Clinical Fellow of the American Cancer Society, and Dr. Lazar is a United States Public Health Service Postdoctoral Fellow.

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were killed on days 3 to 7. In both experiments rats injected with MC only and tumor homogenate only were used as controls.

MATERIALS AND METHODS

Adult, male, non-litter mate Sprague-Dawley rats of similar age and weight were used in each experiment. The average weights were: experiment 1, 384 gm.; experiments 2 and 3, 345 gm., experiment 4, 270 gm.; experiment 5, 262 gm.; and experiment 6, 405 gm. The maintenance amino acid diet[20] was force-fed in experiment 1 three times daily for 8 days starting on the fifth day after transplantation of tumor.

The Murphy Sturm lymphosarcoma was transplanted as previously described.[7, 10] The tumors were measured in two external diameters (a, b) at right angles with a caliper, and the volumes were calculated by the formula:

$$a \times b \times \frac{(a + b)}{2} \times 0.514$$

MC (Methocel, 400 centipoise, U.S.P.) was injected intraperitoneally as 2 ml. of a 2.5 per cent aqueous solution three times weekly on Monday, Wednesday, and Friday, except as noted above in experiments 5 and 6. The effect on the growth of tumor and histologic changes observed are reported separately.[10]

The electrophoretic studies were performed with the Spinco model R apparatus, using Spinco B-2 Veronal buffer, pH 8.6 and ionic strength 0.075, and a constant current of 8 ma. per cell for 16 hours at room temperature. The strips of paper were stained with bromphenol blue and scanned with the Spinco model RB Analytrol. The total protein was determined by the biuret method.[8]

Samples of organs of rats from experiments 5 and 6 were fixed in Carnoy's fluid and stained with methyl green-pyronin.

RESULTS

The electrophoretic patterns of the sera from the untreated tumor-bearing rats showed a decrease of total protein, albumin, and α_1- and γ-globulin, and a marked increase of α_2-globulin (Table 1, Figs. 1 to 4). The two animals that had growing tumors and survived for 28 days showed a marked decrease of β-globulins; their α_2-globulin was lower than had been determined on these animals 16 days previously but was still elevated above base line.

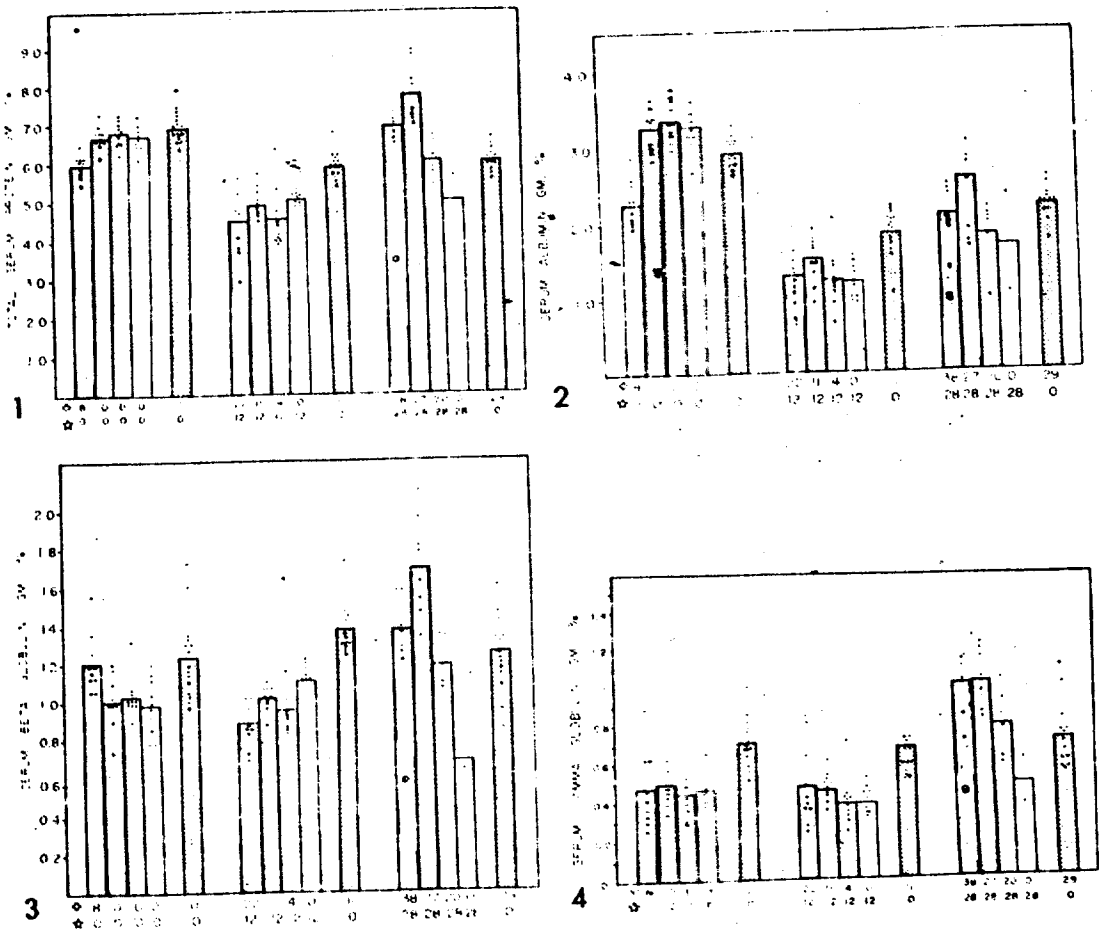
Administration of MC to normal, non-tumor bearing rats, resulted in a decrease of total proteins, albumin, and α_1-globulin. An early decrease of γ-globulin was followed by a return to pretreatment levels. An increase of α_2- and β-globulins was observed in the early period of treatment with MC (Table 2, Figs. 1 to 4).

Some of these animals developed ascites. The amount of ascitic fluid ranged from 0 to 185 ml., with an average of 55 ml. The ascitic fluid was pooled at the end of this experiment and gelatinous clots were removed by centrifugation. The levels of individual protein fractions of the supernatant ascitic fluid were about half the serum protein values. These proteins in the ascitic fluid were, in grams per 100 ml.: total protein, 2.97; albumin, 0.95; α_1-globulin, 0.66;

TABLE 1. SERUM PROTEINS OF UNTREATED RATS BEARING MURPHY STURM LYMPHOSARCOMA[a]

Time of bleeding[b]	No. of rats: Dead of tumor	No. of rats: With [illegible] tumor	No. of rats: With [illegible] tumor	Total protein	Albumin	Globulins: α_1	Globulins: α_2	Globulins: β	Globulins: γ	Calculated tumor volume
				(gm.)						(cc.)
Before				6.76 ± 0.42	3.27 ± 0.08	1.52 ± 0.07	0.51 ± 0.03	0.99 ± 0.04	0.18 ± 0.03	0
12 days after	6	10	0	5.18 ± 0.01	1.19 ± 0.08	1.21 ± 0.01	1.28 ± 0.05	1.11 ± 0.02	0.40 ± 0.03	[illegible]
28 days after	8	2	0	5.09 ± 0.64	1.67 ± 0.57	1.11 ± 0.19	0.78 ± 0.10	0.69 ± 0.05	0.50 ± 0.12	225 ± [illegible]

[a] The figures in all the tables represent the arithmetic mean and standard deviation of the mean.
[b] Before and after tumor transplantation.



FIGS. 1 TO 4. Serum proteins of tumor free and tumor bearing rats treated with methylcellulose. Experiment 1, *single column*; experiments 2 and 3 *adjacent* [illegible]. The first row (4-) indicates days of methylcellulose treatment; the second row [illegible] indicates days after tumor transplantation. The *dot in circle* [illegible] represents animal with nonregressed tumor.

FIG. 1. Changes in total serum protein.

FIG. 2. Changes in serum albumin.

FIG. 3. Changes in serum 3 globulin.

FIG. 4. Changes in serum [illegible] globulin.

TABLE 2. SERUM PROTEINS OF TUMOR FREE RATS TREATED WITH METHYLCELLULOSE

Time of Feeding	No. of Rats	Total Protein	Albumin	Globulin			
				[illegible]	[illegible]	[illegible]	[illegible]
4 before	15	7.06 ± 0.10	2.91 ± 0.05	1.42 ± 0.03	0.76 ± 0.03	1.24 ± 0.06	0.73 ± 0.03
4 after	15	5.18 ± 0.17	1.72 ± 0.08	1.29 ± 0.03	0.93 ± 0.03	1.21 ± 0.05	0.55 ± 0.04
11 after	14	6.02 ± 0.10	1.81 ± 0.08	1.28 ± 0.04	0.86 ± 0.02	1.38 ± 0.03	0.69 ± 0.07
22 after	13	5.87 ± 0.08	1.91 ± 0.07	1.24 ± 0.03	0.78 ± 0.02	1.28 ± 0.03	0.67 ± 0.05
29 after	13	6.10 ± 0.10	2.17 ± 0.06	1.25 ± 0.03	0.71 ± 0.03	1.26 ± 0.05	0.72 ± 0.05
38 after	13	5.67 ± 0.13	1.66 ± 0.05	1.29 ± 0.05	0.76 ± 0.02	1.21 ± 0.03	0.73 ± 0.05

* Number of days before or after beginning treatment with methylcellulose.

TABLE 3. SERUM PROTEINS OF RATS WITH MURPHY STURM LYMPHOSARCOMA, TREATMENT WITH METHYLCELLULOSE (MC) STARTED ON DAY FOLLOWING TUMOR TRANSPLANTATION

Time of bleeding, in days, from:														
Tumor transplantation (Table)	Onset of MC treatment	Dead	Number of rats	Weight of rats, gm	Liver, gm	Albumin	α globulins		β globulin	γ globulin				Calculated tumor volume
Before	Before			6.82 ± 1.12	2.5 ± 0.08	3.45 ± 0.05	0.51 ± 0.03		1.51 ± 0.01	0.51 ± 0.05				
12 after	11 after	1	5	5.0 ± 0.16	1.9 ± 0.04	1.15 ± 0.05	0.88 ± 0.06		1.12 ± 0.02	0.18 ± 0.03	12	±	3	
28 after	27 after	3	0	7.8 ± 0.27	2.5 ± 0.20	3.60 ± 0.05	1.53 ± 0.07		1.70 ± 0.09	1.03 ± 0.05	0			

* One additional rat ... regressed ... other ...

α_2 globulin, 0.11; β globulin, 0.68; and γ globulin, 0.27.

The animals in the MC treated, tumor bearing groups showed certain similarities in the serum proteins (Tables 3, 4, and 5; Figs. 1 to 4). The total proteins, albumin, and α_2 globulin were lower during the period of rapid growth of tumors in the sera of these groups than in tumor free animals that had received comparable amounts of MC. The γ globulin rose to very high levels in all tumor-bearing, MC treated animals at the time of regression of tumor. The sera from tumor bearing rats in the "pretreated" and "simultaneously MC treated" rats showed, after regression of tumor, an elevation of total proteins, α_1, α_2, and β globulins; the recovery of albumin was only partial. The levels of individual serum protein fractions in the MC pretreated rats whose tumors did not regress were lower than the levels seen in the untreated rats bearing tumors.

The rats in experiment 4 were force fed to guarantee equal calorie and protein intake. The rats that were not treated with MC lost 3.2 per cent of their carcass weight. The rats treated with MC gained 2.5 per cent of carcass weight during the 9 days of treatment; ascites contributed to part of this increase in weight. The total protein, albumin, and α and γ globulins were higher in the MC treated rats in which the tumors regressed, as compared with the untreated group or with the rats with non regressed tumors in the treated group (Table 6).

Histologic examination of the spleens of rats from experiment 5 revealed a delay in the appearance of pyroninophilic cells in the MC treated group. The immune reactions, judged by the percentage of pyroninophilic cells and lymphocytes in the red pulp,[18, 19] appeared on the fourth day in the group that received only intravenous tumor homogenate. The immune reaction in the group that received a single intraperitoneal injection of MC 4 days before the intravenous injection of tumor homogenate occurred on the sixth day after injection of tumor homogenate. MC was localized predominantly in the marginal zone of the follicles at first in all groups receiving this drug; on subsequent days it was also present in the red pulp. The pyroninophilic cell response to tumor in the spleens of MC treated rats in experiment 6 was at its peak on the sixth day, but represented only a fraction of the response seen in the rats that did not receive MC (Figs. 5 to 8).

The pyroninophilic cell response in the medullary cords of mesenteric lymph nodes was marked in the groups that received tumor homogenate intraperitoneally. The pyroninophilic cells were present in large numbers on the third day and persisted without change throughout the experiment. The number and distribution of pyroninophilic cells of these lymph nodes in the MC treated and untreated groups were similar. MC could not be demonstrated in the mesenteric lymph nodes of rats receiving the drug intravenously.

DISCUSSION

The results of these experiments show a similarity in the early effects produced by the growth of Murphy Sturm lymphosarcoma and by treatment with MC. Both produce an early increase in β globulin and decrease in γ globulin, and a prolonged

TABLE 4. SERUM PROTEINS OF RATS WITH MURPHY STURM LYMPHOSARCOMA; TREATMENT WITH METHYLCELLULOSE (MC) STARTED 10 DAYS BEFORE TRANSPLANTATION

Time of bleeding, in days, from:		No. of rats			Total protein	Albumin	Globulins				Calculated tumor volume
Tumor transplantation	Onset of MC treatment	Dead of tumor	With growing tumor	With regressed tumor			α	α	β	γ	
					gm. %						cc.
Before	Before				6.65 ± 0.09	2.27 ± 0.06	1.28 ± 0.03	0.82 ± 0.01	1.21 ± 0.05	0.49 ± 0.06	
12 after	22 after	0	10	0	4.60 ± 0.27	1.27 ± 0.[illegible]	1.08 ± 0.05	0.87 ± 0.06	0.89 ± 0.01	0.49 ± 0.07	20 ± 3
28 after	38 after	1[a]	0	7	7.02 ± 0.17	2.07 ± 0.14	1.43 ± 0.05	1.13 ± 0.07	1.38 ± 0.05	1.02 ± 0.07	0
[illegible]			1	0	3.45	0.95	0.85	0.60	0.58	0.46	79

[a] One additional rat died of anesthesia.

TABLE 5. SERUM PROTEINS OF RATS WITH MURPHY STURM LYMPHOSARCOMA; TREATMENT WITH METHYLCELLULOSE (MC) STARTED 8 DAYS AFTER TUMOR TRANSPLANTATION

Time of bleeding, in days, from:		No. of rats			Total protein	Albumin	Globulins				Calculated tumor volume
Tumor transplantation	Onset of MC treatment	Dead of tumor	With growing tumor	With regressed tumor			α	α	β	γ	
					gm. %						cc.
Before	Before				6.88 ± 0.12	3.36 ± 0.09	1.57 ± 0.06	0.45 ± 0.03	1.04 ± 0.03	0.46 ± 0.03	
12 after	4 after	0	10	0	4.65 ± 0.25	1.23 ± 0.11	1.18 ± 0.06	0.96 ± 0.05	0.96 ± 0.04	0.40 ± 0.04	14 ± 2
28 after	20 after	2[a]	0	6	6.12 ± 0.63	1.82 ± 0.18	1.34 ± 0.05	0.97 ± 0.09	1.19 ± 0.05	0.80 ± 0.08	0

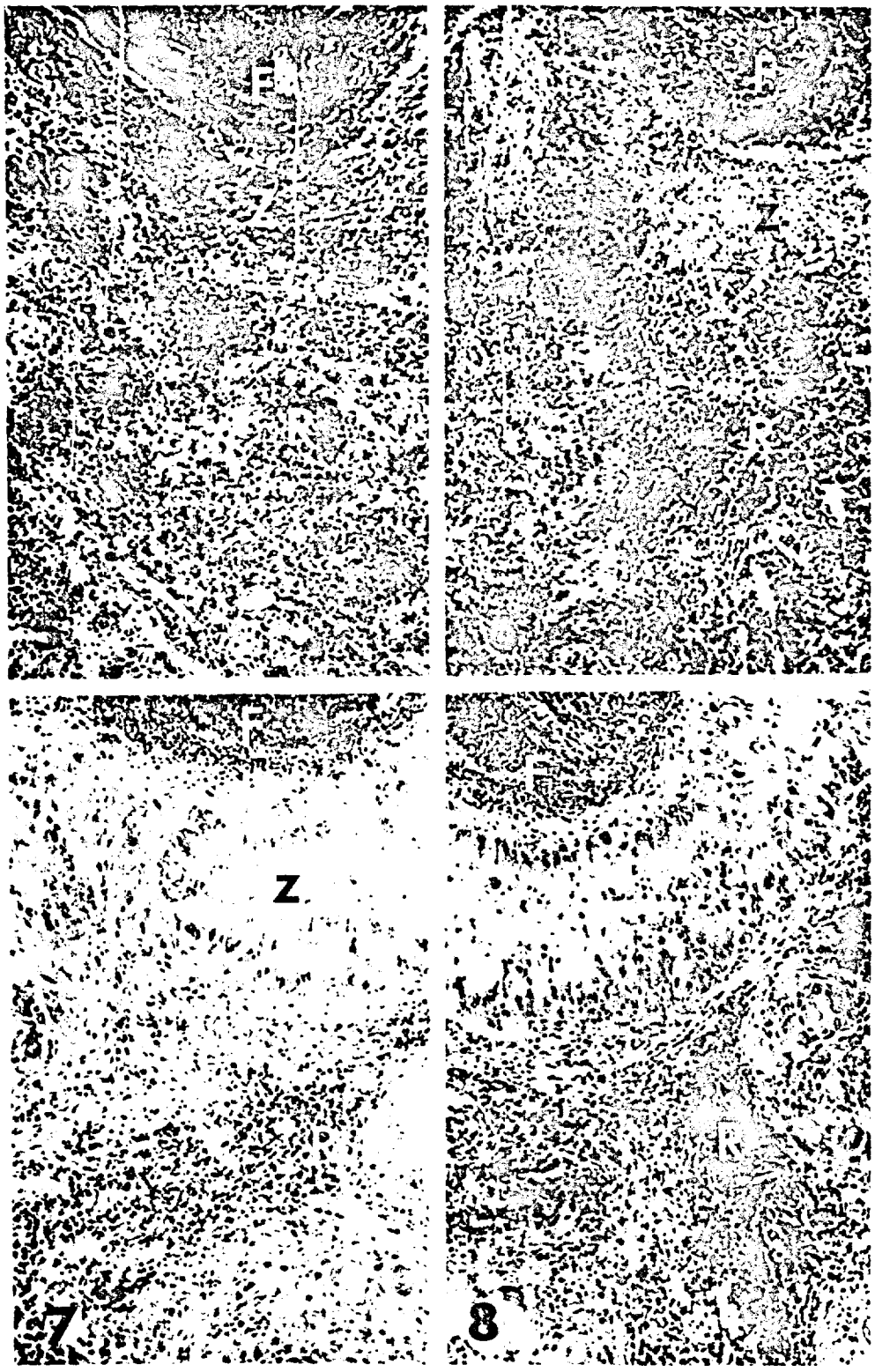
[a] One additional rat died in anesthesia.

TABLE 6. SERUM PROTEINS OF RATS WITH MURPHY STURM LYMPHOSARCOMA; TREATMENT WITH METHYLCELLULOSE (MC) STARTED 5 DAYS AFTER TUMOR TRANSPLANTATION. ANIMALS FORCE-FED A MAINTENANCE AMINO ACID DIET

Time of Bleeding, in days, from:		No. of rats			Total protein	Albumin	Globulins			Calculated tumor volume
Tumor transplantation	Onset of MC treatment	Dead of tumor	With growing tumor	With regressed tumor			α	α	β	
					gm. %					cc.
13 after	9 after	0	0	3	5.48 ± 0.01	1.88 ± 0.13	1.15 ± 0.05	0.67 ± 0.03	1.15 ± 0.02	0.63 ± 0.08	2 ± 1
			2	0	4.46 ± 0.10	1.13 ± 0.17	1.24 ± 0.25	0.78 ± 0.32	0.90 ± 0.26	0.40 ± 0.00	38 ± 3
13 after	None given	0	5	0	4.12 ± 0.16	1.01 ± 0.13	1.13 ± 0.16	0.79 ± 0.04	0.78 ± 0.02	0.50 ± 0.01	41 ± 2

decrease in total proteins and albumin. Changes in serum proteins attributable directly to the growth of Murphy-Sturm lymphosarcoma are the decreases in total protein, albumin, and γ-globulin.[8] The increase in α-globulin and β-globulin levels seen in untreated tumor-bearing rats is probably the result of necrosis and infection of the overlying skin when it becomes invaded by tumor.[8,15] The small tumors in the MC-treated groups, in general, did not invade the skin, and therefore this factor did not affect the serum protein composition.

The effects of MC treatment on the serum proteins of tumor-free rats were previously studied by Fröhlich *et al.*,[2,3] who reported a decrease in total protein, albumin, and α- and β-globulins and an increase in γ-globulins after 33 days of treatment with MC. In our experiments a difference between early and late changes in the serum protein patterns during MC treatment was seen.



Figs. 5 to 8

The absolute values of γ globulins seen in our experiments were much lower than those reported by Fröhlich and his co-workers.

In tumor-bearing rats treated with MC the reversal from rapid growth to regression occurred at about the 11th day after transplantation of tumor.[7] Prediction, based on serum protein patterns, of which tumors would regress and which would grow could not be made at this time.

Although the total serum proteins of rats in which the tumors regressed after treatment with MC were about the same as those prior to the transplantation of tumor, the individual fractions were present in different proportions. The albumin was lower than its pretreatment level, and the α_2-, β-, and γ globulins were higher. The low serum albumin was due, at least in part, to loss of significant amounts of albumin in the urine as part of a nephritic type syndrome resulting from MC induced damage in the kidney[6] and the formation of ascitic fluid.

Effects of antitumor treatment on serum proteins have been reported previously. Serum protein changes in animals bearing Yoshida ascites tumor treated with endoxan and E39 resulted in decrease of γ globulins,[5] and thus differ from the results obtained with MC. These observers attributed elevated α- and β-globulins to necrobiosis of tumor. Other authors[4] observed in man an increase in α- and β-globulins following treatment with triethylenemelamine (TEM); they ascribed these variations to enhancement of nucleoprotein breakdown. In our experiments almost all globulins were elevated in animals with regressed tumors. Elevation of γ globulins is a common finding in cancerous patients;[2] it is not present, however, in animals with grafted tumor of nonviral origin.[3]

The responses of splenic pulp of the rat to single injections of different particulate antigens are quite consistent.[8, 10] By the fourth day after intravenous injection of the antigen, the red pulp becomes crowded with large cells that have abundant pyroninophilic cytoplasm and large vesicular nuclei. Small, dark cells appear between the fifth and eighth days; the serum antibody peak is reached at about day 6.

The regression of tumor after treatment with MC is associated with an increase in γ globulins, which occurs in spite of a delayed appearance of pyroninophilic cells in the spleen. As the tumors in animals treated with MC start to regress on day 11, and the splenic pyroninophilic response occurs on day 6, the process responsible for tumor regression probably is most active between the 6th and 11th days after tumor transplantation. This process may be mediated through the production of antitumor antibody, as suggested by the high levels of γ globulin in rats with regressed tumors. On the 12th day of tumor growth, however, the γ-globulins have low levels, which may reflect a binding of these globulins to tumor.

SUMMARY

Methylcellulose treatment of rats bearing the Murphy-Sturm lymphosarcoma results in a significant percentage of complete regressions of tumors. The electrophoretic studies of the sera from these animals reveal that the dominant changes in the serum proteins during the early growth phase of the tumor could be attributed to the presence of tumor. At the time of regression of tumor the rats treated with methylcellulose have high levels of γ globulin, elevated β globulins, and pretreatment levels of total serum proteins. These changes in methylcellulose-treated animals occur in spite of a delayed immune response to tumor, evaluated by the

FIG. 5. Spleen of control rat. *R*, Red pulp; *Z*, marginal zone; *F*, follicle. Methyl green pyronin; ×200.

FIG. 6. Marked immune response in the red pulp (*R*) following intraperitoneal injection of lymphosarcoma 6 days previously. Methyl green pyronin; ×200.

FIG. 7. Spleen of rats 9 days after intravenous injection of methylcellulose. Giant cells are replacing marginal zone (*Z*) and forming nodules in red pulp (*R*). Methyl green pyronin; ×200.

FIG. 8. Slight immune response in the red pulp following intraperitoneal injection of lymphosarcoma 6 days previously, in rats receiving intraperitoneal methylcellulose 9 days previously. Giant cells are replacing marginal zone (*Z*) and forming nodules in red pulp (*R*). Methyl green pyronin; ×200.

appearance of pyroninophilic cells in the spleen.

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PATHOLOGY

Carcinogenic Studies on Water-Soluble and Insoluble Macromolecules

W. C. HUEPER, M.D., Bethesda, Md.

Numerous experimental investigations of the past decade have established the fact that various synthetic and semisynthetic, water-insoluble carbon and silicon polymers elicit sarcomas in rats and mice at the site of their subcutaneous or retroperitoneal implantation (Table 1).[1-23] Experimental studies of more recent years have shown that this property is shared by two water-soluble carbon polymers, namely, polyvinylpyrrolidone (PVP) and carboxymethylcellulose, when parenterally introduced into rats. Sarcomas developed at the site of their repeated subcutaneous injection in an aqueous solution, while reticulum-cell sarcomas of the liver and peritoneal lymph nodes represented the main neoplastic responses to the subcutaneous and intraperitoneal deposition of PVP in powder form or after its repeated intravenous introduction in saline solution into rats and mice.[24-26]

The target organs of carcinogenesis from water-soluble carbon polymers are under these conditions the tissues in which the macromolecular material is retained and stored and in which according to previous observations with these and other water-soluble synthetic, semisynthetic, and natural carbon polymers (polyvinyl alcohol, methylcellulose, pectin) a marked proliferation of reticuloendothelial cells and histiocytes accompanies the striking storage phenomena as well as the arterial atheromatosis developing after the parenteral administration of these colloids.[27-32] These proliferative and thesaurismotic reactions involving in part also the parenchymal cells of the liver, kidney, and brain and elicited by nonphysiologic macromolecules bear a close resemblance to those present in a number of spontaneous thesaurismoses which are characterized by the retention of lipoids, polysaccharides, mucopolysaccharides, and proteins of normal and abnormal types and of which some are associated with the occurrence of benign or malignant neoplastic lesions. Such physiologic thesaurismoses with neoplastic characteristics or complications are the various lipoidoses, such as essential xanthomatosis; Gaucher's disease; Niemann-Pick disease; the myocardial glycogenosis often accompanied by tumors of the brain, skin, and kidney; the mucopolysaccharidosis known as gargoylism; the microproteinosis associated with myelomatosis, and the megaloglobulinosis of Waldenström frequently associated with cancers of various organs but particularly those of the reticuloendothelial system. The experimental "polymer cancers" developing around the various synthetic and semisynthetic carbon and silicon polymers exhibit moreover a definite relationship to the pulmonary cancers induced in human lungs by the retention and in the vicinity of inhaled asbestos dust, which is a silicon oxide polymer.

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TABLE 1.—*Carcinogenic Macromolecular Carbon and Silicon Polymers*

Name	Description	Investigators
I. Water-Insoluble Polymers		
(a) Hydrocarbon Polymers Synthetic		
Polythene	Polyethylene	Oppenheimer et al., Druckrey et al., Bering & Handler
Lucite	Polymethylmethacrylate	Oppenheimer et al., Laskin et al., Zollinger
Polystyrol	Polyvinylbenzol	Oppenheimer et al.
Ivalon	Cross linked polyvinyl alcohol	Oppenheimer et al.
Dacron	Polyester condensate of terephthalate & ethylene glycol	Oppenheimer et al.
Bakelite	Phenol-formaldehyde condensate	Turner
(b) Halogenated Hydrocarbon Polymers (Synthetic)		
Saran	Polyvinylene chloride	Oppenheimer et al.
PVC, Igelit, Vestolit, Vinnol	Polyvinyl chloride	Oppenheimer et al.
Teflon	Polyfluor (chlor)-olefine	Oppenheimer et al.
Pliofilm	Polymethylmethacrylate chloride	Oppenheimer et al.
Vinyon N, Dynel	Copolymer of vinyl chloride and acrylonitrile	Oppenheimer et al.
(c) Aminized Hydrocarbon Polymers (Polyamides)		
Nylon	Polyhexamethylene diamine adipanide	Oppenheimer et al.
Perlon	Poly-e-caprolactam, polyurethane	Druckrey et al.
(d) Hydrocarbon Polymers (Semisynthetic & Natural)		
Rubber	Processed latex gum	Druckrey et al., Mecke
Cellophane	Processed polyglucose (cellulose)	Oppenheimer et al., Druckrey et al., Körbler & Frank
Linen		
Parchment paper	Processed cellulose	Oppenheimer et al.
Silk		Oppenheimer et al.
Keratin		Oppenheimer et al.
Ivory		Nothdurft
(e) Silicon Polymers		
Silastic	Polydimethylsiloxane processed (silicon rubber)	Oppenheimer et al., Mohr & Nothdurft
II. Water-Soluble Polymers		
PVP	Polyvinylpyrrolidone	Hueper Lusky & Nelson
CMC, Collocel	Carboxymethylcellulose	Lusky & Nelson
Tween 60	Polyoxyethylene sorbitan monostearate	Lusky & Nelson

The discovery of polymer or macromolecular carcinogenesis has created important and urgent scientific as well as practical problems. A major controversy has developed concerning the type and the specificity of the causative mechanism active in the development of these polymer cancers. Some investigators (Druckery, Hueper) favored the view that specific chemical or physicochemical properties of these macromolecular agents are responsible for and active in the carcinogenic process. Such qualities may be related to specific endgroups, to macromolecular peroxide cross linkages, to metabolic fragments or molecular chemical abnormalities, to residual valencies, to trapped free radicals, to complex formation with protein or mucopolysaccharides or with their building stones or to their molecular size and shape, or to the presence of impurities, such as traces of the catalyst used in their production (Haddow, Fitzhugh). Other workers (Nothdurft, Oettel, Oppenheimer et al.) have concluded that the great chemical diversity of the polymers involved in carcinogenesis and the apparent dependence of this property upon the absolute size of the implants and upon the size of the unbroken surface of the implants of water-insoluble polymers and on their physical status—i. e., film, disk, fabric, thread or powder—militates against a specific chemical causal mechanism of polymer carcinogenesis. They have advanced the concept that the carcinogenesis of the polymerized substances depends upon their ability to elicit in the tissues the formation of a fibrous capsule from which in turn as the result of chronic nonspecific irritation and therefrom resulting continued cell proliferation sarcomas originate on a multicentric basis. The tendency toward the formation of a fibrohyaline capsule depends chiefly, according to these investigators, upon the physical status of the polymer, being most marked when polymers are implanted as films and being least developed when the polymer is present in powder form. Polymer carcinogenesis, according to this concept, is a nonspecific physical phenomenon, which is, in the opinion of its supporters (Nothdurft, Oppenheimer et al.), essentially analogous to the sarcogenesis obtained around disks of certain metals follow-

TABLE 2.—*Carcinogenic Metals and Mineral Foils and Powders*

Name	Description	Investigators
Mica	Metal silicates	Oppenheimer et al.
Glass ?	Metal silicate	Oppenheimer et al.
Quartz	Silicon oxide polymer	Druckrey et al.
Asbestos	Metal silicon oxide polymer	Druckrey et al.
Mercury		Druckrey et al.
Silver		Nothdurft
Gold		Nothdurft
Platinum		Nothdurft
Stainless steel	Alloy of nickel, chromium, iron	Oppenheimer et al.
Steel		Oppenheimer et al.
Tantalum		Oppenheimer et al.
Vitallium	Alloy of cobalt & chromium	Oppenheimer et al.
Beryllium		Barnes et al., Gardner; Dutra et al.
Nickel		Hueper
Chromium		Hueper, Schinz
Cobalt		Heath, Schinz
Uranium		Hueper

ing their implantation into the subcutaneous tissue of rats (Table 2).[33-39]

Apart from the distinct scientific importance which these two divergent concepts on polymer carcinogenesis possess, they are also of immediate practical significance, since they must influence the carcinogenic screening techniques applied to polymers, as well as the assessment of possible carcinogenic hazards connected with exposure to the same polymer when present in different physical states and when incorporated in or in contact with consumer goods or when introduced into experimental animals or man by various routes.

The second and practical problem of major importance resulting from these observations on polymer carcinogenesis is closely linked to their use in the human economy. Water-soluble and insoluble macromolecular polymers of various types are being introduced into the human environment to a rapidly increasing degree and are being employed in numerous consumer goods, such as medicinal and cosmetic preparations, medicinal protheses, food and medicinal containers, packagings and coatings, household and sanitary goods, paints, dinnerware, and clothing. The human population, therefore, has frequent and intimate contact with polymers. The observations made on experimental animals, therefore, raises the question whether or not carbon and silicon polymers coming in contact with human tissues by various routes and in different forms may have a carcinogenic effect also upon man. Because of the possibility of a potential carcinogenic hazard to man posed by previous studies on polymers, a greatly extended experimental study of carbon and silicon polymers for elucidating the chemical and physical properties underlying their carcinogenicity and their carcinogenic mechanism was urgently needed.

Experimental Procedure

The experiments to be reported were conducted for these reasons on various water-soluble and insoluble carbon and silicon polymers. Ten PVP's were tested. Of these, four were of technical grade and three were of medicinal type, while three others were specially prepared for this study by American and German manufacturers. These three PVP's had a rather low average molecular weight, ranging from 10,000 to 23,000. Two of them, moreover, had a narrow molecular weight range. The average molecular weights of the entire PVP series ranged from approximately 10,000 to about 300,000. Six of them were made in Germany; three, in the United States, and one, in Great Britain. Different catalysts were used in their production, depending upon the manufacturer.

In an additional experiment, a polyvinyl alcohol preparation of American make and with an average molecular weight of 120,000 was used.

A third series of experiments comprised the testing of various polyglucoses. Eleven different dextrans manufactured by an American pharmaceutical concern and by the Regional Laboratory in Peoria, Ill., were used. These dextrans not only varied in molecular weight which covered a range between 37,000 and several million, but they differed among each other also in regard to their molecular shape. Some consisted of linear molecules, while others showed varying degrees of branching and thus had an almost globular shape. Other polyglucoses tested were a brand of methylcellulose with an average molecular weight of 140,000, an ethylcellulose of rather low molecular weight, and a soluble starch.

In addition to ethylcellouse the water-insoluble polymers studied were as follows: 1. Silastic, which is a processed polymer of dimethlysiloxane. This is vulcanized by the addition of 2,4-

TABLE 3.—*Carcinogenic Tests on Macromolecular Substances—Water-Soluble Polymers*

Chemical	Type	Average Mol. W.	Route	Dose, Mg. Single	Dose, Mg. Total	Species	Animals, No.
PVP 1	Technical	20,000	Subcut.	200	200	C57bl. mouse	50
PVP 2		20,000	Powder				
PVP 3		50,000	Intraper. powder	200	200	C57bl. mouse	50
PVP 4		300,000	Subcut. powder	500	500	Beth. bl. rat	30
			Intraper. powder	500	500	Beth. bl. rat	30
			Intraven. 7% sol.	18.5	150	Beth. bl. rat	15
			Intraven. 7% sol.	2,500	22,000	Dutch rabbit	3-6
PVP 6	Medicinal	50,000	Intraper.	1,000	15,000	Beth. bl. rat	20
PVP 7		50,000	20% sol.				30
			Intraven.	3,500	56,000	Dutch rabbit	6
			7% sol.	3,500	56,000	Dutch rabbit	6
			Subcut. powder	200	200	Beth. bl. rat	30
			Subcut. Rep. powder	200	600	Beth. bl. rat	30
Plasmosan		50,000	3.5% sol.	1,700	12,600	Dutch rabbit	6
PVP 5	Special	10,000	Subcut.	200	200	C57bl. mouse	30
			Powder	500	500	Beth. bl. rat	20
			Intraper.	200	200	C57bl. mouse	30
			Powder	500	500	Beth. bl. rat	20
PVP K17		18,000	Intraper.	500	2,000	Beth. bl. rat	30
PVP K25		23,000	25% sol.	6,250	62,500	Dutch rabbit	6
Polyvinyl alcohol	Technical	120,000	Subcut. powder	500	500	Beth. bl. rat	25
Methylcellulose	Technical	140,000	Subcut. powder	500	500	Beth. bl. rat	25
Soluble starch	Technical		Subcut. 45% sol.	500	500	Wistar rat	30
			Intraper.	500	500	Wistar rat	30
Dextran 1	Linear	200,000	Subcut.	200	200	C57bl. mouse	40
Dextran 2	Linear	100,000	Powder	500	500	Beth. bl. rat	20
Dextran 8	Branched	37,500	Intraper.	200	200	C57bl. mouse	20
Dextran 9	Branched	300,000	Powder	500	500	Beth. bl. rat	20
Dextran 10	Branched	89,400	Intraven.	1,750	17,500 to 35,500	Dutch rabbit	2-4
Dextran 11	Highly br.	71,400	7% sol.				
Dextran 3		Sev. million	Subcut.	200	200	C57bl. mouse	35
Dextran 4	Linear	75,000	Subcut.	200	200	C57bl. mouse	30
Dextran 5	Highly br.		Subcut.	200	200	C57bl. mouse	28
Dextran 6	Highly br.		Subcut.	200	200	C57bl. mouse	13
Dextran 7	Highly br.		Subcut; powder	200	200	C57bl. mouse	9

dichlorbenzoyl peroxide. Through the action of this chemical on the latex the dimethylsiloxane polymer molecule is altered from a straight linear molecule into a net molecule because of the formation of numerous cross linkages between the macromolecular chain molecules. The average molecular weight of the thereby resulting silicone rubber ranges from 300,000 to 400,000. The rubber contains, moreover, as a filler and plasticizer, a specially prepared and very pure silicon dioxide, which in the silicon rubber takes the place of carbon black used in the manufacture of natural or synthetic carbon rubber. 2. Polyethylene. 3. Polyurethane (Mondur TD-80), which is a modified polymer of tolylene-diisocyanate.

Pertinent data relative to the various macromolecular substances used as well as information on the general scheme of experimental investigations applied are presented in Tables 3 and 4.

The various water-soluble macromolecules were investigated for carcinogenic properties by administering them in single or repeated doses either as powders or as aqueous solutions through subcutaneous, intraperitoneal, and intravenous routes so as to determine the possible role which differences in chemical composition as well as in molecular size and shape might play in bringing about these responses. The water-insoluble polymers, on the other hand, were studied for ascertaining whether differences in the physical state of these substances —i. e., whether their presence in the form of cubes, balls, sheets (disks), films, sponges, or powders— is important in determining the occurrence and degree of cancerous reactions to parenterally implanted water-insoluble polymers. Rabbits, rats, and mice were employed for obtaining information on the species-specific character and range of such manifestations.

TABLE 4.—*Carcinogenic Tests on Macromolecular Substances—Water-Insoluble Polymers*

Chemical	Type	Average Mol. W.	Route	Dose, Mg. Single	Dose, Mg. Total	Physical State	Species	Animals, No.
Ethylcellulose	Technical	Low viscosity	Subcut.	500	500	Powder	Beth. bl. rat	25
Polyethylene			Intraper.	65	65	Cube 3.5X3.5X3 mm.	Beth. bl. rat	30
				65	65	Sheet 0.5 mm. thick, disk 12 mm. diam.	Beth. bl. rat	30
				65	65	Film 10X17.5 mm.	Beth. bl. rat	30
				65	65	Powder	Beth. bl. rat	30
Polyurethane			Subcut.	65	65	Sheet 2mm. thick 2 disks 3mm, 5mm. diam.	Beth. bl. rat	30
				65	65	Sponge 25X20X3 mm.	Beth. bl. rat	30
				65	65	Powder	Beth. bl. rat	30
			Intraper.	65	65	Sheet	Beth. bl. rat	30
				65	65	Sponge	Beth. bl. rat	30
				65	65	Powder	Beth. bl. rat	30
Polydimethyl-siloxane (latex) (gum)		High viscosity	Subcut.	300	300	Ball	Beth. bl. rat	30
			Intraper.	300	300	Ball, 5 mm. diam.	Beth. bl. rat	30
Polydimethyl-siloxane rubber (Silastic)			Subcut.	300	300	Cube	Beth. bl. rat	30
			Intraper.	300	300	Cube 5 mm. diam.	Beth. bl. rat	30
Silica			Subcut.	300	300	Powder	Beth. bl. rat	30
			Intraper.	300	300	Powder	Beth. bl. rat	30

The aqueous solutions of the polymers used were prepared with isotonic saline and were autoclaved for insuring their sterility. PVP (Plasmosan), as well as the solutions of PVP K17 and PVP K25, was supplied in ampules. The maximal observation period for mice and rats was two years; for rabbits, up to four years. All survivors were killed at the end of this period. Autopsies were performed on all animals. Histologic examinations of the tissues were made on all animals exhibiting grossly demonstrable pathologic changes or on at least 30% of animals of each series regardless of the presence or absence of such lesions. The organs of all rabbits were studied histologically. Normal untreated animals of the same strain and animals given parenteral implants or injections of various noncarcinogenic vehicles (wool fat, gelatin, tricaprylin) or test chemicals served as controls. They were permitted to live for the same length of time as the animals of the experimental series and were used for determining the type, age, and site distribution of spontaneous tumors.

The neoplastic lesions observed among the animals of the different test series in relation to the species and polymers used and the routes of their administration employed are listed in Table 5.

Experimental Observations

In the assessment of the relative significance of the cancers observed in the various experimental series in relation to the different polymers administered, the following criteria were considered:

(*a*) The absolute and relative number of cancers in a specific test series.

(*b*) The number of animals in a particular test series.

(*c*) The degree of uniformity of neoplastic response in several test series in which the same polymer was given to the same species by different routes.

(*d*) The degree of uniformity of neoplastic responses in test series in which different species were employed and to which the same polymer was administered.

(*e*) The topographical distribution of cancers in specific organs and tissues and their histogenetic types in their relation to the sites of retention and/or storage of the polymer introduced, i. e., the identity of the site of the implanted or stored polymer with the site of the subsequently developing proliferative hyperplastic and precancerous lesions and cancers.

(*f*) The relative frequency of spontaneously occurring cancers of the same type and site in control series of normal untreat-

TABLE 5.—*Neoplastic Reactions in Mice, Rats, and Rabbits Following*

Chemical Molecular Weight	Route Admin.	Species	No. Animals	Sites & Types: Lymph Nodes: Lymphoma	Lymph Nodes: Leukemia	Liver: Ret. Sarcoma	Liver: Cholangioma	Liver: Ret. Sarcoma	Liver: Leukemia	Lung: Adenoma	Lung: Mesothelioma	Heart: Mesothelioma	Breast: Adenofibroma
PVP 1	Subcut.	Mouse	50										
	In. per.		50										
20,000	Subcut.	Rat	30			4		2					
	In. per.		30			4	1						
	In. ven.		15			1		1					
	In. ven.	Rabbit	6										
PVP 2	Subcut.	Mouse	50	3									
	In. per.		50					1					
20,000	Subcut.	Rat	30				2						
	In. per.		30			1							1
	In. ven.		15										1
	In. ven.	Rabbit	4										
PVP 3	Subcut.	Mouse	50										
	In. per.		50	3									
50,000	Subcut.	Rat	30			5		2	1			1	
	In. per.		30				1		1			1	
	In. ven.		15	3									
	In. ven.	Rabbit	3										
PVP 4	Subcut.	Mouse	50	1									
	In. per.		50										
300,000	Subcut	Rat	30			4		2				1	
	In per.		30			6							1
	In. ven.		15			2							
	In. ven.	Rabbit	2										
PVP 5	Subcut.	Mouse	30										
10,000	In. per.		30	1								1	
	Subcut.	Rat	20			1		3					
	In. per.		20			2		1					
PVP 6	In. per.	Rat	20	Unfinished									
	Subcut.	Rat	30			4		1					
50,000	Rep. sbc.		30			1		1					
	In. ven.	Rabbit	6										
PVP 7	In. per.	Rat	30	Unfinished									
50,000	Subcut.	Rat	30					2					
	Rep. sbc.		30			2		1					2
	In. ven.	Rabbit	6										
Plasmosan 50,000	In. ven.	Rabbit	6										
PVP K17	In. per.	Rat	35	Unfinished									
18,000	In. per.	Rabbit	6										
PVP K25	In. per.	Rat	35	Unfinished									
23,000	In. per.	Rabbit	6										
PVA 120,000	Subcut.	Rat	25				1	4			1		2
Methocel 140,000	Subcut.	Rat	25										
Dextran 1	Subcut.	Mouse	40	1	1			2					
200,000	In. per.		20	1									
	Subcut.	Rat	20			2		1					2
	In. per.		20			1							2
	In. ven.	Rabbit	3										
Dextran 2	Subcut.	Mouse	40					1					
100,000	In. per.		20					4		2			
	Subcut.	Rat	20			1						1	1
	In. per.		20				1	2					
	In. ven.	Rabbit	3										
Dextran 3 Sev. million	Subcut.	Mouse	35	1						1			
Dextran : 75,000	Subcut.	Mouse	30			1							

of Tumors

Kidney	Skin		Uterus	Ovary		Adrenal		No. & % Tumors					
								Total		Benign		Malig.	
Carcinoma	Carcinoma	Sarcoma	Carcinoma	Cystadenoma	Carcinoma	Hemangioma	Adenoma	No.	%	No.	%	No.	%
								0					
								0					
						1		7	23	1	3	6	20
	1		2	2				10	33	3	10	7	23
			1					3	20			3	20
								0					
								3	6			3	6
								1	2			1	2
			1	1				4	13	3	10	1	3
			1	3				6	20	5	17	1	3
			1					2	13	2	12	1	1
								0					
								0					
								3	6			3	6
				1		1		11	37	2	7	9	30
			3	1				7	23	2	7	5	16
			2				1	6	40	1	7	5	33
								0					
								1	2			1	2
								0					
	1		1	2				11	37	2	7	9	30
			2					9	30	1	3	8	27
			1					3	20			3	20
								0					
								0					
								1	3			1	3
			2					6	30			6	30
								4	20			4	20
			2					7	23			7	23
			6					8	27			8	27
								0					
								2	7			2	7
			2					7	23	2	7	5	16
								0					
			1					9	36	3	12	6	24
			1					1	4			1	4
								4	10			4	10
								1	5			1	5
			7					7	35	2	10	7	35
			1					4	20	2	10	2	10
								0					
								1	2			1	2
								6	30	2	10	4	20
								3	15	1	5	2	10
			1					4	20	1	5	3	15
								0					
								2	6	1	3	1	3
								1	3			1	3

TABLE 5.—*Neoplastic Reactions in Mice, Rats, and Rabbits Following*

Chemical Molecular Weight	Route Admin.	Species	No. Animals	Sites & Types: Lymph Nodes: Lymphoma	Lymph Nodes: Leukemia	Lymph Nodes: Ret. Sarcoma	Liver: Cholangioma	Liver: Ret. Sarcoma	Liver: Leukemia	Lung: Adenoma	Lung: Mesothelioma	Heart: Mesothelioma	Breast: Adenofibroma
Dextran 5 H. Br.	Subcut.	Mouse	28			3							
Dextran 6 H. Br.	Subcut.	Mouse	13										
Dextran 7 H. Br.	Subcut.	Mouse	9			1							
Dextran 8	Subcut.	Mouse	40	1									
37,000	In. per.		20	1									
	Subcut.	Rat	20				1		1				1
	In. ven.	Rabbit	3										
Dextran 9	Subcut.	Mouse	40			1							
300,000	In. per.		20	1	1								
	Subcut.	Rat	20										
	In. per.		20			2		2					
	In. ven.	Rabbit	3										
Dextran 10	Subcut.	Mouse	40	1				2					
89,400	In. per.		20					4					
	Subcut.	Rat	20			1	1	3					1
	In. per.		20				1	2					
	In. ven.	Rabbit	3	1					1				
Dextran 11	Subcut.	Mouse	40					2	1				
71,400	In. per.		20										
	Subcut.	Rat	20					1					
	In. per.		20			2							1
	In. ven.	Rabbit	3							2			
Starch	Subcut.	Rat	30										6
	In. per.		30			1							1
Eth. cel.	Subcut.	Rat	25							1			
Silastic	Subcut.	Rat	30				2	1			2		1
rubber	Int. per.		30										
Polysil. latex	Subcut.	Rat	30										1
	Int. per.		30			2					2		
Silica	Subcut.	Rat	45			1	1	2			2		
	Int. per.		30					2					1
Polyurethane	Subcut.; int. per. disk; sponge; powder	6×30		Unfinished									
Polyethylene	Int. per. cube; disk; film 4×30; powder			Unfinished									
Normal controls		Rat	200			7		4			1		3
Treated controls		Mouse	840			6							

ed animals or those treated with noncarcinogenic chemicals.

When evaluating the data contained in Table 4 by applying the above listed criteria, carcinogenic responses attributable to the action of the polymer parenterally introduced into mice, rats, and rabbits are present for the following polymers: PVP 1, PVP 3, PVP 4, PVP 5, PVP 6, Dextran 10, Silastic rubber, and PVA. Such a relationship is probable but not definite for PVP 2, PVP 7, Dextran 1, Dextran 2, Dextran 5, Dextran 11, Polysilicone latex, and silica. For all other polymers, although some of them belong to the same general chemical group as the ones adjudged to be carcinogenic, the

a Parenteral Introduction of Water-Soluble and Insoluble Polymers—Continued

of Tumors

Kidney	Skin		Uterus	Ovary		Adrenal		No. & % Tumors					
								Total		Benign		Malig.	
Carcinoma	Carcinoma	Sarcoma	Carcinoma	Cystadenoma	Carcinoma	Hemangioma	Adenoma	No.	%	No.	%	No.	%
								3	10			3	10
								0					
								1	10			1	10
								1	2			1	2
								1	4			1	4
			1					4	20	2	10	2	10
								0					
								1	2			1	2
								2	10			2	10
			2	1				3	15	1	5	2	10
			1					5	25			5	25
								0					
								3	8			3	8
								4	20			4	20
1			1					8	40	2	10	6	30
			1					4	20	1	7	3	13
								2	70	1	35	1	35
								3	7			3	7
								0					
			1					2	10			2	10
			1					4	20	1	7	3	13
								2	66	2	66		
								6	20	6	20		
					1		1	4	12	2	6	2	6
			2					3	12			3	12
		10						16	55	3	10	13	43
								1	3			1	3
		1						2	6	1	3	1	3
			1					5	17			5	17
			1	1				8	18	2	4	6	14
			3					6	20	1	3	5	17
1			4		1			20	10	3	1	17	9
								6	0.7			6	0.7

experimental observations failed to demonstrate carcinogenic properties.

Whenever the cancer rate in a test series was found to be two to three times that of the control series, and especially when such a ratio was present following the introduction of a particular polymer by several routes and into several species and whenever the cancers involved organs and tissues in which the particular polymer had elicited storage phenomena and hyperplastic and precancerous cellular responses related to these reactions, the macromolecular substance in question was considered as possessing carcinogenic properties. The evidence

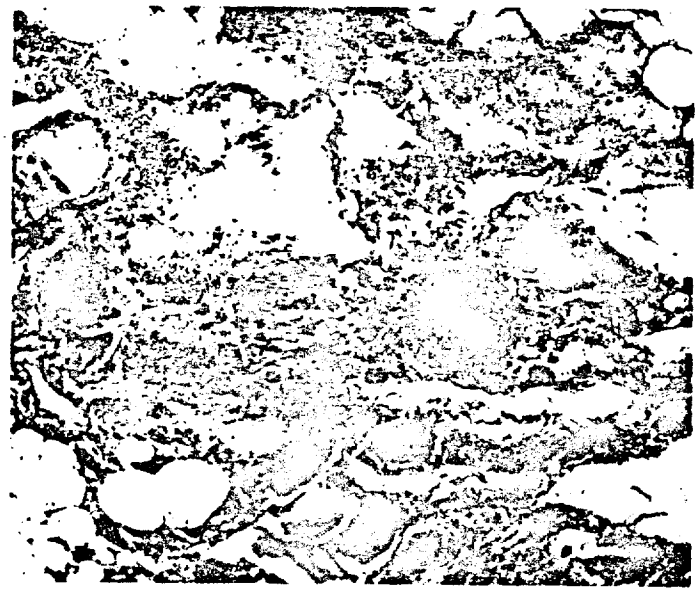

Fig. 1.—Fat tissue with giant cells filled with clumps of blue-stained matter (PVP) (rat).

underlying these conclusions is presented in the data listed in Table 5.

The histopathologic findings pertinent in this respect for the different polymers investigated are as follows:

1. Polyvinylpyrrolidones.—(*a*) Thesaurismotic Reactions: Storage reactions which were characterized by the appearance of bluish-stained material representing probably a PVP protein or mucopolysaccharide complex in usually swollen cells were observed in many organs and tissues of mice, rats, and rabbits, regardless of the molecular weight of the various PVP's tested. The degree of storage reactions depended mainly on the relative amounts of PVP administered and varied considerably between different series of animals and between animals belonging to the same series. In general they were most marked in rabbits, particularly those intravenously injected with Plasmosan and PVP's 6 and 7, i. e., the medicinal PVP's tested. They were somewhat less pronounced in rats and affected least the mice.

Fat tissue at the site of implantation contained often blue-stained phagocytic cells scattered throughout an otherwise normal tissue. Occasionally foci of huge multinu-

Fig. 2.—Meningeal membrane with foam cells with blue tinted cytoplasm (PVP) (rabbit).

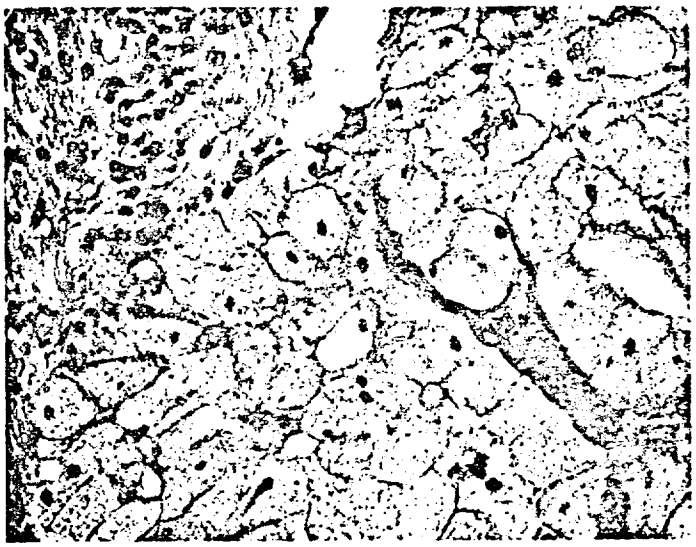

Fig. 3. — Choroid plexus with massive proliferation of foam cells with bluish stained cytoplasm; glia cells with blue cytoplasm in adjacent brain tissue (PVP) (rabbit).

cleated foam cells with bluish cytoplasm were found in the fat tissue (Fig. 1).

Massive accumulations of blue matter containing foam cells were noted in the meninges of rabbits given PVP 6 and 7 and Plasmosan (Fig. 2). In some animals this condition was associated with a distinct increase of histiocytic elements in the meninges. The ependymal cells as well as the cells of the choroid stroma were usually balloon-like, had a foamy and often blue-stained cytoplasm, and formed grape-like proliferations in the ventricles (Fig. 3). Groups of multinucleated giant cells were sometimes present in these masses (Fig. 4), while swollen and blue matter containing glia cells were noted at times in the adjacent brain tissue. In a few rabbits and mice also glia and ganglion cells of the basal ganglionic region exhibited a distinct blue stipling of their swollen cytoplasm. Cells with a bluish-stained cytoplasm were occasionally found also within peripheral nerves.

The lungs not infrequently revealed clusters of blue-stained foam cells located in the interstitial tissue. Giant-cell granulomas often were present in pulmonary capillaries occluding their lumens. The lumens of

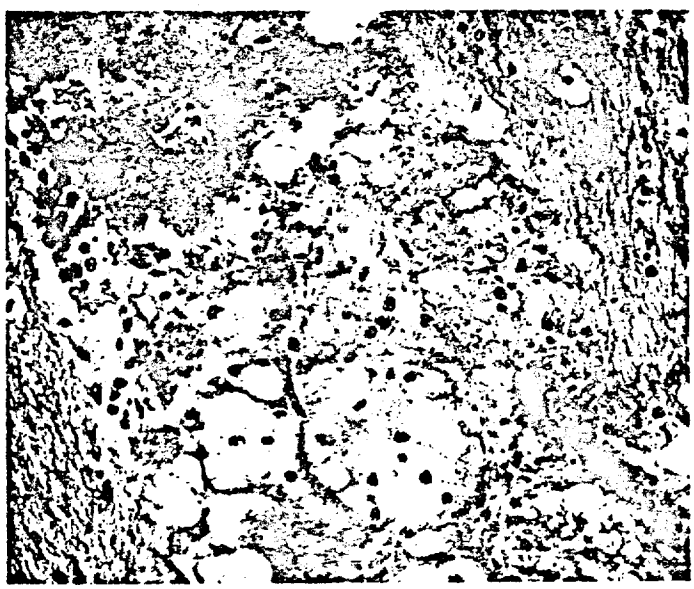

Fig. 4. — Choroid plexus with foam cells and giant cells with smudge-like clumps of blue-stained matter (PVP) (rabbit).

Fig. 5.—Pulmonary artery with an occluding foreign-body giant-cell granuloma (PVP) (rabbit).

medium-sized and large pulmonary arteries sometimes contained similar and often large giant-cell granulomas (Fig. 5).

Retention of PVP in the endothelial cells of the aortic intima of rabbits was not uncommon, producing a blue, bead-like cellular lining and being associated with an increase of endothelial cells. Such reactions sometimes resulted in the production of intimal foam-cell cushions in the ascending aorta, representing an experimental type of atherosclerosis (Fig. 6). Similar reactions were observed in the aortic leaflets as well as in the subendocardial tissue. Myocardial infiltrations with blue-stained foam cells were frequent and were usually most pronounced in the subepicardial area, where they gave rise to multiple focal accumulations of blue-stained foam cells.

Blue-stained matter was often noted in both liver cells and swollen Kupffer cells, which not infrequently were transformed into giant cells. Histiocytic balloon-like swollen blue foam cells were present in the periportal tissue, which occasionally was markedly increased in amount and which contained giant-cell granulomas (Fig. 7).

Mediastinal as well as abdominal lymph nodes revealed at times foci of blue-stained foam cells. Similar alterations occurred in

Fig. 6.—Ascending aorta with an intimal foam-cell cushion containing blue-stained matter (PVP) (rabbit).

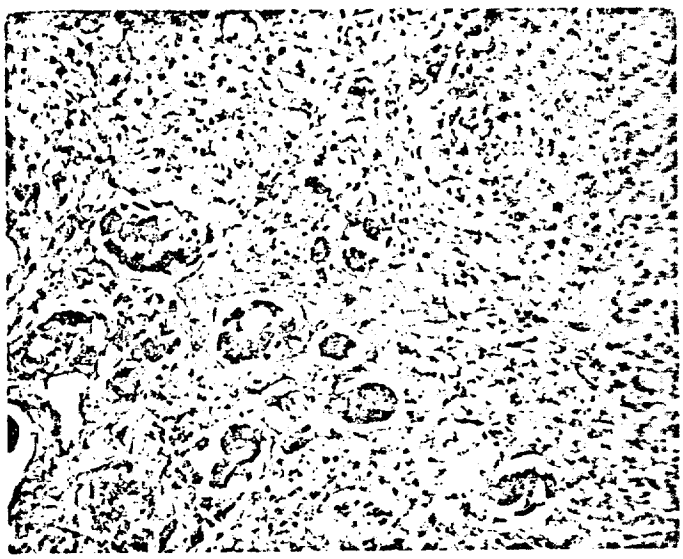

Fig. 7.—Liver of rat with interstitial fibrosis and clusters of foam cells and giant cells containing blue-colored material (PVP).

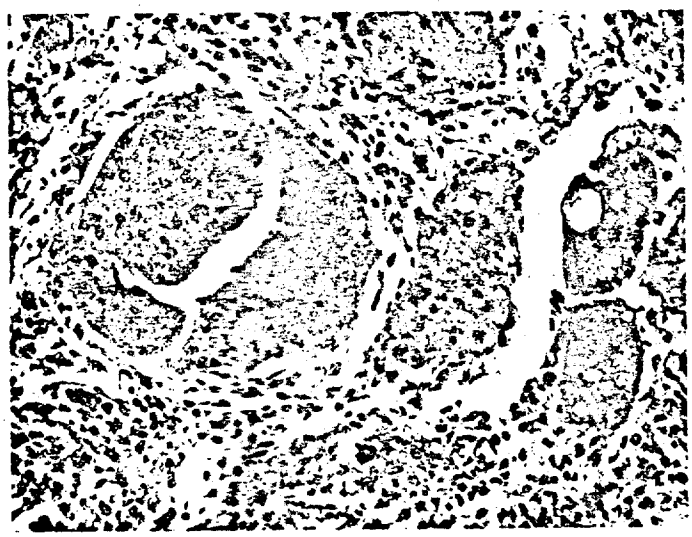

Fig. 8. — Clusters of multinucleated giant cells in the splenic pulp containing blue-stained foam cells (PVP) (rabbit).

Fig. 9.—Kidney of rat with glomeruli composed of foam cells faintly bluish-stained (PVP).

Fig. 10.—Endometrium of rat with interstitial foam cells and giant cells which are deeply blue-stained (PVP).

the spleen, where the pulp contained occasionally masses of huge multinucleated syncytia (Fig. 8), displaying a bluish-stained cytoplasm. "Lakes" of bluish matter apparently originating from a disintegration of reticulum cells were often observed in the bone marrow.

Blue-stained foam cells originating from capillary endothelium were noted in the renal glomeruli (Fig. 9). Bluish matter was contained also in tubular epithelial cells as well as tubular lumens. The tubules affected sometimes showed a hyperchromatic cell lining of syncytial character.

Massive deposits of PVP were often found in the endometrium, where they were located in clusters of foam cells as well as where they were infiltrating the intercellular spaces, thereby coming in close contact with the glandular epithelium (Fig. 10). Blue-stained histiocytes occurred frequently in the ovarian stroma.

It is evident from the evidence present that given adequate amounts of PVP this substance will be distributed diffusely in the body and will be retained in many organs and tissue not only in phagocytic cells but also in the parenchymal cells of several organs, such as the brain, liver, and kidney.

(*b*) Hyperplastic and Precancerous Reactions: Many of the organs and tissues in which PVP was stored displayed hyperplastic reactions which sometimes assumed

Fig. 11.—Alveolar adenomatosis of the lung in a rat (PVP intraperitoneally).

Fig. 10.—Endometrium of rat with interstitial foam cells and giant cells which are deeply blue-stained (PVP).

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Fig. 11.—Alveolar adenomatosis of the lung in a rat (PVP intraperitoneally).

Fig. 12.—Bronchiolar cyst lined by a stratified squamous-cell epithelium with peribronchiolar adenomatosis in a rat (PVP intraperitoneally).

metaplastic as well as "preneoplastic" characteristics if standards of human cancerology are applied. The storage of PVP in ependymal, choroid, and meningeal cells was always accompanied by marked proliferations of the cells affected.

Multifocal adenomatosis of pulmonary alveoli, particularly in the peribronchial areas, was rather frequently encountered in rats (Fig. 11). In several rats these lesions were associated with the occurrence of multiple cysts lined by a stratified squamous epithelium and originating apparently from bronchial structures (Fig. 12). The pleura as well as the epicardium and endocardium of rats exhibited focal proliferations of closely packed hyperchromatic oval cells, which displayed a tendency to invade the underlying tissue and therefore became suspected of possessing potentially malignant properties or of representing actually early cancerous manifestations.

Similar diagnostic difficulties were met in assessing the potential and actual biologic character of the focal as well as diffuse proliferations of Kupffer cells in liver. These cells possessed sometimes a foamy cytoplasm, at other times they represented round or oval cells arranged often in ill-defined nodules or trabeculae (Figs. 13 and 14). Decisions concerning the benign or malignant nature of some of these reactions were by necessity sometimes of a rather arbitrary nature. The spleen and lymph

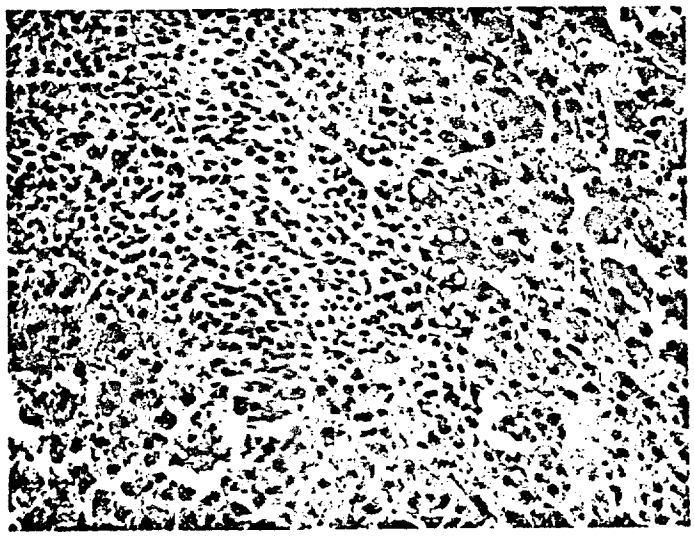

Fig. 13.—Focal Kupffer-cell proliferation in the liver of a rat (PVP subcutaneously).

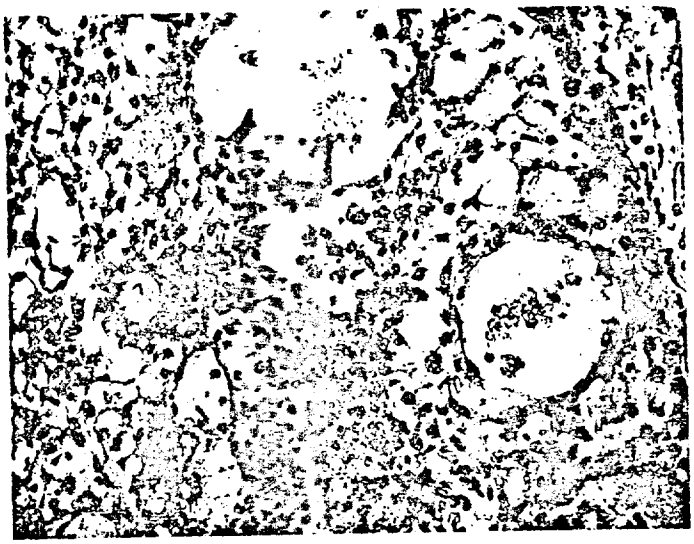

Fig. 14.—Focal Kupffer-cell proliferation of foam cell type in a rat (PVP).

nodes showed in some animals marked proliferations of reticulum cells and an occasional loss of follicular structure.

A marked glandular hyperplasia of the endometrial glands frequently assuming the character of a massive adenomatosis and associated with the formation of cysts, epithelial papillomatosis, and cellular atypia was noted in a considerable number of rats and rabbits. Such glandular proliferations were sometimes coexisting with focal or diffuse squamous-cell metaplasia of the endometrial epithelial lining as well as at times with areas of definite adenocarcinoma or squamous-cell carcinoma (Fig. 15). An unusual degree of cellularity of the endometrial stroma was also observed in some rats with PVP deposits.

Noteworthy in this connection is, moreover, the coexistence of large masses of blue-stained foam cells invading the adjacent muscular tissue located near an osteoid and possibly sarcomatous tumor involving the lower jaw in a rabbit (Fig. 16).

(*c*) Cancerous Reactions: The malignant nature of the lesions adjudged to be cancerous was attested not only by their histologic characteristics but also by their locally invasive growth, their diffuse involvement of the target organ, and often by the frequent presence of widespread metastases.

Fig. 15.—Adenocarcinoma of the uterus in a rat (PVP).

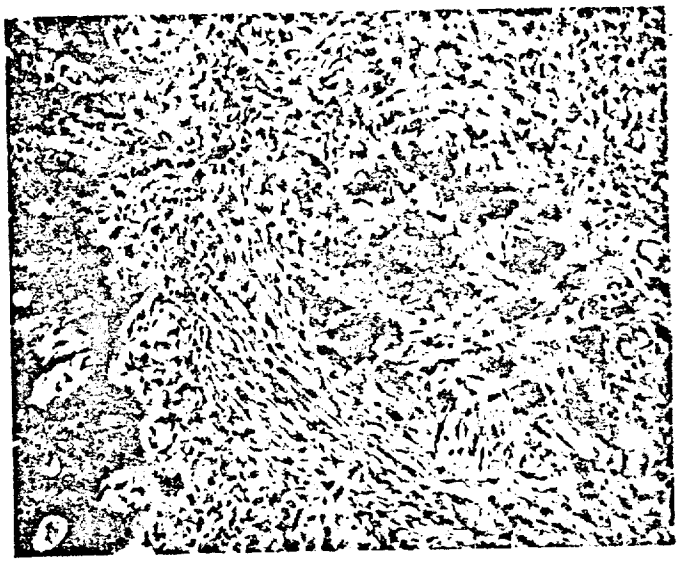

Fig. 16.—Sarcomatoid osteogenic tumor of the lower jaw with groups of phagocytic cells containing blue-stained material (PVP) (rabbit).

17. Endocardial mesothelioma in a rat (PVP).

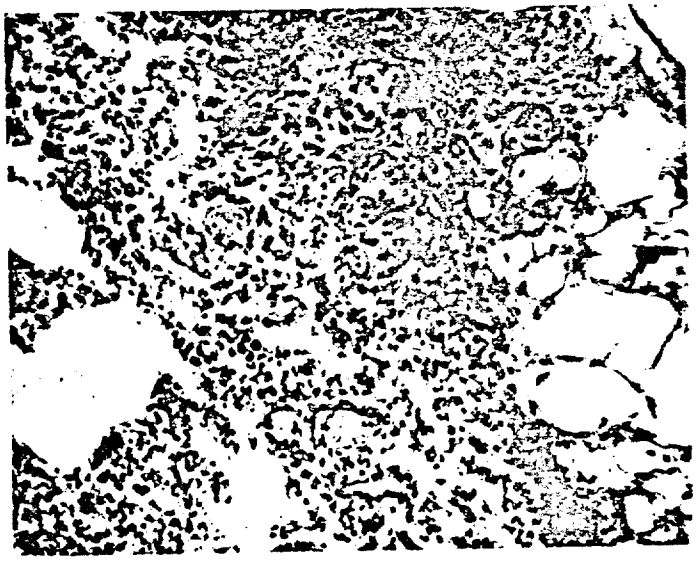

Fig. 18. — Marked mesothelial proliferation with foam cells of the pleura of probably sarcomatous nature in a rat (PVP).

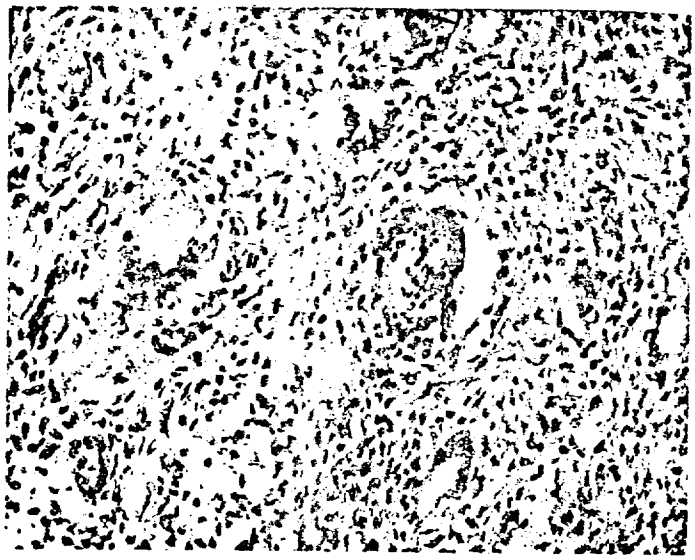

Fig. 19. — Kupffer-cell sarcoma with multinucleated giant cells of the liver in a rat (PVP).

The endothelio- or mesotheliosarcomatous neoplasms originating from the endocardial, epicardial, and pleural lining cells consisted of closely and irregularly packed oval cells invading the myocardium or lung, respectively. They extended along the pulmonary or cardiac vessels into the mediastinum and mediastinal lymph nodes and formed there large white medullary masses (Figs. 17 and 18).

The Kupffer-cell sarcomas displayed usually a similar cellular structure, being composed of strands or masses of oval-shaped cells or of irregularly round cells with a reticulum network. Huge multinucleated giant cells were present occasionally in these cell masses (Fig. 19). In addition to these sarcomatous hepatic lesions a few livers displayed changes of a leukemic character of monocytic or lymphocytic type.

Similar round cellular malignant neoplasms forming nodular masses in the peritoneal lymph nodes and invading the abdominal and thoracic organs apparently originating from the reticulum cells of lymph nodes and only occasionally from those of the spleen. The presence of giant cells in some of these tumors produced a resemblance with histologic changes found

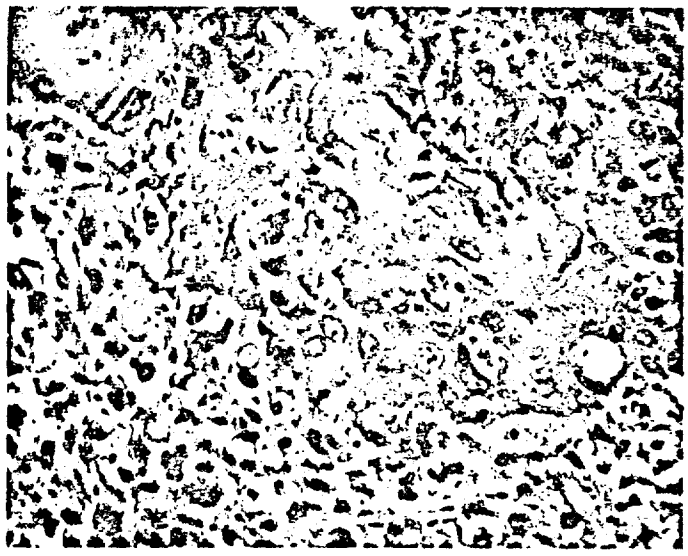

Fig. 20.—Squamous-cell carcinoma and round-cell sarcoma of the uterus of a rat (collision tumor) (PVP).

Fig. 21. — Intracapillary cluster of foreign-body giant cells in the lung of a rabbit (dextran).

in sarcomas derived from Hodgkin's disease in man.

The cancers affecting the uterus of some rats and of one rabbit were either adenocarcinomas or squamous-cell carcinomas or anaplastic carcinomas. Occasionally a mixture of adenocarcinoma and squamous-cell carcinoma or of adenocarcinoma and endometrial round-cell sarcoma was observed (Fig. 20).

Two rats developed squamous-cell carcinomas of the skin in the region of the subcutaneous implantation of PVP.

The histopathologic changes recorded for mice, rats, and rabbits which received PVP's by various parenteral routes demonstrate the topographical and in part also cellular identity of the localization of thesaurismotic, hyperplastic, and cancerous reactions. Statistical as well as histopathologic evidence thus support the view that at least some of the cancers observed in these animals are attributable to the PVP administered.

II. Polyvinyl Alcohol.—The storage phenomena observed were essentially identical in type and distribution with those seen in the PVP animals as well as with those previously reported for dogs and rabbits,[38,39] i. e., polyvinyl alcohol was present in vari-

Fig. 22.—Foreign-body giant-cell granuloma obstructing the lumen of a large pulmonary artery in a rabbit (dextran).

Fig. 23.—Intimal cushion of proliferated endothelial cells in a pulmonary artery of a rabbit (dextran).

ous cellular constituents, such as periportal foam-cell granulomas, isolated and swollen Kupffer cells, proliferated reticulum cells and giant cells of the spleen, and interstitial giant-cell granulomas of the lung. The cancerous reactions likewise resembled the corresponding ones observed among the PVP animals, i. e., Kupffer-cell sarcomas and mesotheliomas.

III. Dextran.—(*a*) Thesaurismotic Reactions: The lungs of rabbits revealed frequently intracapillary giant-cell granulomas, which were sometimes rather cellular and at other times mainly hyaline (Fig. 21). Similar but much larger formations occasionally blocked the lumens of large pulmonary arteries (Fig. 22). In the presence as well as in the absence of such foreign-body granulomas the endothelial lining of arteries exhibited sometimes focal increases of cells which at times produced small crescent-shaped, cellular plaques of the intima (Fig. 23). Apparently older lesions of this derivation appeared as fibrous intimal thickenings (Fig. 24).

Foam-cell accumulations in intrahepatic sinusoids were only occasionally observed in the livers of mice (Fig. 25). In the spleen of one rabbit hyaline masses surrounded atrophic lymph follicles. The same rabbit exhibited also an extensive hyalinosis of the renal glomeruli.

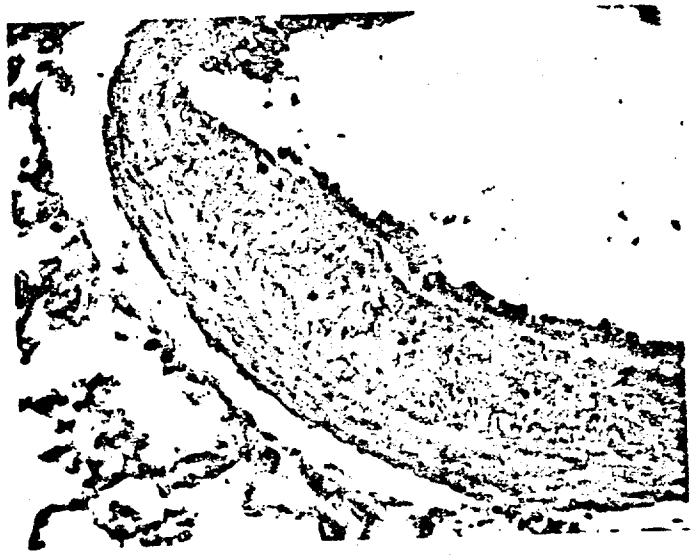

Fig. 24.—Fibrous intimal thickening in a large pulmonary artery of a rabbit (dextran).

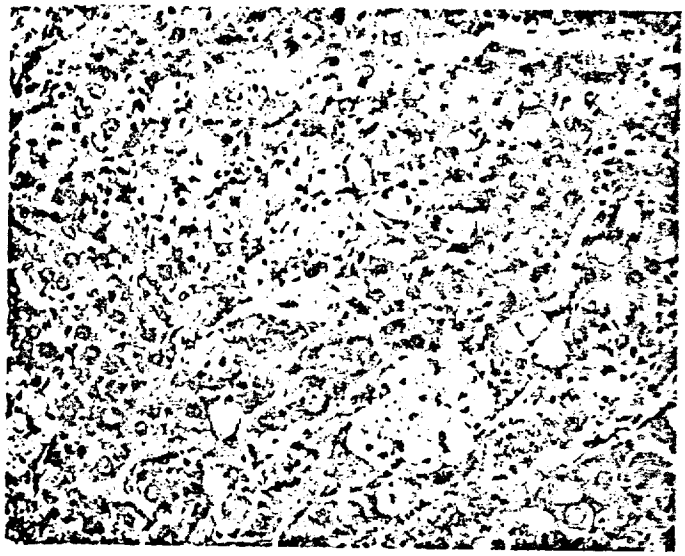

Fig. 25. — Proliferated Kupffer cells with swollen, foamy cytoplasm of a mouse (dextran).

The storage phenomena were in general distinctly less frequent and less marked in animals treated with dextran than in those given PVP's.

(*b*) Hyperplastic and Precancerous Reactions: The hyperplastic and precancerous reactions were correspondingly also less marked and less frequent in dextran animals than in PVP animals. The spleens of some mice and of one rabbit revealed marked mononuclear or reticulum-cell proliferations obliterating the lymph follicular structure of this organ. Two mice showed, moreover, pulmonary adenomas, one of which projected above the pleural surface as a polypous formation (Fig. 26). The lung of one rabbit exhibited a large area of alveolar adenomatosis.

(*c*) Cancerous Reactions: The cancerous reactions in dextran animals resembled in histologic type and in topographical distribution those seen in PVP animals. The various types of cancers found in dextran-treated animals were endothelioma of the endocardium (Fig. 27), myeloid leukemia, reticulum-cell sarcoma of the liver or of the lymph nodes, and carcinoma or carcinosarcoma of the uterus. It is noteworthy that

Fig. 26. — Polypous adenocarcinoma of the pleura of a mouse (dextran).

Fig. 27. — Pericardial endothelioma invading the myocardium of a rat (dextran).

this identity in histogenesis and histological structure of the cancers seen in dextran and PVP animals is shared only in part with those seen in normal control animals, which, while carrying some of the cancers recorded in the experimental animals, show them at a considerably lower incidence rate and lack also the various and frequent developmental and "precancerous" stages seen in the test animals.

IV. Silastic Rubber.—This observation applies also to the cancers found in the internal organs of rats which received implants of silastic rubber or gum into the subcutaneous tissue or into the abdominal cavity. These tumors occurred in these experimental animals at a normal frequency rate. The spindle-cell sarcomas which developed around the subcutaneously implanted cubes of silastic rubber, on the other hand, represent specific reaction products to the processed silicon polymer (Fig. 28). The histologic examination of the capsules surrounding these implants in the subcutaneous tissue and in the abdominal cavity revealed that they consisted originally of oligocellular hyaline membranes which were thicker for implants in the subcutaneous tissue than for those in the abdominal cavity. In some capsules multicentric small cellular foci were found beneath the inner lining which were not in direct contact with the implant. It may be assumed that the subsequent sarcoma formation started from these foci.

Fig. 28.—Fibrosarcoma surrounding a cube of Silastic in the subcutaneous tissue of a rat.

It is remarkable that none of the capsules formed around the abdominal implants developed sarcomas and that such an event was observed in only one of two series of 30 rats each which received implants of the silicon latex. While tumors were not formed around the implants of powdered silica placed in the subcutaneous tissue and into the peritoneal cavity, the relatively large number of cancers of the internal organs observed in rats of these series creates the suspicion that the two phenomena may not entirely be unrelated.

Mention may finally be made of the fact, which is apparent from the descriptions of the histological changes observed in the different experiments, that storage phenomena and hyperplastic reactions were present in animals with and without neoplastic reactions. In these respects animals with tumors did not differ as a group from animals without tumors.

Comment

The evidence presented indicates that under the experimental conditions observed some, but not all, PVPs; some, but not all dextrans; a polyvinyl alcohol, and Silastic are carcinogenic to mice, rats, and perhaps also to rabbits, when they are introduced parenterally. The existence of fundamental differences in carcinogenic properties between polymers of the same type, such as PVPs, was suggested by the divergent results reported previously, since Hueper and Nelson and Lusky obtained sarcomas with PVP introduced into rats, while Hecht failed to do so.

The carcinogenic effect of the water soluble polymers involves several organs and tissues. It becomes manifest mainly in organs in which these macromolecular substances are retained and stored over prolonged periods and where they tend to elicit reactive cellular proliferations which seem to supply the preparatory state for the subsequent development of cancers. In contrast, the carcinogenic effect of water insoluble polymers whenever it is present is limited to the site of implantation, i. e., mainly the subcutaneous connective tissue.

Differences in carcinogenic potency between polymers of the same type and of different types seem to be reflected in variations in the length of the minimal latent period. The minimal preparatory period of cancers for PVPs 1, 2, 3, and 4 was six to eight months. Since about 25% of all cancers among the rats of this experiment occurred during the 7th to 12th months of the exposure time, these particular PVPs exhibited a considerable degree of carcinogenic potency as judged by the length of the minimal latent period. Subsequently studied carcinogenic PVPs, dextrans, polyvinyl alcohol, and silicon rubber, on the other hand, elicited cancers not until the elapse of at least one year following the start of the experiment. A complete lack of carcinogenic response during the first 12 to 15 months of observation has been noted so far for the different polyethylene and polyurethane preparations parenterally introduced by various routes and in different physical forms.

The route of administration which apparently influences the time of onset and the degree of development of fibrous encapsulation of the implanted materials and its relative concentration in the tissues seems to exert a determining influence upon the degree of the carcinogenic effect of water-insoluble polymers, since sarcomas developed at the site of encapsulation of water insoluble polymers much more often in the subcutaneous tissue than in the peritoneal cavity. There was, on the other hand, no consistent difference in the carcinogenic response in internal organs for the various routes of introduction of water-soluble polymers.

When the degrees of carcinogenic response obtained with water-soluble polymers of different molecular size and shape were compared, again no distinct pattern of behavior was discernible. This lack of distinction extended also to reactions elicited by products of commercial grade and those of medicinal grade. The results of experiments with PVP 6 and PVP 7 which were administered in one set in single doses and in a second set in repeated doses suggested, however, that repeated exposures intensified the carcinogenic effect and thus brought to light weak carcinogenic properties which were not apparent for PVP 7 after the introduction of a single dose.

These observations are of fundamental importance because they indicate that the differences in carcinogenic behavior of different polymers of the same general type

are apparently related to much more complex differences than those represented by mere variations in average molecular weight or the ratio of branched to linear member groups in a polymer. The marked carcinogenic effect of the Silastic molecule possessing cross linkages of peroxide nature in comparison to the at best weakly carcinogenic one of the linear latex molecule provides perhaps a clue to a future study of this intricate problem. It is noteworthy in this connection that Mark,[40] working with polyvinyl macromolecules, recently pointed out that certain polymers can have different properties if they have been prepared under different conditions, not because of head-to-tail, head-to-head isomerism and not because they represent different degrees of branching, but simply because the substituents are differently arranged in space as one progresses along the chain and affect the configuration of the chain as a whole. It is, therefore, possible that the industrial use of different catalysts which determine the character of the end-groups and of different production methods in the preparation of polyvinylpyrrolidones may account for the differences observed in carcinogenic potency of PVPs of different manufacture (PVP 2 and PVP 7 of German manufacture; PVP 1 and PVP 6 of American origin).

Support of this concept of a chemospecific basis for the carcinogenic action of polymers and macromolecular condensates is provided by the recent observation of Richmond,[41] showing that an iron-dextran complex when parenterally introduced into rats was carcinogenic, while the dextran alone was not. A similar connotation may be attached to the finding that the carcinogenic polyamide "Perlon" (e-amino-caprolactam), when used as a filling of human pleural cavities, undergoes gradual disintegration of its fibrils and that in fact the fibrils varied structurally when viewed under the microscope before they were implanted. This observation indicates that at least some polymers (Mondur TD-80) do not seem to be stable in a biologic medium; nor are they chemically uniform. These facts may account for the variability of the anatomic reactions to various Perlon products, since some elicit a marked foreign-body reaction and fibroblastic proliferation, while others seem to be almost inert in this respect (Wolter).

The action of a chemical mechanism in the production of anatomic reactive lesions to polymers is, moreover, suggested by the development of granulomatous reactions in the lungs of man following the inhalation of PVP-containing hair lacquers (Bergmann, Flance, and Blumenthal) and of similar pulmonary changes in rats after the intratracheal injection of dust of Perlon and Nylon (polyhexamethylene adipamid [Massmann and Pilgrim]). The pulmonary reactions in rats were progressive and later on complicated by the development of extensive adenomatous hyperplasias originating from bronchial epithelium. Although these adenomatous responses were considered by the investigators as unrelated to the inhalation of the polyamides and although cancerous pulmonary reactions were not observed, these findings nevertheless show that some finely dispersed and powdered polymers exert distinct biologic effects in the organs of contact which must be ascribed to chemical interactions and not to the action of mere surface forces. There is no sound reason for assuming that the marked fibrosing and scar tissue development stimulating action of polyethylene films utilized in human surgical practice has any other than a chemical basis (Yeager and Cowley). The serious toxic reactions, such as alopecia, thrombocytopenia, diarrhea, and anorexia, elicited by dextran sulfate in man, are doubtlessly responses to chemical phenomena (Tudhope et al.). In fact, Tudhope compares these effects of dextran sulfate with those produced by whole-body irradiation and by exposure to mechlorethamine hydrochloride (nitrogen mustard), colchicine, and other antimitotic substances and thereby refers to chemicals and physical agents which often have ambivalent qualities, i. e., they are also carcinogenic under proper conditions.

There can be no doubt that some water-soluble as well as water-insoluble polymers are capable of producing in the organism of man and experimental animals toxic, proliferative, and even carcinogenic reactions, which in their general behavior and type cannot be distinguished from those elicited by other chemicals. The fact that evidently only some but not all polymers of the same general type seem to be carcinogenic has its well known analogue in the observation that only some and not all derivatives of certain polycyclic aromatic hydrocarbons or of aromatic amines or of azo compounds are carcinogenic and that often some specific and often apparently minor aspect of their chemical composition as well as molecular configuration are the determining factors in this respect.

Apart from a direct chemical interaction of these polymers, particularly the water-soluble variety, with cellular constituents, there exist two additional mechanisms through which these metabolically rather inert macromolecules may act upon cells. Their intracellular and extracellular storage in excessive amounts doubtlessly interferes in the long run with the normal vital cellular activities by hindering intracellular and extracellular reactions and fluid and chemical exchange processes, thereby causing cellular degeneration and death. These, in turn, may be followed by reactive regenerative cell proliferation. Such possible effects may involve the blood-brain barrier, owing to the accumulation of PVP in the choroid plexus, meninges, and adjacent cerebral tissue, and thereby impair cerebral function. They may affect the nutrition of the arterial walls as well as the liver and kidney function. Storage of PVP in the spleen, resulting in secondary reticulum-cell proliferation, may exert an influence upon erythrocytic destruction rate and thereby account for the delayed appearance of anemia often developing as a manifestation of hypersplenism in excessively stored animals (macromolecular hematic syndrome).

Since PVP has a definite affinity to form complexes with proteins, dyes, various drugs, and other chemicals, i. e., it exerts an "embathic" effect, its marked accumulation in the endometrium raises the question whether it has also an affinity to sex hormones and may elicit through the mechanism of local hyperestrogenism, endometrial hyperplasia, and carcinoma.

Concerning the merits and the validity of the theory on carcinogenesis by surface action and capsule formation of polymer films, which is also applied to the carcinogenic action of metal foils, it may be pointed out that cancers can readily be produced in rats by the implantation of certain powdered metals, such as uranium and nickel, and have been produced also in rabbits with chromium powder (Schinz). Since similar cancerous responses can also be elicited by the subcutaneous implantation of certain metal compounds, such as calcium chromate, in rats or by the intravenous injection of beryllium compounds into rabbits, it is very certain that the process of metal carcinogenesis does not depend on any nonspecific physical phenomenon related to surface forces but is attributable to specific chemical effects of these metallic elements and their proper biologic availability to the tissues.

It is, moreover, obvious that the carcinogenic effect of water-soluble polymers is not dependent upon the presence of a certain number of square millimeters of unbroken surface of polymer film.

The mere formation of a capsule around implanted polymer films also cannot be considered as the specific prerequisite for a subsequent cancerous development, since the balls of silicon latex subcutaneously implanted were as effectively encapsulated as the cubes of silicon rubber. Nevertheless, sarcomas mainly developed around the rubber cubes and not around the latex balls, indicating that cancerous responses in the capsule tissue depend on an additional and specific carcinogenic factor associated with the polymer. If indeed the formation of cellular scar tissue in rats would represent a fertile basis of the production of cancers, it is surprising that the chronic organizing

pneumonias not infrequently seen in rats do not seem to give rise to carcinomas or sarcomas of the lung, unless a specific carcinogenic agent is active. While at the present time a satisfactory explanation for the differences in cancerous responses to various polymers when implanted as intact film, perforated film, fabric, thread, or powder cannot be given, it is most unlikely that for the reasons given the hypothesis of Nothdurft and of Oppenheimer on polymer carcinogenesis is the correct one.

Finally, comments are in order concerning the applicability of the experimental observations to man, i. e., whether or not health and cancer hazards may result from any exposure to the growing number of synthetic and semisynthetic macromolecular substances for occupational or medicinal reasons or as the result of handling, using, or ingesting the large number of consumer goods either containing these chemicals or having been in contact with them.

The various storage phenomena produced by water-soluble polymers which have been observed not only in experimental animals but for the plasma extenders, especially PVP, also in man (Busch, Traenckner,[29] Gropper et al., Hartmann and Behrmann,[28] Wrage, Upham et al., Ravin et al.) in general have been considered as of little consequence as to any acute or chronic effects upon the function of the organs affected. This conclusion is probably correct unless highly excessive amounts of these agents, particularly PVP, are administered repeatedly over prolonged periods of time. The observations of the present experiments reported add to the list of already known thesaurismotic reactions from which possible delayed health hazards may result under exceptional conditions. These are the storage phenomena in the choroid plexus, the glia and ganglion cells of the brain, and the atheromatous and atherosclerotic arterial reactions seen in rabbits given injections of PVP and dextrans. If these observations indicate that the medicinal and cosmetic use of water-soluble polymers should not be practiced without caution and discrimination, this warning receives increased emphasis by the demonstration of carcinogenic effects elicited by both water-soluble and insoluble polymers. While there exist without any doubt justifiable and legitimate medical and surgical indications for the use of such macromolecular agents, the present uncertainty of their potential carcinogenic action in man upon parenteral or respiratory introduction demands that the calculated risk related to the disease to be treated outweighs definitely any possible delayed risk to health which may be created by a polymer administered.

Although at present no valid evidence exists that the use of such polymers in certain protheses, such as dentures and contact lenses, which have prolonged and intimate contact with mucous membranes, is actually related to an increased occurrence of cancer in the exposed tissues, it seems to be wise to conduct during the coming years carefully controlled surveys on the cancer incidence among persons thus exposed for determining the actual innocuousness of these plastic devices.

Similar considerations may well be extended to the medicinal use of ion-exchange resins (polyamine-methylene resin, carbacrylamine resin) administered by mouth for increasing the fecal excretion of sodium in cardiac edema or as an antacid in the treatment of gastric hyperacidity. Definite attention should be given in this respect also to the incorporation of macromolecular chemicals into other medicinal and cosmetic products, such as ointments, creams, and laxatives. Although the scientific character of the experimental evidence of the carcinogenesis of polymers does not justify any undue alarm concerning the use of such materials in food products, for the impregnation or coating of food containers, or for the coating or wrapping of foodstuffs, it appears to be a sound precautionary measure to keep all workers employed in the production, processing, and handling of polymers or plastics under proper medical supervision for insuring an early discovery

of any cancers possibly attributable to contact with these substances.

Conclusions

1. Certain water-soluble polymers, such as polyvinylpyrrolidone, polyvinyl alcohol, and dextran elicit upon parenteral introduction into rats and mice, and perhaps also in rabbits, sarcomas originating from organs and tissues in which these macromolecular substances are retained and stored, i. e., the reticuloendothelial tissues. Cancers from other tissues, such as uterus and skin, observed in rats may be directly or indirectly caused by these substances.

2. Polymers of the same general type but produced by different procedures and varying among each other in molecular weight and configuration differ greatly in their carcinogenic potency, i. e., some are noncarcinogenic, others weakly carcinogenic, and again others definitely carcinogenic.

3. The experiments conducted have failed so far to provide any information on the physical or chemical molecular factors which may account for these differences.

4. Experiments with silicon rubber and silicon latex showed that the rubber having a net molecule with many cross linkages possesses marked carcinogenic properties when subcutaneously implanted into rats, while the latex having a linear molecule is at best weakly carcinogenic.

5. The total evidence on hand concerning the biologic action of synthetic and semisynthetic polymers parenterally introduced into man and animals favors the view that their toxic, pharmacologic, and carcinogenic effects are mainly related to chemical properties and reactions rather than to their physical characteristics.

6. The existence of actual cancer hazards to man from water-soluble and insoluble polymers possibly associated not only with their parenteral introduction but also with their administration by the cutaneous, respiratory, and oral routes is at present problematical. The experimental evidence on hand, however, is sufficiently definite and serious for requiring distinct caution in the medicinal and cosmetic use of these products.

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STORAGE AND TISSUE DISPOSAL OF CARBOXYMETHYLCELLULOSE INJECTED INTO RATS

GAËTAN JASMIN [1] and PIERRE BOIS

Départements d'Anatomie Pathologique et de Pharmacologie, Université de Montréal.

ABSTRACT

Experiments were performed to determine, by histologic means, storage, distribution and tissue disposal of different types of carboxymethylcellulose (CMC) administered parenterally into rats. Each CMC preparation was injected at a concentration of 1.6% in a volume of 1 ml, either intravenously, intraperitoneally or subcutaneously. The material was readily evidenced in various organs in which it was phagocytosed, by using acidified alcian blue or cresyl violet. The localization of each CMC preparation varies according to the route of administration and the size of the particles. It is assumed that CMC is metabolized within the reticular cells.

Various polymers have been investigated in recent years for their possible clinical value. The search for synthetic plasma expanders, drug vehicles, and retardants has resulted in a number of macromolecular agents that serve many purposes. Some of these substances apparently proved to be detrimental if given in large doses, as was shown by the studies of Hueper (1). However, this finding should not preclude the possible existence of useful polymers for parenteral therapy.

The molecular weight seems to be an important factor; dextran preparations that exceed 75,000 cause intolerance reactions in man (2), presumably as a result of capillary damage. In laboratory animals, the tolerance to parenterally administered macromolecules varies with the species. Injection of relatively low molecular weight dextran (20,000) or ovomucoid (29,000) causes an anaphylactoid reaction in rats (3, 4); the dog, on the other hand, remains insensitive to both substances (5), but reacts severely to polyvinylpyrrolidone (32,000 MW), which is innocuous to rats (6, 7).

Perhaps the most serious aspect of the problem is the fact that some relatively inert macromolecules, when given in sufficiently large doses and for comparatively long periods of time, produce cancer in laboratory animals (8, 9). It is still too early to decide if these observations made in chronically overdosed animals may also apply in man. In the course of our investigations on the cancerigenic properties of various carboxymethylcellulose (CMC) preparations (10), their storage and tissue disposal have been evaluated, following a brief treatment with these agents. This study has been greatly facilitated by the fact that the CMC particles, which accumulate in phagocytes, stain selectively with alcian blue and cresyl violet.

MATERIAL AND METHODS

Sprague Dawley female rats, weighing between 100 and 160 gm were used; they were fed Purina Fox Chow and had free access to tap water. Three different CMC

[1] Research associate of the Medical Research Council of Canada.

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preparations were investigated. The first two, supplied by Nordic Biochemicals, consist of water soluble 4% ammonium salts; they differ from each other in that one is an ultracentrifuged preparation, while the other is not. Thus, the first substance was designated as "supernatant" and the latter as "coarse". The supernatant has an approximate molecular weight of 25,000. The third preparation used in this study is a sodium salt of CMC, equally soluble in water. It is graded as CMC-70 Low and supplied by Hercule Laboratories. It has a viscosity of 35 centipoises at 2% concentration and its substitution rate is 0.7. The approximate molecular weight is 40,000. All CMC preparations were injected at a concentration of 1.6% in groups of 6 rats.

In the first experiment, 1 ml of each CMC solution was injected either once intravenously, or twice intraperitoneally or subcutaneously during 2 consecutive days. Animals were killed 48 hours after the last injection. Organs were dissected and fixed in Susa solution for histologic examination. Paraffin sections were stained in acidified 1% alcian blue or 0.2% cresyl violet (11). Other dyes were also used, such as toluidine blue (12), aldehyde fuchsin (13), Hale's colloidal iron (14) and periodic acid Schiff (15). The results of those experiments are given in Table I. The extent of the phagocytosis in tissue was appraised microscopically, according to an arbitrary scale of 0 to ++++.

TABLE I

Stain affinity of CMC-laden phagocytes

Cresyl violet	Positive
Toluidine blue	"
Aldehyde fuchsin	"
Alcian blue	"
Hale	"
PAS	Negative

Animals of the second experiment received 1 ml of the Nordic "supernatant" CMC intraperitoneally, twice, on the first day of the experiment. Animals were killed at various times (2-60 days) in order to evaluate the rate of disappearance of CMC. The liver, spleen and adrenals served for this purpose. On sections stained with alcian blue, fields were pen-marked at random and phagocytic cells were counted in these areas at magnification of 400X.

RESULTS

The observations arising from the first experiment are summarized in Table II. The intravenously injected Nordic "supernatant" fraction tends to localize mostly in the liver. Particles, as expected, were seen in the Kupffer cells, especially at the periphery of the lobules (Fig. 1). Yet, some CMC-containing phagocytes could also be detected in the spleen, lymph nodes and lungs, but in smaller proportion. When CMC is given intraperitoneally, the phagocytic activity is as intense in the lymphatic organs as in the liver; some CMC particles were also found along the sinusoids of the adrenal medulla, but none in the renal parenchyma. Following subcutaneous injection, CMC is phagocytosed in the derma as reported previously (10) and very little can be evidenced in the liver and the spleen. Other CMC preparations, Nordic "coarse" or Hercule, injected in-

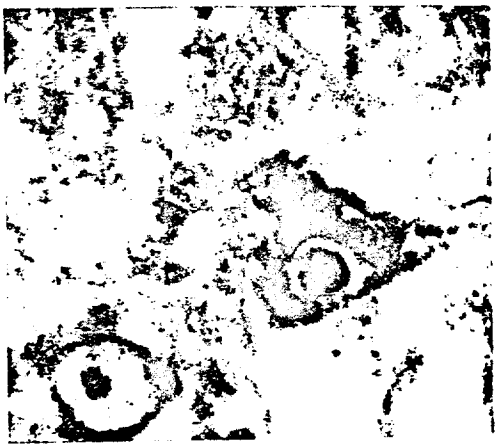

Fig. 1: Kupffer cell containing CMC as evidenced by cresyl violet x 2000.

TABLE II

Distribution of carboxymethylcellulose in various organs 48 hours after parenteral injection.

Preparation	Route	Mean phagocytic reaction (0 to ++++)					
		Liver	Spleen	Lung	Lymph nodes	Adrenals	Kidney
CMC	i.v.	++	+	±	+	0	0
Nordic	i.p.	+++	++	0	++	+	0
"Supernatant"	s.c.	+	+	0	0	0	0
CMC Nordic "Coarse"	i.v.	+	+	+++	0	0	0
CMC Hercule	i.v.	+	±	+	0	0	0

traperitoneally, were largely taken up by the lung reticulo-endothelial cells and only a few phagocytes were found in the liver and spleen.

The results of the second experiment (Table III) indicate that the number of CMC-containing phagocytes steadily decreases with time and, furthermore, the concentration of particles within each individual cell tends to diminish: the total cell count was reduced by approximately ⅓ after 6 days. The presence of CMC particles was also noted in the cytoplasm of the liver cells.

TABLE III

Rate of disappearance of carboxymethylcellulose in various tissues

Days following treatment*	Mean phagocytic cell count per field of .35 mm diam.		
	Liver	Spleen	Adrenals
2	18	35	6
8	12	34	6
15	8	29	4
30	6	24	3
60	6	14	1

*1 ml of CMC Nordic "supernatant" 1.6% injected i.p. on the first day.

DISCUSSION

The phagocytic response to parenterally administered CMC depends upon the route of administration. There is a greater dissemination of particles when the intravenous route is used: the distribution varies according to the size of these particles, the larger being found mostly in the lung whereas the smaller localize in the liver. Following intraperitoneal injection, CMC is slowly absorbed by the lymphatic vessels and phagocytosis predominates in abdominal organs. Curiously, the kidney is not involved in this process; thus it is believed that polysaccharide droplets found in the tubular epithelium in various types of renal pathologic changes (16) characterize a degenerative rather than a phagocytic reaction. When CMC is administered subcutaneously, very little reaches the circulation as a result of intense phagocytosis in the derma.

It is somewhat unexpected that an inert substance such as CMC, which allegedly is not metabolized, eventually disappears from tissues in which it is phagocytosed. The substance very probably undergoes a certain breakdown by some enzymatic system within the

reticular cells. This possibility is substantiated by the fact that, after a certain period of time, some smaller CMC particles were found in the hepatic cells. In the present investigation, the animals were studied for 60 days and, during that time, the amount of CMC-containing phagocytes was reduced by ⅔; most probably, the clearance would be complete within two additional months. Further histochemical studies would be required to investigate tissue disposal of injected CMC.

ABRÉGÉ

Une étude histologique fut effectuée chez le rat sur la phagocytose, la distribution et l'élimination de divers types de carboxyméthylcellulose (CMC) administrés parentéralement. Chaque préparation de CMC, en solution aqueuse de 1.6%, fut injectée à raison de 1 ml par voies intraveineuse, intrapéritonéale ou sous-cutanée. Les particules phagocytées au niveau de divers organes furent démontrées à l'aide du bleu alcian et violet de crésyl acidulés. Il fut observé que la CMC variait dans sa distribution selon la voie d'administration et la dimension des particules. L'élimination progressive de la CMC laisse supposer que cette substance est métabolisée par les cellules réticulo-endothéliales.

ACKNOWLEDGMENTS

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O. G. Fitzhugh

Compilation for 9th & 10th Report

TOXICOLOGICAL EVALUATION OF SOME ANTIMICROBIALS, ANTIOXIDANTS, EMULSIFIERS, STABILIZERS, FLOUR-TREATMENT AGENTS, ACIDS AND BASES

Food and Agriculture Organization of the United Nations
World Health Organization

1967

Joint FAO/WHO Expert Committee on Food Additives, report

"title"

Cellulose Verens.

SODIUM CARBOXYMETHYLCELLULOSE

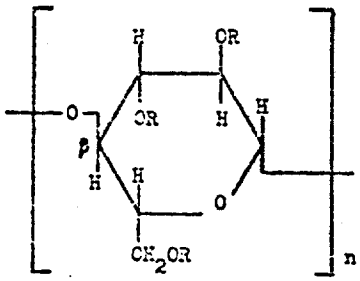
Synonyms: Sodium cellulose glycolate; Na-CMC; CMC

Chemical name: Sodium salt of a carboxymethyl ether of cellulose.

Chemical formula: $\left[C_6H_7O_2(OH)_x(OCH_2COONa)_y\right]_n$

where x = 2.00-2.40
y = 1.00-0.60
x + y = 3.00

Structural formula



where R = H or CH_2COONa

Molecular weight:
Unsubstituted structural unit : 162.14
Monosubstituted structural unit : 242.16
Macromolecules : from about 21 000 (n about 100) up to 500 000 (n about 2000)

Definition: Sodium carboxymethylcellulose is the sodium salt of a carboxymethyl ether of cellulose, where the substitution degree ranges from 0.20 to 1.00. After drying at 110° to constant weight it contains not less than 99.5 per cent. of sodium carboxymethylcellulose.

Description: Sodium carboxymethylcellulose is a white or a slightly yellowish, almost odourless and tasteless hygroscopic powder, consisting of very fine particles, fine granules or fine fibres.

Uses: Thickener, binder, emulsifier, stabilizer and colloidal suspending agent in salad dressing, fruit pie fillings, baked goods, dietetic foods and many others.

Biological Data

This additive was evaluated by the Joint FAO/WHO Expert Committee on Food Additives in its Seventh Report (FAO/WHO, 1964). Since its publicatic some new experimental work has been carried out on these compounds. This a other work not included in the Seventh Report is presented and discussed in this monograph.

Long-term studies

Mouse. Groups of 50 male and 50 female mice were maintained for up to 100 weeks on ad lib. diets containing 0, 0.1 and 1 per cent. of sodium carboxymethylcellulose. There was no apparent difference in mortality and tumour incidence between the groups (Imperial Chemical Industries, 1966).

Rat. Groups of 50 male and 50 female rats were maintained for up to 2 years on ad lib. diets containing 0, 0.1 and 1 per cent. of sodium carboxymethylcellulose. No difference in mortality and tumour incidence was appar between the groups (Imperial Chemical Industries, 1966).

Comments

The additional information supports the previous evaluation of sodium carboxymethylcellulose.

Evaluation

Level causing no toxicological effect

Mouse. 10 000 ppm in the diet, equivalent to 1500 mg/kg body-weight/da

Rat. 10 000 ppm in the diet, equivalent to 500 mg/kg body-weight/day

Estimate of acceptable daily intake for man

	mg/kg body-weight
Unconditional acceptance	0-30[1]
Conditional acceptance:	Higher levels may be used for dietet or calorie control purposes

REFERENCE

Imperial Chemical Industries (1966) Unpublished reports submitted to WHO.

[1] As sum of total cellulose derivatives