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PECTIN

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PECTIN

Summary

For centuries man has known that boiling certain fruits with water and sugar results in a jelly upon cooling. Bracconnot was one of the first to realize that this depended on the extraction of colloidal substances from the fruit. In 1825 he named it "pectin" from a Greek word meaning to congeal (270). Basically, pectins are polymers of D-galacturonic acid units with rhamnose, arabinose, galactose, and traces of other sugars incorporated. The varied degree of esterification by methoxyl groups produces diverse gelling properties important to the food industry. One of the richest sources of pectin is the lemon or orange rind, with apple pomace used as a secondary source (291).

Pectin does not appear to be absorbed by rats, dogs, or man. After feeding rats citrus pectin Viola et al. (447) found 100% of pectin ingested to be represented as non-digested pectin and non-volatile decomposition products in the feces. Werch and Ivy (469) reported that galacturonic acid, a metabolite of pectin, was not absorbed in appreciable quantities from the human and canine ileum or canine colon. Pectin passed through the stomach and part of the small intestine of dogs was recovered without loss by Kertesz (211).

Studies on rats, dogs, and man have shown pectin to be highly digestible. In 1969 Gaedeken (137) reported an apparent digestibility of 97.1% and 93.4% and an apparent digestible energy of 95.8% and 92.2% for apple pectin given as 12 and 24% of the diet. The digestible energy of pure pectin was established as 3.4 to 3.6 kcal/g. A utilization coefficient of 5.6% and 20.3% of the gross energy for the 12% and 24% rations was derived. However, studies by Viola et al. (447) in 1970 indicated a specific digestible energy of -1.6 kcal/g for citrus pectin and a coefficient of digestible energy of 84%.

Early in the 1940's Werch and Ivy (468) discovered that when pectin was fed in a mixed diet to dogs, 90% of the pectin was decomposed but only 50% was decomposed by fasted dogs. Pectin had a decomposition factor of 90% when fed in a mixed diet to humans, but only 85% when given alone. Decomposition was increased by lengthening the retention time. Gilmore (143) noted that the higher the methoxyl content of pectin, the greater the intestinal breakdown in rats.

Detailed studies by Gaedeken (137) on rats revealed that apple pectin is decomposed by intestinal flora into galacturonic acid or other organic acids capable of resorption, and finally to carbon dioxide and water. Since galacturonic acid can be absorbed only in the small intestine, monogastric animals do not utilize this pectin derivative. Gaedeken stated that intense breakdown activity prevents the absorption of intermediate metabolites. Complete pectin breakdown was proportionately smaller the higher the pectin content of the diet, probably due to a shift in intestinal pH.

As early as 1940 Kertesz (211) believed pectin to be attacked only in the large intestine by bacterial enzymes; trypsin and pepsin had no effect on pectin in vitro. Pectin incubated with dog feces was rapidly decomposed. Werch et al. (471) isolated pectin-degrading bacteria from dog feces and found them to belong to Aerobacillus, Lactobacillus, Micrococcus, and Enterococcus. Formic and acetic acids were the chief products, with a very small amount of galacturonic acid.

In their seventeenth report, the Joint FAO/WHO Expert Committee on Food Additives (130) reported an acceptable daily intake for man (ADI) to be 0-25 mg/kg for amidated pectin and not limited for non-amidated pectin on a temporary basis.

Two long term studies of the effects of dietary pectin on rats have been performed. Cohen et al. (87) fed groups of 9 two-month-old rats a basal diet plus 20% pectin, granulated sugar, sucrose, fructose, cerelose, dextrin, galactose or lactose for 18 months. The pectin-fed rats grew the least but had an increased water consumption and fecal production. In a second study by Cohen et al. (88), groups of two-month-old rats consumed ground rat chow plus 0 and 20% citrus pectin N.F. Although the test rats weighed 200 g less at the end of the 18 month period, they appeared quite healthy.

Growth depression was the only effect noted in short term studies performed on chicks and rats. Feeding groups of 10 one-day-old chicks 0, 2, and 4% pectin for 20-21 days resulted in a growth depression of 87-92% for the 2% level and 89% for the 4% level. Becker et al. (40) found 10% sugar beet pectin to retard growth in chicks. Kratzer et al. (241) noted a depressed weight gain in 10 chicks fed 2% pectin for 3 weeks. Similar effects were found by Viola et al. (447) in weanling rats fed 10% citrus pectin-S (55% esterified) or 10% pectin-M (65% esterified), along with a reduced food intake, and a reduced protein utilization for the pectin-M group. After feeding 150 weanling male rats 5% citrus pectin L.M. (3-5% methoxyl) and 5% citrus pectin N.F. (10-12% methoxyl) for up to 2, 4, 6 and 8 weeks, Gilmore (143) found the pectin N.F. rats to consume less and weigh less than the pectin L.M. group.

Ershoff and McWilliams (108) fed groups of 4-6 young male and female rats 0, 2.5, 5 and 10% pectin N.F. or nickel pectinate for 8 weeks. All the animals in the 10% nickel pectinate group died within 10 days; other groups were unaffected.

Two groups of young rats were given 2 and 3 g/kg doses of plantaglucide, (pectin from the plantain plant) by Obolentseva and Khadzhai (326) for 28 days with no untoward effects. Becker et al. (40) reported no ill effects in rats from the feeding of 1.5 g pectin daily for 11 days.

The following special studies were performed. Pectin lowered the severity of atherosclerotic lesions in male rabbits only fed a basal diet plus 5% pectin and 1% cholesterol; mortality was highest in the control group (5% cellulose and 1% cholesterol during the 11-month period (122). Two studies by Fisher et al. (126, 123) have shown dietary pectin to reduce atherosclerotic lesions in chicks fed cholesterol, too.

Borgman et al. (68) found dietary citrus pectin N.F. to reduce gallstones in rabbits induced by a 40% casein- 15% olive oil diet; citrus pectin-low methoxyl aggravated the condition.

Obolentseva and Khadzhay (326) reported that plantaglucide administered internally to rats reduced the occurrence of ulcers following intramuscular injection of phenylbutazone.

As regards acute toxicity data, only one LD_{50} was found. Obolentseva and Khadzhay (326) reported the i.p. LD_{50} of plantaglucide (pectin from the plantain plant) to equal 1.7 g/kg for mice. Richter (316) injected 750-3000 mg/kg of citrus or apple pectin into mice, resulting in marked hyperpnea. Histological examination 4-6 weeks after 2-3 injections revealed liver and kidney lesions. Ionescu-Stoian et al. (185) found 2 g/kg of quince pectin administered i.v. to be fatal to 5 out of 5 mice, 1.8 g/kg to be fatal to 3 out of 5 mice, and doses of 1-1.6 g/kg to be well tolerated. Hyperemia of the kidneys, pancreas, liver, lungs, and brain was discovered. When injected i.v. into rabbits under similar conditions, blood coagulation time decreased by 70-80% at a dose of 36-46 mg/kg, by 50% for 30 mg/kg and by 30% for 20 mg/kg (185).

Splenomegaly and splenic and renal lesions in rabbits were reported by Small et al. (395) after the intravenous injection of unbuffered pectin solutions (450 mg/injection up to a total of 1500 mg/kg). No lesions were present 4 days after the injection of buffered pectin solutions. Microscopic examination of rabbits given 25-75 mg/kg pectin i.v. showed a slight swelling of the hepatic cells at the higher doses (160).

No anaphylactic response was elicited in dogs given i.v. 170-350 mg/kg pectin 4 times over a 26-day period (160). Hueper (180) noted fluctuations in the erythrocyte count, packed erythrocyte value, hemoglobin value, erythrocyte sedimentation rate, and leukocyte count following intravenous injection of 14, 29, and 50 mg/kg pectin into dogs.

Many studies have demonstrated that dietary pectin reduces serum cholesterol levels in man, swine, and rats. Keys et al. (213) reported that dietary citrus pectin N.F. (15 g/day) reduced serum cholesterol levels by 5% of a control value in middle-aged men. Pectin lowers serum cholesterol only in the presence of dietary cholesterol, according to Fisher et al. (125). After feeding twice as much dietary cholesterol to men as in the Keys et al. (1961) study, a more pronounced hypocholesterolemic effect was noted. Palmer and Dixon (334) have confirmed these results.

Fisher et al. (127) noted the requirement for dietary cholesterol in swine, too. Serum cholesterol, triglyceride, and phospholipid levels in swine were raised in a study by Fausch and Anderson (112), but here pectin was fed without cholesterol.

Based on studies with rats, Leveille and Sauberlich (261) suggested that pectins hypocholesterolemic property stems from the inhibition of bile acid and cholesterol absorption. Earlier, Hyun et al. (182) had demonstrated that pectin administered per os reduced cholesterol-^{14}C

absorption in rats by 7%. However, Phillips and Brien (340) disagreed; they felt some other factor was involved.

Wells and Ershoff (465) reported that pectin reduced serum cholesterol levels in rats, but not in guinea pigs, hamsters, or rabbits. They pointed out that the rat is an omnivore, while the others are herbivores.

Anderson and Bowman (12) noted that citrus pectin was a stronger hypocholesterolemic agent for rats than tomato pectin, possibly because the citrus pectin had a higher methoxyl content. According to Ershoff and Wells (110) pectin preparations with a methoxyl content of 5% or less are not hypocholesterolemic, but pectin N.F. (10.7% methoxyl) is.

Arkhipova and Zorina (20) have shown orally administered sunflower pectin to be a useful prophylactic against poisoning by lead, mercury, manganese, and beryllium in rats. Doses of 1.5-2 g/kg-day pectin for animals and 8-10 g/kg-day for man produced no side effects.

Viola et al. (447) observed poor protein utilization in rats fed 10% pectin-M (65% esterified) and reduced protein digestibility in those fed 10% pectin-S (55% esterified); fat and calcium absorption was decreased for both pectins.

Plantaglucide was found to increase gastric secretion in fistulated dogs (326). X-ray studies have indicated that this substance also slows the migration of stomach contents to the small intestine in rats. It was also found to reduce edema produced by the injection of formalin and dextrin in mice and rats.

After examining the literature, Bender (43) found pectins anti-diarrhetic effect to be linked to a shift in intestinal pH back to a normal level and the production of certain organic acids pathogenic to the invading microflora.

PECTIN

Chemical Information

I. Nomenclature

A. Common Name

Pectin

B. Chemical Name

Pectin

C. Trade Name

None

D. Chemical Abstracts Registry Number

PM9000695 Pectin

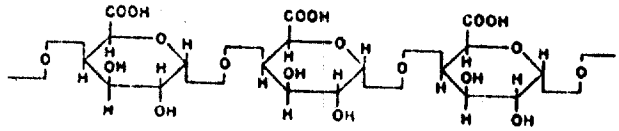
977000671 Pectin, citrus

II. Empirical formula

Not Applicable

III. Structural Formula

Pectins are polymers made up of chains of D-galacturonic acid units joined by an alpha (1 → 4) glycosidic linkage (436). Rhamnose, arabinose, galactose, and traces of other sugars are integral parts of the pectin molecules; proportions vary from different sources. The carboxyl groups are either free or esterified with methyl groups. In natural pectins 20-60% of the carboxyl groups are esterified (291). Structures having this galacturonic acid alpha (1 → 4) backbone will be called "pectic acid"; the salts, "pectates"; and the methyl ester, "pectin" (354). Total hydrolysis yields D-galacturonic acid, methyl alcohol, and small amounts of galactose and arabinose (291).



Pectic acid

IV. Molecular Weight

20,000 - 40,000

V. Specifications

Food Chemicals Codex

Ash (Total)	Not more than 10%
Degree of amide substitution of low ester pectin	Not more than 40%
Degree of esterification of low ester pectin	Not more than 50%
Degree of esterification of high ester pectin	Not less than 50%
Galacturonic acid	Not less than 35%
Gel power of low ester pectin	Not less than 95% and not more than 105% of the labeled gel power
Jelly grade of high ester pectin	Not less than 95% and not more than 105% of the labeled jelly grade
Limits of impurities	
Acid-insoluble ash	Not more than 1%
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 12%
Sodium methyl sulfate	Not more than 0.1%

VI. Description

A. General Characteristics

Pectin occurs as a coarse or fine powder, yellowish-white in color, practically odorless, and with a mucilaginous taste.

B. Physical Properties

Pectin is almost completely soluble in 20 parts water, forming a viscous solution containing negatively charged, very hydrated particles. It is acid to litmus. Pectin is insoluble in alcohol or in diluted alcohol and in other organic solvents. It will dissolve more readily in water, if first moistened with alcohol, glycerol, or sugar syrup, or if first mixed with 3 or more parts of sucrose. Also, pectin is more stable in acid than in alkaline solution.

C. Stability

Store in well-closed containers.

VII. Analytical Methods

The official method for the separation of pectin from fruits and fruit products depends on the precipitation of pectin by alcohol. This precipitate is then saponified with dilute sodium hydroxide, filtered, dried, weighed, ignited, and reweighed. The loss of weight equals the pectic acid. A method is also presented for the removal of pectic acid from cacao bean products (27).

Monselise determined the pectin content of citrus products by saponification of the sample with dilute sodium hydroxide, filtration, and centrifugation of the filtrate. The volume of the pectic acid precipitate is converted into percent pectin by utilizing a graph prepared with standardized pectin solutions (308).

VIII. Occurrence

Plants

Pectins are found in the roots, stems, and fruits of plants, both in cell walls and intercellular layers, and seem to function as a cementing substance. One of the richest sources of pectin is lemon or orange rind which contains about 30% of this polysaccharide. Apple pomace is a secondary commercial source (291).

Biological Data

I. Acute Toxicity

Substance	Animal	Sex & No.	Route	Dosage (mg/kg) Body Weight	Measurement	Ref.
Plantaglucide*	Mice	Unknown	i.p.	1700	LD_{50}	326

* Plantaglucide is the granulated powder of pectins taken from the leaves of the large plantain.

Mice

Obolentseva and Khadzhai reported an LD_{50} equal to 1.7 g/kg for mice given plantaglucide i.p. as a 5-10% aqueous solution. Plantaglucide is the granulated powder of pectins from the leaves of the large plantain (326).

Ionesu-Stoian et al. administered i.v. 1, 1.2, 1.4, 1.6, 1.8, and 2 g/kg quince pectin in a 4% isotonic solution to groups of 5 mice. While doses of 1-1.6 g/kg were well tolerated, 3 mice died from 1.8 g/kg and all five mice died from 2 g/kg. Death occurred after lateral decubitus, followed by spasms, tachycardia, and tachypnea. Histopathological examination revealed hyperemia in the kidneys, pancreas, liver, lungs, and brain. Intumescence of the striated muscle was reported. Because of the presence of vascular multiple thrombosis in all decreased animals, the authors suggested that pectin induced a modification of blood coagulability (185).

Richter injected intravenously 750 to 3000 mg/kg (0.5 to 2.0 ml of a 3% solution) of autoclaved apple, autoclaved citrus, on non-autoclaved citrus pectin into 2 male Swiss albino mice each. Three injections were given over a 2-week period. Immediately following injection some had marked hyperpnea, but recovered within 1 hour. They lost weight (5-7 gm) 1 month after initiation of the experiment. They were then examined histologically. In a 2nd experiment, 6 mice received 1 ml of the autoclaved citrus pectin, and 6 received 1 ml of autoclaved apple pectin. A second injection was given 1 week later; these mice were autopsied at 6 weeks. An unspecified number of mice were given pectin i.p. but no lesions were found. Splenomegaly was present in several of the animals. Focal necrosis was present in most of the mice in the hepatic parenchymea, plus granuloma formation. Focal degenerative changes were seen in the renal tubules (361).

Rabbits

In order to determine the hemostatic effect of pectin, groups of 2

rabbits (1.5-2.9 kg) were given i.v. 20, 30, 36, 40, and 46 mg/kg quince pectin. Pectin decreased coagulation time by 70-80% when 36-46 mg/kg was given, by 50% for 30 mg/kg, and by more than 30% for 20 mg/kg. Maximum hemostatic activity was established at 30 minutes, continued 45-75 minutes, and returned to normal gradually after 105 minutes. The leukocyte count increased 2 hours after dosing and continued for 48 hours. Within 72 hours, the value was normal (185).

Small et al. injected intravenously 1.5% autoclaved pectin solutions, buffered (18) and unbuffered (15), into New Zealand white and Himalayan rabbits. Three control rabbits were also examined. Pectin solutions were given daily in 30 ml doses (450 mg) until a total of 100 ml/kg (1500 mg/kg) had been administered. Two or three animals from each group were killed at intervals from 1 to 101 days. Livers, kidneys, spleens, hearts, and lungs were weighed, and the first three organs were examined histologically. Splenomegaly and splenic lesions were noted in rats given unbuffered pectin up to 9 days past last injection. The buffered pectin rabbits autopsied 4 days after treatment revealed no lesions. Renal lesions were observed in 2 of the unbuffered group (395).

Twelve rabbits of six and eight pounds each were given 1% pectin solution intravenously, 250-500 mg/kg. Doses were repeated at 1 and 2 weeks. No reactions were noted. Rabbits given 500-750 mg/kg of the same solution showed a slight swelling of the hepatic cells (160).

Guinea Pigs

Hartman et al. injected i.v. 3 guinea pigs with 1, 1.5, and 2 ml of a 1% pectin solution (13, 20, 27 mg/kg). At the end of 14 days, the injections were repeated. No reactions were reported (160).

Dogs

Hueper injected i.v. (14, 29, and 50 mg/kg) pectin into 3 dogs. Immediately a brief transitory increase in the erythrocyte count occurred, followed by a drop to somewhat below the original level. This trend was reversed for the two dogs receiving 14 and 29 mg/kg, but progressive in the dog receiving 50 mg/kg. The hemoglobin and packed erythrocyte values followed these fluctuations. The erythrocyte sedimentation rate increased significantly. The rate returned to about normal at 8 hours, increased again at 12 hours and remained high for another 6-8 days. Normal rates were found at 14 days. A leukopenic reaction was noted for 6 to 8 hours at the 2 highest doses (180).

Three dogs were injected 4 times over a 26-day period with 17-35 mg/kg pectin in a 1% solution. No anaphylactic symptoms were manifested. Liver function tests showed no tendency toward cumulative storage or blockage (160).

II. Short-Term Studies

Chicks

Vohra and Kratzer fed a basal diet to groups of 10 day-old, Arbor-Acres broiler type chicks with pectin added at 0, 2, and 4%. Growth was depressed to 89% of the control group in the 4% group after 20 days. In a 21 day trial the 4% pectin group was depressed to 78%. The 2% group had values of 92 and 87% for the 20 and 21 day trials, respectively. Growth inhibition was completely overcome if the pectin was first reacted with pectinase. When pectin and the enzyme were fed together, growth inhibition was only partly overcome (451).

Ten chicks were fed a basal diet containing 2% pectin for 3 weeks. Results were compared to a group on a 2% cellulose diet. Growth was depressed, but feed efficiency was unaffected. Nitrogen retention, fat absorption, and metabolizable energy were not altered. Growth depression was not overcome by high-fat or high protein diets (241).

Becker et al. fed groups of four New Hampshire chicks (850 g) a standard diet plus 0 and 10% dried sugar beet pectin for a preliminary feeding period of 6 days and an 8 day analysis period. Pectin at the 10% level was tolerated, but growth was retarded. About half was excreted in the feces; the energy balance showed a general digestive depression (40).

Rats

Viola et al. fed groups of 6 weanling rats (male and female) of the Charles River C. D. strain diets containing 0, 5, and 10% Pectin-S and 10% Pectin-M for 10 days. Pectin-S is a slow-setting, 55% esterified citrus pectin; Pectin-M is a medium-rapid setting, 65% esterified pectin. The basal diet was a low in protein, containing 10% casein. The effect on growth is shown in the following table:

Level	Relative Food Intake %	Relative Weight Gain	Protein Efficiency Ratio
0	100	100	3.2 ± 0.10
(S) 10%	82.5	71.5	2.75 ± 0.12
(M) 10%	70.5	52.0	2.3 ± 0.14
(S) 5%	97.0	108.5	3.6 ± 0.20

Growth was not proportional to food intake, indicating a depressed protein utilization for Pectin M (447).

A basal diet containing 25% casein was fed to 150 weanling male rats of the Sprague-Dawley strain. The two experimental diets contained 5% citrus pectin N.F. (10 to 12% methoxyl) or 5% citrus pectin L.M. (3 to 5% methoxyl), replacing 5% of the sucrose. Animals were sacrificed at

intervals of 2, 4, 6, and 8 weeks. The pectin N.F. fed rats consumed less diet and gained less weight. The pectin N.F. appeared to share the responsibility for slowing weight gain; this effect required 5 weeks to surface. Magnesium, phosphorus, and zinc in livers of the pectin L.M. fed rats increased during the study's last 4 weeks coupled with marked accumulation of copper in the kidney and later in the liver.

Groups of 4-6 male and female rats (30-35 g) were fed 0, 2.5, 5, and 10% pectin N.F. or 0, 2.5, 5, and 10% nickel pectinate for 8 weeks. The basal diet contained 63-74% sucrose and 22% casein; controls were fed purina dog chow. No effect on growth or gross appearance was revealed, except for the 10% nickel pectinate group. These animals lost weight steadily and died in roughly 10 days. No evidence of bacteriostasis as indicated by growth retardation or nutritional deficiencies was observed for either pectin or nickel pectinate groups (108).

Two groups of young rats weighing 70-80 g (10 animals per group) were given plantaglucide internally daily in 2 and 3 g/kg doses for 28 days. Plantaglucide is the granulated powder of pectins from the leaves of the large plantain plant. No visible changes were noted in the state and weight of the animals (326).

Groups of 4 adult male rats were fed 0 and 1.5 g dried sugar beet pectin daily for a total of 11 days. Becker et al. (40) found no untoward effects.

III. Long-Term Studies

Rat

Cohen et al. fed groups of 9 two-month-old rats a basal diet plus 0 or 20% pectin, granulated sugar, sucrose, fructose, cerelose (glucose), dextrin, galactose, or lactose for 18 months. The pectin-fed group grew the least, reflecting a decreased caloric intake. Water consumption and stool output varied inversely with weight gain. Water consumption was also found to be linearly related to dried stool residue; animals drank 15 g water per 1 g stool. As regards liver lipid composition, the ester cholesterol content varied from 3% for pectin and cerelose rats to 10% in the lactose rats. Phospholipids were 60-70% for pectin, granulated sugar, and galactose groups, but 40% for the lactose group (87).

A group of 8 two-month-old, Sprague-Dawley rats were fed a diet of ground rat chow plus 20% citrus pectin N.F. and 5 consumed the basal diet only. Animals were bled by tail vein at 9, 14 and 18 months. The pectin rats grew less, presumably because of the low digestibility and absorption of pectin. The protein efficiency ratio was comparable for both groups. The test animals were smaller by some 200 g, but were healthy and had the appearance of 6-month-old animals. Water consumption increased, as did stool residue. The relative liver weights were slightly lower for the test group, and relative testicle weights were slightly higher for the test group. The final serum cholesterol levels were lower in the pectin group (88).

IV. Special Studies

Atherosclerosis Reducing

Rabbits

Fisher et al. fed groups of 8-13 male and female young Californian rabbits a cholesterol supplemented (1%) diet plus either 5% pectin or 5% cellulose. After 11 months, surviving animals were executed, and the aortas and hearts were examined histologically. All the animals except the pectin-fed males lost weight between 6 and 11 months. Mortality increased during this period with more rabbits dying on the control diet (46%) than on the pectin diet (35%). Serum cholesterol values were lower in the pectin fed group than in the control group, for males only. Pectin lowered the severity of atherosclerotic lesions in the thoracic and the abdominal aorta of the males only. Rabbits on the pectin regimen had an increased fecal excretion of lipids and sterol (122).

Chicks

Fisher et al. studied the effect of pectin in an atherosclerosis inducing diet in day old Cornell Leghorn cockerels. Two groups of 39 were fed a basal diet which included 0.5% cholesterol (whole egg powder) plus 0 or 5% pectin. At 7 weeks pectin was decreased to 3% because growth had been quite depressed. Although pectin-fed chicks grew more slowly, their final weight (24 weeks) was comparable to control weights; these birds also consumed more food per weight gain. Pectin fed birds had significantly lower levels of plasma cholesterol, and they eliminated more lipid and cholesterol in their excreta. Histological examination of the heart and aorta revealed a decreased incidence and severity of atherosclerosis in pectin cockerels (123).

In studying avian atherosclerosis, Fisher et al. found that dietary pectin retarded spontaneous atherosclerosis. Two groups of 30 two-year-old cockerels were fed a corn-soybean ration containing 15% protein, plus 5% pectin N.F. or 5% non-nuitritive fiber for 18 months. Pectin-fed birds had significantly fewer atherosclerotic plaques in the abdominal aorta than control birds, measured histologically. Dietary pectin increased food consumption, but decreased weight gain by two-thirds; serum and liver cholesterol levels were also raised (126).

Gallstone - Reducing

Rabbits

New Zealand white rabbits (2-2.5 kg) were placed on a gallstone inducing diet containing 40% casein and 15% olive oil for 24 weeks. At 12 weeks, an unspecified number of rabbits were given daily 0, 100 g of citrus pectin, N.F., or 100 g of citrus pectin, low methoxyl. At the 24th week rabbits were sacrificed and the liver and gallbladder were examined. The animals remained in good physical condition and gained weight normally throughout the experimental period. In the control group the hepatic fatty degeneration was moderate to severe as well as an increase in gallstone occurrence. The control rabbits had an increase of gallstones in the period between 12th and 24th weeks. The

citrus pectin, low methoxyl, caused more severe degeneration than in controls. Liver weights were comparable for all groups. Citrus pectin, low methoxyl, had no effect on gallstone dissolution, but citrus pectin N.F. produced some dissolution (68).

In a second experiment, the gallstone-producing diet was fed and agents added for 12 weeks. Groups of 5 male and 5 female rabbits were given 0 and 100 g of citrus pectin, N.F. Growth was normal and physical condition was good during the 12 week period. Citrus pectin N.F. reduced gallstone formation as compared with the control group (68).

Anti-Ulcer

Rats

In order to evaluate the anti-ulcer activity of plantaglucide (the powdered pectin from the leaves of the large plantain), Obolentseva and Khadzhay induced ulcers in rats by injecting phenylbutazone intramuscularly. Dosage and results are presented in the following table:

Group	Dose in g/kg	# of rats	# of rats surviving	# of rats with ulcers (in %)	# of ulcers	Paul's index	Anti-ulcer activity
Control	—	23	13	100	314	34	—
Plantaglucide	0.1	10	8	87	66	7.2	3.3
	0.5	9	7	71	36	3.6	6.6
	1.0	19	17	30	67	1.2	20.0

Length of study was not specified; plantaglucide was administered internally. Under the influence of plantaglucide, no loci of necrosis or inflammations at the injection site were observed. The authors concluded that plantaglucide had a distinct anti-ulcer effect (326).

Biochemical Aspects

I. Breakdown

The pectic enzymes may be classified into pectin methylesterases which hydrolyze the methyl ester linkages, the polygalacturonases which split the glycosidic bonds between galacturonic acid molecules, and the pectin trans-eliminases. The latter enzymes, only discovered within the past few years, bring about non-hydrolytic cleavage of alpha-(1 → 4) linkages forming unsaturated derivatives of galacturonic acid. Some of the enzymes previously considered to be polygalacturonases may turn out to be transeliminases since both show the same effect of reducing the viscosity of pectin and producing one molecule of reducing sugar for each alpha-(1 → 4) linkage broken. Further divisions exist with the pectic enzymes depending upon whether they act on the methylated or on the free polygalacturonic acid substrates, and whether they attack internal linkages.

These pectic enzymes occur in many plants and microorganisms. Pectinases used commercially come from the Aspergillus niger group of fungi and a mixture of several enzymes is used. Pectic enzymes are destroyed in a product to preserve the viscosity or are added to it to provide a clarity (436).

Temperatures over 50 degrees during production (except for short intervals as in pasteurization), high storage temperatures, low or high pH's, and access to free air during storage lead to degradation of pectin. Damage by gamma rays from cobalt-60 has been discussed; apple and carrot tissue is softened in steps from 0.5 to 2.5 X 106 rep. and the extracted pectin material is much degraded (43).

II. Absorption - Distribution

After feeding weanling rats 0, 5, and 10% citrus pectin-S (55% esterified) and 10% citrus pectin-M (65% esterified), Viola et al. found 100% of the ingested compound to occur as undigested pectin and non-volatile decomposition products in the feces. This indicated that neither form of pectin was absorbed (447).

Galacturonic acid, a metabolite of pectin, is not absorbed in appreciable quantities from the human and canine ileum or canine colon. Pectin is not excreted in the urine as galacturonates in quantities measurable by the Kapp method. The decomposition of citrus pectin in the colon in young men on a mixed diet did not significantly change the excretion of formic acid, acetic acid, and reducing substances in the urine and the feces (469).

III. Metabolism and Excretion

After feeding male Wistar rats apple pectin, Gadeken reported an apparent digestibility of 97.1 and 93.4% and an apparent digestible energy of 95.8 and 92.1% for 12 and 24% dietary pectin, respectively. Carbon and nitrogen were retained at the 24% level indicating depressed

digestion. Using gaseous exchange methods, he reported that pectin was decomposed by intestinal flora into galacturonic acid or other organic acids capable of resorption, and finally to carbon dioxide and water. Most of the carbon administered in pectin-augmented rations could be found as CO_2. Since galacturonic acid is absorbed in the small intestine, monogastric animals can't use it. The intense breakdown activity prohibited the absorption of any large amount of possible intermediate metabolites. Complete pectin breakdown was proportionatly smaller the higher the pectin content of the diet. Gadeken suggested that when increasing amounts of pectin were decomposed, the resulting increase in the formation of galacturonic and other organic acids shifted intestinal pH, which inhibited floral activity. This enabled the animals to resorb a greater proportion of the intermediates; however, actual resorption could not be measured (137).

Gadeken reported the digestible energy of pure pectin to be 3553 cal/g for the 12% ration and 3415 cal/g for the 24% ration. After adjustments were made for digestive depressions and variations in live body weight, he reported a utilization coefficient of 5.6 and 20.3% of the gross energy for 12 and 24% rations, respectively (137).

In their feeding studies on weanling rats, Viola et al. determined a specific digestible energy of -1.6 kcal/g for citrus pectin-M and -1.65 for citrus pectin-S when fed at a 10% level. M indicates 65% esterified and S indicates 55% esterified. The coefficient of digestible energy was 84.1 and 84.8% for pectin-S and pectin-M, respectively (447).

When citrus pectin (140 g) was fed with a mixed diet to dogs for seven days, 90% of the pectin was decomposed; only 50% was decomposed by fasted dogs. Decomposition increased when the pectin was retained longer and when fed in a mixed diet rather than alone. Humans fed a mixed diet had a decomposition factor of 90%. But, fasting man decomposed more pectin (85%) than the fasting dog. Ileostomies revealed that pectin decomposed chiefly in the colon and that bacterial enzymes are involved rather than animal enzymes (468).

Gilmore fed Sprague-Dawley rats diets containing 5.0% citrus pectin N.F. (10-12% methoxyl) and 5.0% citrus pectin L.M. (3 to 5% methoxyl) for 8 weeks. Examination of the feces revealed that less than 1% of pectin N.F. could be recovered while 20-23% of the pectin L.M. could be recovered (143).

In 1940 Kertesz reported that saliva and gastric juice do not act enzymatically on pectin, based on studies with dogs and man. Pectin passed through the stomach and part of the small intestine of a dog was recovered without loss. Trypsin and pepsin had no effect on pectin in vitro, but pectin incubated with feces was rapidly decomposed. Kertesz suggested that ingested pectin is not attacked until it reaches the large intestine where it is completely hydrolyzed by bacterial enzymes (211).

Bacteria capable of decomposing pectin in vitro were isolated from the feces of a dog fed pectin alone for a week. The more active bacteria belonged to Aerobacillus, Lactobacillus, Micrococcus, and Enterococcus. Berkefeld filtrates of these organisms contained heat labile, enzyme-

like substances such as a pectinase-like, liquefying enzyme and/or pectase-like, coagulating enzyme. Formic and acetic acids were the chief products, with some galacturonic acid. The quantity of galacturonic acid formed was either very small or broken down quickly (471).

IV. Effects on Enzymes and Other Biochemical Parameters

Keys et al. found serum cholesterol values to decrease 5% below a control value (15 g cellulose diet) when middle-aged men were fed 15 g citrus pectin N.F. per day for several weeks. The diets were similar in calories, fats, and cholesterol, but differed in the carbohydrate source. In one diet, a considerable amount of carbohydrate was provided by legumes and matched with sugar in another. Pectin reduced cholesterol levels for both diets (213).

Fisher et al. reported that pectin lowers blood cholesterol only in the presence of dietary cholesterol. Data was cited from Fisher et al. (1964) in which chickens fed a cholesterol free diet showed no hypocholesterolemic response from pectin, but cholesterol feeding reversed this response. Experiments on humans revealed a more pronounced hypocholesterolemic effect than that found by Keys et al. (1961); twice as much cholesterol was fed in the Fisher study. Pectin exerted a similar response in rabbits, as well (125).

Palmer and Dixon reported that daily administration of 6-10 g pectin N.F. per os reduced serum cholesterol in 12 out of 16 adult male humans over a 4 week period. Subjects consumed their usual diet throughout. They received citrus pectin capsules in 2 g increments in 6 four-week-test periods followed by a 10 week period without treatment; some received Alphacel placebos (334).

Fisher et al. reported a hypocholesteremic effect of pectin in a diet containing cholesterol in swine. Four groups of either six yorkshire pigs (8-week old; both sexes) or 4 sixteen-week old male Hampshire pigs received either 5% pectin or 5% cellulose in the presence or absence of dietary cholesterol (egg powder). Food intake, body weight, and plasma cholesterol were determined during the 4-week study. Pectin had no effects on the swine consuming a cholesterol-free diet. But pigs on the pectin plus cholesterol diet were hypocholesterolemic when compared to those on the cholesterol diet. Pigs on the cholesterol plus pectin diet gained less weight than cholesterol fed animals (127).

Fausch and Anderson fed groups of 5 Minnesota No. 3 weanling barrow swine a basal diet plus 0 and 5% citrus pectin. Two more groups received diets containing 5% fat, 5% fat plus and 5% citrus pectin. Pectin was added during the last 4 weeks of the 12 week study. At the 10th week pigs fed pectin had significantly higher serum cholesterol levels than those on the basal diet; pigs fed pectin plus fat had even higher cholesterol levels, indicating a synergism between pectin and fat. Pectin and fat fed separately had a similar effect on cholesterol. Serum triglycerides and phospholipid levels increased in pectin-fed pigs. The hepatic cholesterol level of those receiving both pectin and fat was significantly greater than in control animals, but not from those receiving pectin or fat. Weight gain, feed consumption, and feed efficiency were unaffected (112).

Leveille and Sauberlich fed male rats 1% cholesterol with and without 5% pectin for 28 days. Pectin lowered plasma and liver cholesterol levels. In vitro experiments revealed that pectin decreased taurocholic acid (a bile acid) transport by about 50%. Similar evidence was noted when both pectin and cholestyramine, and inhibitor of bile acid absorption, were fed. Isotopic studies showed cholesterol absorption to be depressed by dietary pectin. Leveille and Sauberlich suggested that pectin is hypocholesterolemic because of the inhibition of bile acid and cholesterol absorption, rather than an effect on intestinal microflora (261).

Isotopic studies by Hyun et al. indicated that oral pectin reduced cholesterol -4-C^{14} absorption by 7% from control values in male Wistar rats (182).

Phillips and Brien fed weanling rats either a ground cube diet or a semipurified casein-sucrose diet, supplemented with either 1% cholesterol, 2 or 3% pectin, or both for 18-21 days. Pectin was hypocholesterolemic only when the diet contained cholesterol. No limiting effect on vitamin A absorption or utilization was reported, as is found upon administration of cholestyramine. Cholestyramine inhibits cholesterol absorption. However, since pectin does not affect vitamin A metabolism, pectin must be hypocholesterolemic through some other mechanism (340).

Wells and Ershoff found that citrus pectin counteracted the hypercholesterolemia induced by cholesterol feeding in rats, but not in guinea pigs, hamsters, or rabbits. Male rabbits, guinea pigs, hamsters, and rats were fed a basal diet plus 0, 1% cholesterol, or 1% cholesterol plus 5% pectin N.F. (1 of each species per diet). The guinea pigs were sacrificed at 4 weeks and the other animals at 6 weeks. Serum and liver cholesterol and liver total lipids increased in all animals consuming 1% cholesterol, except for rats fed cholesterol plus 5% pectin (465).

Anderson and Bowman found citrus pectin to be a better hypocholesterolemic agent than tomato pectin for rats on a casein-sucrose diet. This may be due to the differences in methoxyl content of the two pectins; citrus pectin was 9.2% methoxylated while tomato pectin was only 6.5% methoxylated. There was a trend toward heavier kidney weights in the pectin-fed rats, especially with tomato pectin. The citrus pectin group had significantly smaller adrenals than the other groups (12).

Ershoff and Wells found that pectin preparations with a methoxyl content of 5% or less were not hypocholesterolemic for rats fed cholesterol. However, pectin N.F. (10.7% methoxyl) exerted a high degree of hypocholesterolemic activity (110).

Arkhipova and Zorina have shown sunflower pectin to be a useful prophylactic against poisoning by lead, mercury, manganese, and beryllium. Following intubation of lead nitrate, lead removal from the gastrointestinal tract was higher in rats fed pectin than in rats given no pectin. Similar results were found for guinea pigs that received 200 mg in one month and for industrial workers who consumed a high-pectin marmalade for 2 months. In rats given mercury nitrate per os, mercury removal from the G. I. tract was improved by orally administered pectin over a 5-day period. Other rats were fed pectin for 1 month after a

single dose of mercury; the mercury content of the blood and kidneys was reduced. Pectin doses of 1.5-2 g/kg-day for animals and 8-10 g/kg-day for men produced no side effects (20).

When rats were fed 0 and 10% pectin-M (65% esterified) and 5 and 10% pectin-S (55% esterified), Viola et al. observed poor protein utilization for pectin M. Pectin-S had reduced protein digestibility but protein utilization was normal. Both pectins reduced fat absorption at the 10% level. Calcium absorption was depressed by both types of pectin (447).

Obolentseva and Khadzhai reported that gastric secretion was enhanced by plantaglucide in dogs. Plantaglucide is the granulated powder of pectin from the leaves of the large plantain. Dogs with stomach fistulas received 1 g plantaglucide in 50 ml of water 30 minutes prior to a s.c. injection of 0.5 ml of a 0.1% histamine solution. Under the influence of plantaglucide, 2 out of 3 dogs (30 experiments) increased the amount of gastric fluid released by histamine 2.5 times. In all 3 dogs, the total and free acidity rose by 20-23% (326).

X-ray studies indicated that plantaglucide slowed the migration of stomach contents to the small intestine in 22 male rats. Doses of 0.1-1.0 g/kg were orally administered 20-30 minutes prior to giving the irradiated mass. Doses of 0.1 and 1.0 g/kg slowed evacuation by 2 and 3.5 times respectively (326).

Obolentseva and Khadzhai studied the effect of plantaglucide on edema produced by injection of formalin and dextrin in mice and rats. Oral doses of 0.5 and 1.0 g/kg were given before and after the edema-inducing agents. Edema was reduced for both agents; plantaglucide showed a definite anti-inflammatory effect (326).

Bender summarized the work on pectin as an antidiarrhetic in medicine. Referring to articles printed between 1940 and 1942, he stated that "pectin moves rapidly to the large intestine, coating the mucosa with unchanged polysaccharide. There, it is used as a carbon source by the normal flora. The lower fatty acids, especially acetic and formic, are the end products; the intermediate D-galacturonic acid is also formed, producing the normal complement of acids that stabilizes the pH of the colon. These acids are also bacteriostats against most pathogens at normal bowel pH. Since disorders associated with abnormal and undesirable flora are usually accompanied by a higher-than-normal pH, the inflow of pectin and subsequent return to normal pH by its digestion adversely affects the growth of the pathogens and encourages the normal flora to return to full activity. There are two apparent causes for this return to normalcy, the lowering of pH and the production of certain acid radicals at levels which can not be tolerated by most of the pathogens. However, some effects remain which are not explained by this mechanism, such as the extremely prompt but usually only temporary relief which results from a single dose, with the need for two or more doses for complete relief, the almost immediate reversal of the fatigued feeling to one of well-being, and the inhibiting effect of pectin on certain viruses. The mechanism of its action against diarrhea obviously has not yet been completely determined." (43).

V. Drug Interaction

No Information Available

VI. Consumer Exposure Information

Pectin is used as a gelling agent, thickener, stabilizer, and emulsifier. In 1959 Benders' review of the use of pectin by the food industry estimated that 85-95% was used in the manufacture of jams and jellies. Confectioner jelly, low-sugar jelly, puddings, and fruit preserves contain pectin, too. Various milk products are thickened with pectin to produce a smooth curd and whey, imitation custard, or pudding texture. It is used in food coatings for candies as well as cheese rind. Pectin is an efficient stabilizer for frozen strawberries, eggless custard, domestic ice creams, fondant, meringue, bread and cake, mayonnaise, and French dressing (43).

PECTIN

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Comparative Cholesterol-Lowering Activity of Citrus and Tomato Pectin (33630)

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Citrus pectin, when included in a cholesterol-containing diet, was reported to be an effective hypocholesterolemic agent in several species (1–3). The purpose of the present study was to evaluate the cholesterol-lowering activity of tomato pectin compared with that of citrus pectin.

Methods and Procedure. Forty-eight male weanling rats were randomly assigned to one of the four different rations shown in Table I. All rats were individually caged and allowed *ad libitum* access to feed and water during the 4-week feeding period. On the twenty-eighth day of the study, all rats were weighed and quickly killed in random sequence with a Harvard model 130 RM decapitator and the liver, kidneys, adrenals, and a blood sample collected from each animal. Serum and hepatic cholesterol were determined by a modification of the technique of Mann (4) and total serum and hepatic lipid measured gravimetrically after the Folch-washed tissue homogenate was evaporated to dryness (5).

Results and Discussion. The performance of the four groups of rats as indicated by body weight gain (Table II) was essentially identical. The pectin-fed animals (Rations 3 and 4) consumed slightly more feed; however, body weight gain expressed in terms of feed eaten did not reveal any ration effects. Liver weights of the rats receiving Ration 1 were significantly lighter ($p < 0.01$) than those of the cholesterol-fed animals regardless of whether or not pectin was included in the ration. There was a trend toward heavier kidney weights in the pectin-fed rats, particularly in the rats fed tomato pectin ($p < 0.01$). Rats receiving citrus pectin in the ration were observed to have adrenals which were significantly smaller than any other group ($p < 0.01$). The ration effects on serum and hepatic lipids are presented in Table III.

TABLE I. Experimental Rations.

Ingredient	Ration no. 1	2	3	4
Sucrose	59.0	58.0	53.0	53.0
Vitamin-free casein	24.0	24.0	24.0	24.0
Soybean oil	10.0	10.0	10.0	10.0
Salt mixture (USP XIV)[a]	5.0	5.0	5.0	5.0
Vitamin mix[b]	2.0	2.0	2.0	2.0
Cholesterol (USP)		1.0	1.0	1.0
Citrus pectin[c]				5.0
Tomato pectin[d]			5.0	
	100.0	100.0	100.0	100.0

[a] Nutritional Biochemicals Corporation.

[b] Vitamin Diet Fortification Mix, Nutritional Biochemicals Corporation.

[c] Kindly supplied by Mr. Grant H. Palmer, Pharmaceutical Coordinator, Sunkist Growers, Corona, California.

[d] Prepared by Dr. Walter L. Thompson, Senior Fellow, Heinz Fellowship, Mellon Institute, Pittsburgh, Pa.

Serum cholesterol and total lipid levels in the citrus pectin-fed rats were the lowest of any treatment group including the rats receiving the cholesterol-free ration. Feeding tomato pectin resulted in serum cholesterol levels which were approximately the same as the rats fed Ration 1. Cholesterol-fed rats not receiving pectin had serum cholesterol levels which were significantly elevated ($p < 0.05$) above all other treatment groups.

Highly significant differences in hepatic cholesterol levels were noted between each of the four treatment groups. Rats fed Ration 2 had hepatic cholesterol levels which were almost seven times higher than in those animals receiving the cholesterol-free ration. Compared with rats fed Ration 2, hepatic cholsterol levels were effectively reduced in both the citrus (−53%) and tomato (−27%) pectin groups.

TABLE II. Comparative Effects of Citrus and Tomato Pectin on Performance and Organ Weights in Cholesterol-Fed Rats.

Variable	Ration no. 1	2	3	4	SE
Initial wt. (g)	83	87	83	86	±1.0
Final wt. (g)	269	283	276	277	±8.4
Body wt. gain (g)	186	196	193	191	±7.8
Total feed eaten (g)	447	455	464	473	NA[a]
Feed eaten/g gain	2.40	2.33	2.40	2.47	NA[a]
Liver wt. (% body wt.)	5.14[b]	5.90	5.62	5.58	±0.111
Kidney wt. (% body wt.)	0.77	0.78	0.87[b]	0.80	±0.015
Adrenal wt. (% body wt. × 10^3)	1.55	1.49	1.46	1.24[b]	±0.063

[a] Pooled samples.
[b] $p < 0.01$.

TABLE III. Comparative Effects of Citrus and Tomato Pectin on Serum and Hepatic Cholesterol Levels in Cholesterol-Fed Rats.

Variable	Ration no. 1	2	3	4	SE
Serum total lipid (mg/100 ml)	423	544	467	389	NA[a]
Liver total lipid (g/100 g)	5.1	7.9	6.7	6.5	NA[a]
Serum cholesterol (mg/100 ml)	83	96[b]	84	80	±4.2
Liver cholesterol (mg/100 g)	205[c]	1360[c]	1003[c]	639[c]	±96.2

[a] Pooled samples.
[b] $p < 0.05$.
[c] $p < 0.01$.

The results of this study indicate that citrus and tomato pectin significantly lower both serum and hepatic cholesterol levels in the cholesterol-fed rat. Citrus pectin is the more effective of the two pectins in this regard. Ershoff and Wells (6) have reported that the cholesterol-lowering effects of apple and citrus pectins is associated with the methoxyl content. The fact that the methoxyl content of the citrus pectin (9.2%) was somewhat higher than that of the tomato pectin (6.5%) may explain the observed difference in cholesterol-lowering activity.

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USE OF PECTIN AS A PROPHYLACTIC FOR POISONING DUE TO METALS (A CLINICAL-EXPERIMENTAL STUDY)

Arkhipova-'65
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One important problem of occupational health and the occupational disease clinic is answering questions of poisoning prophylaxis.

As is well-known, a number of industrial poisons (lead, mercury, manganese) are capable of accumulating in the organism and forming deposits in various organs.

Studies directed toward discovering the means of preventing the absorption of metals from the gastrointestinal tract are very limited. Besides, in industrial situations, especially in industries associated with dust formation, the entry of metallic dust per os is possible. Many metals (lead, mercury, manganese) are excreted in large amounts from the bile in the intestine, from which they are reabsorbed into the blood. A prophylactic against original resorption, or against entry of toxic metals from the intestine and for the acceleration of metal removal through the gastrointestinal tract may prevent or decrease deposition of metals in the organism and, consequently, prevent of lessen the development of poisoning. As a prophylactic against poisoning, it is important to look for preparations of a type which may be used daily by the simplest means - per os, immediately upon manufacture. Such substances are the pectins which are a component part of many food products.

In research on pectin transference in experiments on animals and people under industrial conditions, it was established that pectin, even in significant doses, does not cause secondary phenomena (the dosage for animals was 1.5-2 g/kg, for people 8-10 g/kg or more of pectin pro die).

Experimental studies of the sorption properties of pectin in vitro have shown that pectin will absorb lead, mercury, manganese, and beryllium, but not vanadium. Sunflower pectin possesses the best sorptive properties; apple pectin absorbs metals significantly worse.

The experimental studies in vivo established the definite prophylactic action of pectin in lead poisoning elicited in animals (rats, guinea pigs) by intragastric introduction of lead nitrate. In rats which received lead and pectin, lead removal through the gastrointestinal tract during the whole experimental period (three weeks) was higher than in animals which received lead without pectin. In guinea pigs which received pectin with their food in amounts of 200 mg during the course of a month, together with toxic doses of lead, the occurrence of lead poisoning was less obvious (Figure 38). Thus in animals which received lead without pectin, reticulocytosis was observed (55%), the amount of basophilic granular erythrocytes was increased (32.8%), and hemoglobin was lowered (from 75.8 +or - 2% to 64.2 + or - 2.2%). In animals which received pectin as a prophylactic measure, alterations in the blood were significantly less noticeable.

TABLE 38. ALTERATIONS IN PERIPHERAL BLOOD IN GUINEA PIGS WHICH RECEIVED PECTIN

Group of Exper. Animals		Control	Ones Which Received Lead	Ones which Received Lead & Pectin
# of animals		10	10	10
Hemoglobin in %	Bef. Exper.	78.1±1.4	77.7±0.93	75.8±2.0
	After. Exper.	77.4±1.5	64.2±2.2	68.6±2.0
Reticulocytes in %	Bef. Exper.	9.2	10.7	12.4
	After Exper.	10.4	55.3	15.3
Basophilic granular erythrocytes in %	Bef. Exper.	---	—	—
	After. Exper.		32.8	3.5

Thus reticulocytosis was practically absent, the number of basophilic granular erythrocytes did not increase significantly, and the decrease in hemoglobin was less.

The prohylactic effect of pectin in the experiment was also made apparent in relation to mercury deposition. In white rats which received mercury nitrate per os, containing Hg^{203}, mercury removal through the gastrointestinal tract was less than in animals which received Hg^{203} and pectin in 60 mg (through a probe) in the course of five days.

In rats on a pectin diet for a month after a single administration of $Hg^{203}(NO_3)_2$, a significant difference was noted in Hg^{203} content in the blood and kidneys.

In the kidneys of animals which received pectin, Hg^{203} content was 44% of the control's, and 40% in the blood, i.e. a definite, statistically significant decrease occurred in mercury deposition within the organism.

On the basis of experimental studies conducted by the Institute of Occupational Hygiene and Occupational Disease, Academy of Medical Sciences, USSR, jointly with the Department of Occupational Diseases of the Central Institute for the Advanced Training of Physicians, instructions were worked out for the use of food products containing a large amount of pectin.

In one of the factories investigated, workers received marmalade with a high pectin content for two months. At the beginning of pectin administration, and again twice during the two months, lead content was studied in the urine and feces, hemoglobin content was determined as well as that of erythrocytes, reticulocytes, basophilic granular erythrocytes, and leucocytes in peripheral blood. The studies conducted before pectin administration established that among those examined, there was no instance of lead posioning, which was shown by the absence of basophilic granular erythrocytes, reticulocytosis, and a relatively high hemoglobin content.

TABLE 39. LEAD REMOVAL USING PECTIN

#	Persons Examined	Age in years	Period of work with Pb expos. (in yrs)	Amt. of lead in urine (in mg/liter) Studies Ist	2nd	3rd	Amt. of lead in feces (in mg/kg) 1st	2nd	3rd
1	B.	40	16	0	—	0	0.03	—	2.5
2	V.	38	10	0.01	0.01	0.01	0.06	0.4	0.4
3	G.	36	9	0	0.4	0.02	0.14	1.1	0.6
4	D.	46	9	0.03	0	0	0	0.27	0.05
5	Z.	22	1	0	—	0	0.05	0.69	0.61
6	K.	50	16	0	—	0	0.1	—	0.57
7	P.	32	2	0.1	—	0	0.08	—	2.4
8	R.	47	7	0.028	0.02	0.04	0.015	0.2	1.0
9	B.	35	12	0.01	0	0	0.02	0	0

As is evident from Table 39, lead content determined by the chromate method varied in urine from 0 to 0.13 mg/liter, and in feces from 0 to 0.14 mg/kg. Therefore among those examined it was promarily experienced workers who were carriers of Pb.

The receipt of pectin in the majority of studies caused an increase in lead removal through the gastrointestinal tract. In some cases this increase was extremely significant (an increase of 80, 30, and 12 times). In only one case was there no increase in lead removal and in one case a decrease was noted.

In using pectin in the clinic, the dynamics of lead removal could be traced in the urine only, which showed in all cases either a decrease or the absence of lead. At the same time a decrease was noted in the amount of

basophilic granular erythrocytes and reticulocytes in the blood. The data obtained on the decrease in lead removal from urine could be considered as indirect evidence of redistribution of the means of lead removal after giving pectin. With pectin administration, no secondary phenomena or complications of any sort were noted.

Therefore, pectine-like substances may be used in industries in which workers come in contact with lead and mercury. Pectin increases lead and mercury removal from the organism.

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Применение пектина для профилактики интоксикации металлами (клинико-экспериментальные исследования)

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Одной из важных проблем гигиены труда и клиники профболезней является разработка вопросов профилактики интоксикаций.

Как известно, ряд промышленных ядов (свинец, ртуть, марганец) способен накапливаться в организме и образовывать депо в различных органах.

Исследования, направленные на изыскание средств, предупреждающих всасывание металлов из желудочно-кишечного тракта, весьма ограничены. Между тем в производственных условиях, особенно в производствах, связанных с пылеобразованием, возможно попадание металлической пыли per os. Кроме того, многие металлы (свинец, ртуть, марганец) в большом количестве выводятся с желчью в кишечник, откуда всасываются обратно в кровь. Профилактика первичной резорбции, а также обратного поступления токсических металлов из кишечника, ускорение выведения металлов через желудочно-кишечный тракт могут предупредить или уменьшить депонирование металлов в организме, а следовательно, предупредить или ослабить развитие интоксикации. Для профилактики интоксикаций важно изыскать препараты такого рода, которые могут быть применены повседневно, самым простым путем — per os, непосредственно на производстве. К таким веществам относятся пектины, являющиеся составной частью многих пищевых продуктов.

Исследованием переносимости пектина в эксперименте на животных и на людях в условиях производства установлено,

что пектин даже в значительных дозах не вызывает побочных явлений (для животных 1,5—2 г/кг, для людей по 8—10 г/кг и более пектина pro die).

Экспериментальные исследования сорбционных свойств пектина in vitro показали, что пектин сорбирует свинец, ртуть, марганец и бериллий, но не сорбирует ванадий. Лучшими сорбционными свойствами обладает подсолнечниковый пектин, значительно хуже сорбирует металлы яблочный пектин.

Экспериментальными исследованиями in vivo установлено определенное профилактическое действие пектина при свинцовой интоксикации, вызываемой у животных (крысы, морские свинки) внутрижелудочным введением азотнокислого свинца. У крыс, получавших свинец и пектин, выведение свинца через желудочно-кишечный тракт во все время исследования (3 недели) было выше, чем у животных, получавших свинец без пектина. У морских свинок, которые в течение месяца наряду с токсическими дозами свинца получали с кормом пектин в количестве 200 мг, явления свинцовой интоксикации были менее выраженными (табл. 38). Так, у животных, получавших

Таблица 38

Изменения в периферической крови морских свинок, получавших пектин

Группа подопытных животных		Контроль	Получавшие свинец	Получавшие свинец и пектин
Количество животных		10	10	10
Гемоглобин в %	До опыта	78,1±1,4	77,7±0,93	75,8±2,0
	После опыта	77,4±1,5	64,2±2,2	68,6±2,0
Ретикулоциты в ‰	До опыта	9,2	10,7	12,4
	После опыта	10,4	55,3	15,3
Базофильнозернистые эритроциты в ‰	До опыта	—	—	—
	После опыта	—	32,8	3,5

свинец без пектина, наблюдался ретикулоцитоз (55‰), увеличивалось количество базофильнозернистых эритроцитов (32,8‰), снижался гемоглобин (с 75,8±2% до 64,2±2,2%). У животных, получавших в качестве профилактического средства пектин, изменения в крови были значительно менее выражены. Так, практически отсутствовал ретикулоцитоз, число базофильнозернистых эритроцитов увеличилось незначительно, падение гемоглобина было меньшим.

Профилактическое влияние пектина в эксперименте было выявлено также в отношении депонирования ртути. У белых крыс, которым вводилась per os азотнокислая ртуть, содержащая Hg^{203}, выведение ртути через желудочно-кишечный тракт

было меньшим, чем у животных, получавших Hg^{203} и пектин по 60 мг (через зонд) в течение 5 дней.

У крыс, находившихся на пектиновой диете в течение месяца после однократного введения $Hg^{203}(NO_3)_2$, отмечена значительная разница в содержании Hg^{203} в крови и почках.

В почках животных, получавших пектин, содержание Hg^{203} составляет 44% от контроля, в крови—40%, т. е. имеет место определенное, статистически достоверное уменьшение депо ртути в организме.

На основании экспериментальных исследований, проведенных Институтом гигиены труда и профзаболеваний АМН СССР совместно с кафедрой профзаболеваний ЦИУ, была разработана Инструкция по применению пищевых изделий, содержащих повышенные количества пектина.

На одном из заводов рабочие в течение 2 месяцев получали мармелад с повышенным содержанием пектина. До начала приема пектина, а также дважды в течение 2 месяцев исследовалось содержание свинца в моче и кале, определялось содержание гемоглобина, эритроцитов, ретикулоцитов, базофильнозернистых эритроцитов и лейкоцитов в периферической крови. Исследованиями, проведенными до приема пектина, установлено, что среди обследуемых не было случаев выраженной интоксикации свинцом, о чем свидетельствовало отсутствие базофильнозернистых эритроцитов, ретикулоцитоза и относительно высокое содержание гемоглобина.

Таблица 39

Выведение свинца при применении пектина

№ п/п	Обследованные лица	Возраст в годах	Стаж работы со свинцом в годах	Количество свинца в моче в мг/л			Количество свинца в кале в мг/кг		
				исследования					
				первое	второе	третье	первое	второе	третье
1	Б.	40	16	0	—	0	0,03	—	2,5
2	В.	38	10	0,01	0,01	0,01	0,06	0,4	0,4
3	Г.	36	9	0	0,4	0,02	0,14	1,1	0,6
4	Д.	46	9	0,03	0	0	0	0,27	0,05
5	З.	22	1	0	—	0	0,05	0,69	0,64
6	К.	50	16	0	—	0	0,1	—	0,57
7	П.	32	2	0,1	—	0	0,08	—	2,4
8	Р.	47	7	0,028	0,02	0,04	0,015	0,2	1,0
9	Б.	35	12	0,04	0	0	0,02	0	0

Как видно из табл. 39, содержание свинца, определяемое хроматным методом, в моче колебалось от 0 до 0,13 мг/л, в кале от 0 до 0,14 мг/кг. Таким образом, среди обследованных

имелись преимущественно стажированные рабочие, которые являлись носителями свинца.

Прием пектина у большинства исследованных вызвал увеличение выведения свинца через желудочно-кишечный тракт. В некоторых случаях это увеличение было весьма значительным (в 80—30—12 раз). Лишь в одном случае не отмечено увеличения выведения свинца, а в одном случае наблюдалось уменьшение.

При назначении пектина в клинике удалось проследить в динамике выведение свинца только с мочой, которое показало во всех случаях либо уменьшение, либо отсутствие свинца. В то же время отмечено уменьшение количества базофильно-зернистых эритроцитов и ретикулоцитов в крови. Полученные данные о снижении выведения свинца с мочой можно рассматривать как косвенное доказательство перераспределения путей выведения свинца после дачи пектина. При приеме пектина не отмечалось каких-либо побочных явлений и осложнений.

Таким образом, пектиновые вещества могут быть применены в производствах, где имеет место контакт работающих со свинцом и ртутью. Пектин усиливает выведение свинца и ртути из организма.

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Studies of Pectin Substances as a Component of Animal Feed

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3. Communication

Further experiments with pectin-containing feed rations for chicks, rats and sheep.

The first experiments on feeds, which were reported on in communication 2 in this journal, had attempted to determine approximately the compatibility, the digestibility of the gross energy, and the feed effects of pectin. In the meantime, the elaboration of determination methods for pectin, or its main component, galacturonic acid, made it possible to carry out similar experiments in which the material balance of pectin in digestion could be determined. The experiments, which will be reported on in the present communication, were again carried out on chicks as well as on rats and finally on small cud chewers (sheep).

In the first series of experiments, the utilization of pure pectin by chicks was tested. Eight New Hampshire chicks of equal size and the same development (average starting weight 850 g) were chosen on the basis of preliminary tests and separated into two groups. The animals of the standard group I received a granulated ready-made feed ("Ibeka" - young hen feed) of the following composition: 15% wheat bran, 15% oats, 15% barley, 10% albumen concentrate, 10% corn, 10% tapioca, 10% feed wheat, 5% wheat aftermeal, 5% corn starch residue, 2% Vitamin A-, D_3-, B_2- premix, 2% shredded bone feed, 1% mixture of minerals. In the feed of the experimental group II, 10% of this normal feed was replaced by pectin and commercially common, pure dried pectin (83.6% Tr.S.) from sugar beets. In order to ensure the complete consumption of the pectin, it was mixed with the basic feed before the granulation.

Analysis of the basic feed gave the following values: 90.7% dried substance and in the dried substance: 17.8% raw albumen, 6.6% raw fiber, 4.3% raw fat, 6.5% raw ashes; gross energy 4.405 kcal/g. In this basic feed, there was a natural content of galacturonic acid of 4.35%. The experiment itself consisted of a six-day preliminary feeding period and an eight-day analysis period. It went smoothly, yet the excrement of the animals fed with increased pectin obviously contained certain amounts of unaltered pectin. The results can be gathered in detail from the following summary. All values are calculated on an average and per day.

I. Normal Group

Daily feed consumption: 75.0 g basic feed containing 68.0 g dried material, 2.95 g uronic acid (natural pectin content), 299.5 kcal gross energy.

II. Experimental Group

Daily feed consumption: 67.5 g basic feed + 7.5 g pectin. Contained in the daily feed were: 61.20 g dried material from the basic feed, 6.27 g of dried material from pectin, 2.66 g uronic acid from the basic feed, 3.94 g uronic acid from the pectin addition, 269.5 kcal gross energy from the basic feed, 18.4 kcal gross energy from the pectin addition.

Table 1

Metabolic Balance

	I. Normal Group	II. Experimental Group
Daily intake	20.5 g	8.2 g
Energy in feed	299.5 kcal	287.9 kcal
Energy in excretions	109.3 "	117.1 "
Convertible energy	190.2 "	170.8 "
From basic feed		171.2 "
From pectin addition		—0.4 "
Uronic acid in feed	2.95 g	6.60 g
Uronic acid in excrements	1.69 g	3.46 g
Uronic acid converted	1.26 g	3.14 g
From basic feed		1.14 g
From pectin addition		2.00 g
In % of the pectin addition		51%

The results determined here confirm the earlier experiments on the incomplete utilization of pectin additions in the feed of chickens. A component of 10% pectin in the total feed ration even leads to a certain depression in the effectiveness of the feed. Finally, it is certain that the utilization of pectin by chicks is questionable, and a substantial nutritive value from the part broken up in the digestive tract is not to be expected. Hence, pectin corresponds, in the nourishment of chickens, to the other plantlike structural substances such as cellulose, lignin and pentosane.

With rats, only a few experiments were carried out for completion of the earlier ones and, indeed, for the time being, the intake and compatability of pure free galacturonic acid was to be tested. Experimental animals were male Wistar rats of about 200 g in weight. The addition of 2 g galacturonic acid monohydrate daily to 10 g normal feed was, however, so poorly endured that the experiment had to be broken off after a few days. The animals became strongly diarrhetic and refused further feed intake.

For the next series of experiments, smaller additions of galacturonic acid were used. Experimental animals were again male, approximately adult rats in groups of 4 animals each. The normal group I received daily (exactly weighed) 10 g basic feed consisting of 85% wheat starch, 12% fish meal, 3% Waldhof dried yeast, with 3000 I.E. vitamin A and 400 I.E. vitamin D_3 per kg. The animals of group II received, in addition, 1.5 g of dried pectin daily and those of group III 1 g galacturonic acid. The excreted feces and urine were separately caught in special glass flasks and collected daily. The feces were carefully dried at 80°, the urine washed with water in a 250-ml graduated flask, which served as a collector, and preserved in toluene. At the end of the experiment, the graduated flasks were filled with water, the contents mixed thoroughly, and aliquot parts removed and taken for analysis.

After a four day preliminary feeding period followed the seven day long analysis period. In the animals of group III, the addition of galacturonic acid caused diarrhea in the first two days, after which things returned to normal and, during the true experimental time, the consistency of the feces was normal. During the experiment, no troubles came up and the animals consumed completely the amounts of feed brought to them.

In Table 2, the material balances of galacturonic acid in the digestibility experiments are given for the three groups. It is apparent that the determination method of McCready is not useable for such experiments. While the decarboxylation gives correct values for pure galacturonic acid and pure pectin, it yields completely false results for pectin-type materials, e.g. normal feed and, above all, feces; the values are much too high. The basis, namely the occurrence of apparent pectin values through carbon dioxide formation from other organic substances, have been put forth in the first communication. The specific galacturonic acid analysis according to the new method of Harnisch, however, gives reliable results. The galacturonic acid part of the additions, also with dried pectin, turns out to be highly digestible and useable. Galacturonic acid itself was, to be sure, not tolerated as well, which certainly indicates that the free acid strongly irritates the intestines. However, the galacturonic acid quantities freed gradually from the high molecular weight pectin has no such effect. After the supply of free galacturonic acid, the elimination of certain amounts in the

urine of the animals was established. This undoubtedly refers back to the more rapid resorption than was present with pectin. The latter caused no increase worth mentioning of galacturonic acid in urine.

Table 2

Galacturonic Acid Balance in the Trial Period (7 Days)

Additions (daily)	Normal Group none	1. Experimental Group 1.5 g pectin	2. Experimental Group 1 g galacturonic acid
Uronic acid in feed	0.462 g	6.132 g	7.462 g
Uronic acid in the addition	—	5.670 g	7.000 g
Uronic acid in feces	0.055 g	0.501 g	0.084 g
Uronic acid in urine	0.029 g	0.035 g	0.107 g
Uronic acid digested	0.407 g	5.631 g	7.378 g
From basic feed		0.407 g	0.407 g
From addition		5.224 g	6.971 g
In % of addition		92.1%	99.6%
Uronic acid converted	0.378 g	5.596 g	7.271 g
From basic feed		0.378 g	0.378 g
From addition		5.218 g	6.893 g
In % of addition		92.0%	98.5%

Experiments with Sheep

The main interest with regard to utilization of the pectin substance in feed covers the nourishment of ruminants. In the following, a few experiments carried out on sheep will be described briefly. In these, the utilization of pectin substances in basic feed (hay) as well as in a commercially common pectin concentrate and in dried chips, as a relatively pectin-rich feed, were studied. The sheep that were used numbers 1, 2 and 3 were Merino meat sheep. They were about 1½ years old and accustomed to experiments of this sort. Four distinct experiments were undertaken, one as a basic feed experiment, the others as difference experiments with supplements of dried pectin or dried chips. In addition to the quantitative recording of feed and excrement, which is necessary for the determination of the digestible energy, the urine was also carefully collected. After a preliminary experiment in which the animals were checked on equal behavior and the reliability of

the apparatus and other technical arrangements were secured, the four real experiments followed at short intervals between each other. Each experiment consisted of a preliminary feeding period of 10 or 11 days and a 10-day analysis period.

As basic feed, a clover grass hay drawn from a large apparatus was given, half in a coarsely torn state, half as a fine chaff. The latter allowed a good mixing of the supplements of pectin, etc. to the basic feed. This was especially necessary with the pure pectin preparation since the latter began to swell right at consumption. The intake of the highest amount (200 g per animal and day) without loss would hardly have been possible otherwise.

The daily collected feces were immediately conserved by drying at 70-80°. The urine was caught in a graduated flask filled somewhat with toluene and filled daily to a certain volume. From this, aliquot parts were taken, united with a collection assay and conserved with mercuric chloride.

Basic Feed Experiment

Feed Ration: 1000 g clover grass hay; analyzed values: 91.6% dried substance in which 10.2% raw albumen, 38.0% raw fiber, 2.3% fat, 8.1% ash and 41.4% N-free extracts; gross energy 4.252 kcal/g; uronic acid content 11.1%. The experiment went smoothly in very warm weather. The animals showed no substantial weight changes.

Uronic Acid and Energy Balances per Animal and Day

	Sheep #1	Sheep #2	Sheep #3
Uronic acid in feed	101.7	101.7	101.7 g
Uronic acid in feces	34.4	33.4	32.9 g
Uronic acid in urine	0.26	0.27	0.26 g
Digested	67.3	68.3	68.8 g
Digestibility	66.2%	67.1%	67.6%
Energy in feed	3904	3904	3904 kcal
Energy in feces	1901	1839	1965 kcal
Energy in urine	146	140	146 kcal
Digestible energy	2003	2065	1939 kcal
Digestible energy in %	51.3%	52.9%	49.7%
Converted energy	1857	1925	1793 kcal
Converted energy in %	47.6%	49.3%	44.6%

2. Experiment

Daily Feed Ration: 100 g hay + 100 g dried pectin of the following composition: 85.4% dried substance; in this 3.3% raw albumen, 1.0% raw fiber, 0% fat, 17.8% ash and 78.4% N-free extracts; gross energy 2.930 kcal/g; uronic acid content 62.9%. The experiment went smoothly.

Uronic Acid and Energy Balances per Animal and Day

	Sheep #1	Sheep #2	Sheep #3	
Uronic acid in feed	155.4	155.4	155.4	g
Uronic acid in feces	38.3	37.6	37.5	g
Uronic acid in urine	0.27	0.29	0.28	g
Digested	117.1	117.8	117.9	g
From basic feed	67.3	68.3	68.8	g
From supplement				
Pectin	49.8	49.5	49.1	g
In % of addition	92.7%	92.2%	91.5	%
Energy in feed	4154	4154	4154	kcal
Energy in feces	2027	1922	2028	kcal
Energy in urine	123	122	125	kcal
Digestible energy	2127	2232	2126	kcal
From basic feed	2003	2065	1939	kcal
From addition	124	167	187	kcal
In % of addition	49%	67%	75	%

3. Experiment

Daily Feed Ration: 1000 g hay + 200 g dried pectin. The experiment went exceptionally smoothly. Sheep #1 had, however, a weight loss of 2.2 kg, so that one is to assume that disturbances of digestion and metabolism occurred. The other animals showed no substantial weight changes.

Uronic Acid and Energy Balances

	Sheep #1	Sheep #2	Sheep #3	
Uronic acid in feed	209.1	209.1	209.1	g
Uronic acid in feces	34.0	38.6	37.8	g

Uronic Acid and Energy Balances (continued from page 5)

	Sheep #1	Sheep #2	Sheep #3
Uronic acid in urine	0.38	0.35	0.36 g
Digested	175.1	170.5	171.3 g
From basic feed	67.3	68.3	68.8 g
From the addition	107.8	102.2	102.7 g
Digestibility of the addition	(104)	95.1%	95.6 %
Energy in feed	4404	4404	4404 kcal
Energy in feces	1970	1915	2028 kcal
Energy in urine	128	119	90 kcal
Digestible energy	2434	2489	2376 kcal
From basic feed	2003	2065	1939 kcal
From addition	431	424	437 kcal
In % of the energy of the addition	86%	85%	87 %

4. Experiment

Daily Feed Ration: 1000 g hay + 400 g dried chips of the following composition: 91.0% dried substance; in this 9.1% raw albumen, 17.9% raw fiber, 1.1% fat, 6.4% ash and 65.5% N-free extracts; gross energy 4.037 kcal/g; uronic acid content 21.6%. In this experiment, difficulties arose toward the end in the feed uptake of the somewhat too amply measured rations. The additions of dried chips (400 g with 364 g dried substance, 78.6 g uronic acid and 1629 kcal gross energy) were completely consumed in each case. With the hay (basic feed), however, there was a certain amount which was returned. The experimental results are therefore to be taken with reservations. All three experimental animals gained weight. Sheep #1 = 1.4 kg; Sheep #2 = 2.7 kg and Sheep #3 = 1.7 kg.

Uronic Acid Balances

	Sheep #1	Sheep #2	Sheep #3
Uronic acid in feed	165.5	173.6	178.3 g
Uronic acid in feces	41.0	45.0	43.1 g
Digested	124.5	128.6	135.2 g
From basic feed	57.5	61.3	67.4 g
From supplement	67.0	67.3	67.8 g
In % of supplement	85.2%	85.6%	86.3 %

Discussion of the Findings

The roughly 10% pectin part present in a coarse feed medium, namely clover grass hay of average quality, was digested to about 66% in animal experiments. In subsequent experiments with additions of 10% of a dried pectin preparation, an apparent digestibility of 92% was obtained; and, with the introduction of additions of 20% pectin to the original basic feed ration, another increase of the pectin digestion to about 96% was determined. Here a gradual action of ruminant flora on pectin probably entered and a consequently stronger formation of pectin-decomposing fermentation. The pectin part of dried chips, with over 20% relatively pectin-enriched material, was (likewise in animal experiments) up to 85% digested, but to a somewhat lesser extent than in pure pectin.

These findings nevertheless still do not give definitive information whether and to what extent the pectin decomposed in the gastrointestinal canal has been transformed into reabsorbable products and products useable in metabolism. The primary product of pectin in question, galacturonic acid, appears to be hardly reabsorbed as such in ruminants, but rather transformed further. In any case, with the highest pectin addition, no significant increase of uronic acid, which is present only in traces, was evident in urine. This is probably due to the separation of glucuronic acid and is independent of the nutrition.

Test of the Solubility of Pectin "in vitro" by Means of Fresh Ruminant Liquid

In these studies, the artificial ruminant technique[1], which was used several times earlier, was applied to freashly drawn ruminant fluid, obtained once after a pure hay feeding of the experimental animals and the other times after the conclusion of the experiment with the highest supplement of pure pectin. Occasionally, exactly 3 g of air-dried excrement were exposed to the effect of ruminant fluid in a fermenting vessel and then the residue analyzed for pectin. The results are given in the following table:

Table 3

Solubility of Pectin of Different Materials in Ruminant Fluid after Different Feedings

Material	Uronic Acid Content	Solubility I.	Solubility II.
Dried Pectin	54%	99.6%	97.4%
Dried chips	23	91.8	83.8
Clover grass hay	10	69.6	55.0
Dried grass silage	12	87.9	77.9
Dried feed beets	11	86.5	80.7
Dried young pasture grass	7	86.0	—
Dried old pasture grass	7	81.2	—
Wheat straw	6.6	65.7	—
Mixture 90% hay + 10% dried pectin	14	83.6	70.4

[1]Details described in the dissertation of D. Heilmann, Kiel 1959

It was characteristic of both types of ruminant fluids used that the juice (I) present after pure hay feeding had a significantly greater ability to dissolve cellulose than that (II) obtained after a longer feeding with higher doses of pure pectin. Each sample was added at least twice, at most however three and four times and analyzed.

Two findings from these determinations are of special interest. The solubility of pectin is very strongly dependent on the form of the material, i.e. its occurrence in plant tissue. With the juice-feed medium, to which basically the dried chips are also to be counted, proto-pectin of the primary cell wall and of the cell juice prevails since because of its high degree of esterfication and its slight binding, it can easily be dissolved. The pectin found mainly in the inbetween cell wall of the coarse feed material is, to the contrary, much less soluble, which is mainly due to the strong binding to other tissue substances and to the strong intermolecular binding of the pectin molecule. With the isolated pectin of the technically pure preparation, this plays no role; it is in each case easily and practically completely soluble. A longer feeding with easily dissolved pectin changes the ruminant flora, whose disintegration capability diminishes appreciably.

Summary and Conclusions

Further experiments with pectin-containing feed rations were carried out on chicks and rats, and new experiments with sheep. Among other things (using newer, more reliable methods) material balances for galacturonic acid are reported.

The experiments with chicks showed that the inclusion of 10% dried pectin in the total feed ration is tolerated. The pectin addition is, however, incompletely decomposed; about half is excreted in the feces. The energy balance showed even a general digestive depression; there was a corresponding retardation of the weight development of the experimental animals as compared to the normal group. Hence, it is conclusively established that pectin materials, as well as the other plant-like tissue substances, can not be used by chicks.

In the experiments with rats, it was first of all established that pure, free galacturonic acid is poorly tolerated, although because of its easy solubility, it is practically completely digested. Definite, although small, amounts of uronic acid were found in the urine of the animals, which did not occur during the feeding of dried pectin which was likewise highly digested.

With the sheep, the utilization of pectin substances in clover grass hay, in a commercially common pectin concentrate and in dried chips was investigated. The roughly 10% pectin-content of clover

grass hay was digested to 66%. For an addition of 10% dried pectin, the apparent digestibility was 92% and, with a later addition of 20% pectin, still somewhat higher at 96%. The more than 20% pectin content of dried chips was digested to 85%. In no case in these experiments was the elimination of galacturonic acid in urine established.

Parallel to these experiments, determinations of solubility of pectin substances of different materials were carried out "in vitro" with the ruminant fluid of the experimental animals. A very clear graduation of pectin solubility was shown according to the form of the existing material and according to the activity of the ruminant juice. Pectin preparations and juice-feed medium contain the pectin in easily soluble form, while the pectin of coarse feeds is much less soluble due to its strong binding in the cell walls. Longer feedings with easily soluble pectin probably leads to a significant change of the ruminant flora. The capability for decomposition of cellulose decreases, which also leads to a depression in ability to dissolve pectin of the coarse feed.

From all experiments in this series, essentially the following can be deduced. Pectin substances are relatively easily soluble in digestive juices, and the dissolved pectin is evidently extensively decomposed. This is mainly to be traced to bacterial activity. Pectin is, therefore, apparently easily digested. Whether along with that there is a reduction to products capable of being reabsorbed and whether the decomposition products of pectin are used to an extent worth mentioning in metabolism in higher animals is at the least uncertain with animal types with a simple stomach system, indeed quite directly in doubt. The situation is different with regard to ruminants. Here, the pectin substances as well as the remaining carbohydrates are decomposed by the bacteria flora of the ruminant, which also makes possible indirect utilization similarly to that of substances otherwise foreign to the body, such as cellulose and pentosane. For a judgement of the true nutritional value of pectin substances the qualitative findings obtained so far and the only determination of the part of the feed that is not again excreted do not suffice. The decisions can bring here only direct nutritional value determinations according to classical methods.

Before such experiments are undertaken, we intend to determine more exactly the fate of pectin substances in ruminants even with the help of detailed microbiological studies, which in the meantime have begun.

Overall, one can now assume with some degree of certainty that the pectin content of organic substances of feeds takes a form in an intermediate place between the true tissue substances and the regular nutritional carbohydrates such as starch and sugar. However, in spite of the relative ease of solubility, the pectin contents are closer to the former; also cellulose, hemicellulose and pentosanes. A utilization in metabolism is therefore to be assumed at the earliest in the nutrition of ruminants.

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Untersuchungen über Pektinstoffe als Bestandteile von Futtermitteln

3. Mitteilung

Weitere Versuche mit pektinhaltigen Futterrationen bei Hühnern, Ratten und Hämmeln

Die ersten Fütterungsversuche, über die in der 2. Mitteilung dieses Archivs berichtet wurde, hatten den Zweck verfolgt, Verträglichkeit, Verdaulichkeit der Bruttoenergie und Futterwirkung der Pektinstoffe wenigstens annähernd festzulegen. Die zwischenzeitliche Ausarbeitung zuverlässiger Bestimmungsmethoden für Pektin bzw. seinen Hauptbestandteil, die Galakturonsäure, machten es möglich, ähnliche Versuche durchzuführen, bei denen auch die Stoffbilanz für Pektin in der Verdauung erfaßt werden konnte. Diese Versuche, über die in der vorliegenden Mitteilung berichtet werden soll, wurden noch einmal an Hühnern, sowie an Ratten und schließlich an kleinen Wiederkäuern (Hammeln) durchgeführt.

In der ersten Versuchsserie wurde die Ausnutzung von reinem Pektin bei Hühnchen geprüft. 8 New-Hampshire-Hähnchen von gleicher Größe und gleichmäßiger Entwicklung (mittleres Anfangsgewicht 850 g) wurden auf Grund von Vorversuchen ausgewählt und auf zwei Gruppen verteilt. Die Tiere der Normalgruppe I erhielten ein gekörntes Fertigfutter („Ibeka"-Junghennenfutter) in folgender Zusammensetzung: 15% Weizenkleie, 15% Hafer, 15% Gerste, 10% Eiweißkonzentrat, 10% Mais, 10% Tapiokamehl, 10% Futterweizen, 5% Weizennachmehl, 5% Maisstärkerückstände, 2% Vitamin A-, D_3-, B_2-Vermischung, 2% Futterknochenschrot, 1% Mineralstoffmischung. Im Futter der Versuchsgruppe II wurden 10% dieses Normalfutters durch Pektin und zwar handelsübliches reines aus Zuckerrüben gewonnenes Trockenpektin (83,6% Tr. S.) ersetzt. Um den vollständigen Verzehr des Pektins zu sichern, wurde es vor dem Körnen mit dem Grundfutter vermischt.

Die Analyse des Grundfutters ergab folgende Werte: 90,7% Trockensubstanz und in der Trockensubstanz: 17,8% Roheiweiß, 6,6% Rohfaser; 4,3% Rohfett; 6,5% Rohasche; Bruttoenergie 4 405 kcal/g. In diesem Grundfutter war ein natürlicher Gehalt an Galakturonsäure von 4,35%. Der Versuch selbst bestand aus einer sechstägigen Vorfütterungs- und einer achttägigen Analysenperiode. Er verlief glatt, jedoch zeigte sich bei der besonders an Pektinzulage gefütterten Tiere anscheinlich zu hohen Mengen unveränderten Pektins. Im einzelnen sind die Ergebnisse aus der nachfolgenden Übersicht zu ersehen. Alle Werte sind jeweils auf ein Durchschnittstier und einen Tag berechnet.

I. Normalgruppe
Täglicher Futterverzehr: 75,0 g Grundfutter, darin 68,0 g Trockenmasse, 2,95 g Uronsäure (natürlicher Pektingehalt), 299,5 kcal Bruttoenergie.

II. Versuchsgruppe
Täglicher Futterverzehr: 67,5 g Grundfutter + 7,5 g Pektin. Im täglichen Futterwaren enthalten: 61,20 g Trockenmasse aus Grundfutter; 6,27 g Trockenmasse aus Pektin; 2,66 g Uronsäure aus Grundfutter; 3,94 g Uronsäure aus Pektinzusatz; 269,5 kcal Bruttoenergie aus Grundfutter; 18,4 kcal Bruttoenergie aus Pektinzusatz.

Tabelle 1

Stoffwechselbilanzen

	I. Normalgruppe	II. Versuchsgruppe
tägliche Zunahme	20,5 g	8,2 g
Energie im Futter	299,5 kcal	287,9 kcal
„ in Ausscheidungen	109,3 „	117,1 „
umsetzbare Energie	190,2 „	170,8 „
davon aus Grundfutter		171,2 „
„ aus Pektinzulage		− 0,4 „
Uronsäure im Futter	2,95 g	6,60 g
„ in Ausscheidungen	1,69 g	3,46 g
„ umgesetzt	1,26 g	3,14 g
davon aus Grundfutter		1,14 g
„ aus Pektinzulage		2,00 g
in % der „		51%

Die hier festgestellten Resultate bestätigen die früheren Versuche über die unvollkommene Ausnutzung von Pektinzulagen im Futter bei Hühnern. Ein Anteil von 10% Pektin in der Gesamtfutterration führte sogar zu einer gewissen Depression in der Futterwirkung. Endgültig steht fest, daß die Verwertung der Pektine beim Huhn fraglich ist und ein wirklicher Nährwert selbst aus den im Verdauungskanal aufgeschlossenen Anteilen nicht zu erwarten ist. Damit entspricht das Pektin in der Ernährung des Huhnes den anderen pflanzlichen Gerüstsubstanzen wie Cellulose, Lignin und Pentosane.

An Ratten wurden nur wenige Versuche zur Ergänzung der früheren durchgeführt und zwar sollte zunächst Aufnahme und Verträglichkeit reiner freier Galakturonsäure geprüft werden. Versuchstiere waren männliche Wistar-Ratten von je etwa 200 g Gewicht. Die Zulage von täglich je 2 g Galakturonsäuremonohydrat zu 10 g Normalfutter wurde jedoch so schlecht vertragen, daß der Versuch nach wenigen Tagen abgebrochen werden mußte. Die Tiere bekamen starke Durchfälle und verweigerten die weitere Futteraufnahme.

Bei den nächsten Versuchsreihen wurden deshalb geringere Zulagen an Galakturonsäure angewandt. Versuchstiere waren wieder männliche ausgewachsene Ratten in Gruppen von je 4 Tieren. Die Normalgruppe erhielt täglich genau zugewogen 10 g Grundfutter, bestehend aus 57% Weizenmehl, 12% Fischmehl, 3% Waldhof-Trockenhefe, mit 3000 I. E. Vitamin A und 400 I. E.

Vitamin D_3 je kg vitaminiert. Die Tiere der Gruppe II erhielten dazu je 1,5 g Trockenpektin täglich und die der Gruppe III je 1 g Galakturonsäure. Die ausgeschiedenen Kot- und Harnmengen wurden in besonderen Glaskölbchen getrennt aufgefangen und täglich gesammelt. Der Kot wurde schonend bei 80° getrocknet, der Harn mit Wasser in als Sammelgefäße dienende 250-ml-Meßkolben gespült und durch Toluol konserviert. Am Schluß des Versuchs wurden die Meßkolben mit Wasser aufgefüllt, der Inhalt gut durchgemischt und davon aliquote Teile zu den Analysen entnommen.

Nach einer viertägigen Vorfütterungsperiode folgte die sieben Tage dauernde Analysenperiode. Bei den Tieren der Versuchsgruppe III verursachte die Zulage von Galakturonsäure in den ersten beiden Tagen Durchfälle, danach normalisierte sich dies wieder und in der eigentlichen Versuchszeit war die Konsistenz des Kotes normal. Während des Versuchs traten keinerlei Störungen auf, auch verzehrten die Tiere die ihnen zugewogenen Futtermengen vollständig.

In der nachstehenden Tabelle 2 sind die stofflichen Bilanzen der Galakturonsäure bei den Verdauungsversuchen der drei Gruppen aufgeführt. Es hatte sich gezeigt, daß die Bestimmungsmethode nach McCready auf solche Versuche nicht anwendbar ist. Während die Decarboxylierung bei reiner Galakturonsäure und reinem Pektin richtige Werte ergab, lieferte sie bei pektinarmen Material, d. h. beim Normalfutter und vor allem beim Kot völlig falsche, viel zu hohe Werte. Die Gründe, nämlich das Auftreten scheinbarer Pektinwerte durch Kohlendioxydbildung aus anderen organischen Substanzen sind schon in der ersten Mitteilung dargelegt worden. Die spezifische Galakturonsäureanalyse nach der neuen Methode von Harnisch erbrachte dagegen verläßliche Resultate. Der Galakturonsäureanteil der Zulagen — auch beim Trockenpektin — erwies sich als sehr hoch verdaulich und umsetzbar. Galakturonsäure selbst wurde allerdings schlechter vertragen, was sicher darauf beruht, daß die freie Säure den Darm stark reizt, während die aus

Tabelle 2

Galakturonsäurebilanzen in der Versuchsperiode (7 Tage)

Zulage (täglich)	Normalgruppe keine	1. Versuchsgruppe 1,5 g Pektin	2. Versuchsgruppe 1 g Galakturonsäure
Uronsäure im Futter	0,462 g	6,132 g	7,462 g
„ in der Zulage	–	5,670 g	7,000 g
„ im Kot	0,055 g	0,501 g	0,084 g
„ im Harn	0,029 g	0,035 g	0,107 g
„ verdaut	0,407 g	5,631 g	7,378 g
davon aus Grundfutter		0,407 g	0,407 g
„ „ Zulage		5,224 g	6,971 g
in % der „		92,1%	99,6%
Uronsäure umgesetzt	0,378 g	5,596 g	7,271 g
davon aus Grundfutter		0,378 g	0,378 g
„ „ Zulage		5,218 g	6,893 g
in % der Zulage		92,0%	98,5%

hochmolekularem Pektin allmählich frei werdenden Galakturonsäuremengen keinen derartigen Effekt haben. Nach der Zufuhr freier Galakturonsäure wurde auch die Ausscheidung gewisser Mengen im Harn der Tiere festgestellt, was zweifellos auf die schnellere Resorption zurückzuführen ist, die hier gegenüber dem Pektin vorlag. Letzteres bewirkte kein nennenswertes Ansteigen der Galakturonsäure im Harn.

Versuche an Hammeln

Das Hauptinteresse hinsichtlich einer Verwertung der Pektinstoffe im Futter erstreckt sich auf die Ernährung von Wiederkäuern. Im Folgenden werden kurz einige an Hammeln durchgeführte Versuche beschrieben, in denen die Ausnutzung der Pektinstoffe im Grundfutter (Heu) sowie in einem handelsüblichen Pektinkonzentrat und in Trockenschnitzeln, als einem relativ pektinreichen Futtermittel, untersucht wurde. Die verwendeten Hammel Nr. 1, 2 und 3 gehörten der Merino-Fleischschafrasse an. Sie waren etwa 1½ Jahre alt und an die Durchführung derartiger Versuche gewöhnt. Es wurden vier Einzelversuche unternommen, einer als Grundfutterversuch, die übrigen als Differenzversuche mit Zulagen von Trockenpektin bzw. Trockenschnitzeln. Neben der für die Feststellung der verdaulichen Energie notwendigen quantitativen Erfassung von Futter und Kot wurde auch der Harn sorgfältig gesammelt. Nach einem Vorversuch, in dem die Tiere auf gleichmäßiges Verhalten geprüft und die Zuverlässigkeit der Apparaturen und sonstigen technischen Einrichtungen sichergestellt wurde, erfolgten die vier eigentlichen Versuche in kurzen Abständen aufeinander. Jeder Versuch bestand aus einer Vorfütterungsperiode von 10 oder 11 Tagen Dauer und einer zehntägigen Analysenperiode.

Als Grundfutter wurde ein aus einem größeren Vorrat entnommenes Kleegrasheu zur Hälfte in grob gerissenem Zustand, zur anderen Hälfte als feines Häcksel gegeben. Letztere Maßnahme erfolgte, um die Zulagen an Pektin usw. gut mit dem Grundfutter vermischen zu können. Dies war insbesondere bei dem reinen Pektinpräparat notwendig, da letzteres schon beim Verzehr zu quellen begann. Die Aufnahme der höchsten Mengen (200 g je Tier und Tag) wäre sonst kaum ohne Verluste möglich gewesen.

Der täglich gesammelte Kot wurde unverzüglich durch Trocknung bei 70–80° konserviert. Der Harn wurde in einem mit etwas Toluol versehenen Meßkolben aufgefangen und täglich auf ein bestimmtes Volumen aufgefüllt, worauf aliquote Teile entnommen, zur Sammelprobe vereinigt und mit Sublimat konserviert wurden.

Grundfutterversuch

Futterration: 1000 g Kleegrasheu; Analysenwerte: 91,6% Trockensubstanz in dieser 10,2% Roheiweiß; 38,0% Rohfaser; 2,3% Fett; 8,1% Asche und 41,4% N-freie Extraktstoffe; Bruttoenergie 4,252 kcal/g; Uronsäuregehalt 11,1%. Der Versuch verlief bei sehr warmer Witterung glatt. Die Tiere zeigten keine wesentlichen Gewichtsveränderungen.

Uronsäure- und Energiebilanzen je Tier und Tag

	Hammel Nr. 1	Hammel Nr. 2	Hammel Nr. 3
Uronsäure im Futter	101,7	101,7	101,7 g
„ im Kot	34,4	33,4	32,9 g
„ im Harn	0,26	0,27	0,26 g
verdaut	67,3	68,3	68,8 g
Verdaulichkeit	66,2 %	67,1 %	67,6 %
Energie im Futter	3904	3904	3904 kcal
„ im Kot	1901	1839	1965 kcal
„ im Harn	146	140	146 kcal
verdauliche Energie	2003	2065	1939 kcal
„ „ in %	51,3 %	52,9 %	49,7 %
umsetzbare Energie	1857	1925	1793 kcal
„ „ in %	47,6 %	49,3 %	44,6 %

2. Versuch

Tägliche Futterration: 1000 g Heu + 100 g Trockenpektin folgender Zusammensetzung. 85,4% Trockensubstanz; in dieser 3,3% Roheiweiß; 1,0% Rohfaser; 0% Fett; 17,8% Asche und 78,9% N-freie Extraktstoffe; Bruttoenergie 2,930 kcal/g; Uronsäuregehalt 62,9%. Der Versuch verlief glatt.

Uronsäure- und Energiebilanzen je Tier und Tag

	Hammel Nr. 1	Hammel Nr. 2	Hammel Nr. 3
Uronsäure im Futter	155,4	155,4	155,4 g
„ im Kot	38,3	37,6	37,5 g
„ im Harn	0,27	0,29	0,28 g
verdaut	117,1	117,8	117,9 g
davon im Grundfutter	67,3	68,3	68,8 g
„ im zugelegten Pektin	49,8	49,5	49,1 g
in % der Zulage	92,7%	92,2%	91,5 %
Energie im Futter	4154	4154	4154 kcal
„ im Kot	2027	1922	2028 kcal
„ im Harn	123	122	125 kcal
verdauliche Energie	2127	2232	2126 kcal
davon aus Grundfutter	2003	2065	1939 kcal
„ „ Zulage	124	167	187 kcal
in % der Zulage	49%	67%	75 %

3. Versuch

Tägliche Futterration: 1000 g Heu + 200 g Trockenpektin. Der Versuch verlief äußerlich glatt. Hammel Nr. 1 hatte jedoch eine Gewichtsabnahme von 2,2 kg, so daß anzunehmen ist, daß Störungen in Verdauung und Stoffwechsel aufgetreten sind. Die anderen Tiere zeigten keine wesentlichen Gewichtsveränderungen.

Uronsäure- und Energiebilanzen

	Hammel Nr. 1	Hammel Nr. 2	Hammel Nr. 3
Uronsäure im Futter	209,1	209,1	209,1 g
„ im Kot	34,0	38,6	37,8 g
„ im Harn	0,38	0,35	0,36 g
verdaut	175,1	170,5	171,3 g
davon im Grundfutter	67,3	68,3	68,8 g
„ in der Zulage	107,8	102,2	102,7 g
Verdaulichkeit der Zulage	(104)	95,1%	95,6 %
Energie im Futter	4404	4404	4404 kcal
„ im Kot	1970	1915	2028 kcal
„ im Harn	128	119	90 kcal
verdauliche Energie	2434	2489	2376 kcal
davon aus Grundfutter	2003	2065	1939 kcal
„ aus Zulage	431	424	437 kcal
in % der Energie der Zulage	86%	85%	87 %

4. Versuch

Tägliche Futterration: 1000 g Heu + 400 g Trockenschnitzel von folgender Zusammensetzung. 91,0% Trockensubstanz; in dieser 9,1% Roheiweiß; 17,9% Rohfaser; 1,1% Fett; 6,4% Asche und 65,5% N-freie Extraktstoffe; Bruttoenergie 4,037 kcal/g; Uronsäuregehalt 21,6%. Bei diesem Versuch ergaben sich gegen Ende Schwierigkeiten in der Futteraufnahme der etwas zu reichlich bemessenen Ration. Die Zulage an Trockenschnitzeln (400 g mit 364 g Trockensubstanz, 78,6 g Uronsäure und 1629 kcal Bruttoenergie) wurde in jedem Fall vollständig verzehrt. Beim Heu (Grundfutter) ergaben sich indessen gewisse Futterreste, die zurückgewogen wurden. Die Versuchsergebnisse sind dadurch mit Vorbehalt aufzunehmen. Alle drei Versuchstiere nahmen an Gewicht zu. Hammel Nr. 1 = 1,4 kg; Hammel Nr. 2 = 2,7 kg und Hammel Nr. 3 = 1,7 kg.

Uronsäurebilanzen

	Hammel Nr. 1	Hammel Nr. 2	Hammel Nr. 3
Uronsäure im Futter	165,5	173,6	178,3 g
„ im Kot	41,0	45,0	43,1 g
verdaut	124,5	128,6	135,2 g
davon aus Grundfutter	57,5	61,3	67,4 g
„ aus Zulage	67,0	67,3	67,8 g
in % der Zulage	85,2%	85,6%	86,3%

Diskussion der Befunde

Die in einem Rauhfutterteil, nämlich Kleegrasheu mittlerer Qualität, vorhandenen Pektinanteile von etwa 10% wurden im Tierversuch zu etwa 60% verdaut. In ausführlichen Versuchen mit Zulagen von 10% eines Pektinkonzentrates ergab sich für dieses eine scheinbare Verdaulichkeit von 92% und bei

der Fortführung mit Zulagen von 20% Pektin zur ursprünglichen Grundfutterration wurde noch einmal eine Erhöhung des Pektinaufschlusses auf etwa 96% festgestellt. Hier hat wahrscheinlich eine allmähliche Einstellung der Pansenflora auf Pektin und eine stärkere Bildung pektinspaltender Fermente eingesetzt. Der Pektinanteil von Trockenschnitzeln, einem mit über 20% relativ pektinreichen Material, wurde (ebenfalls im Tierversuch) zu 85% verdaut, also in etwas geringerem Umfang als in reinem Pektin.

Diese Ermittlungen geben allerdings noch keinen endgültigen Aufschluß darüber, ob und in welchem Ausmaß das im Magendarmkanal zersetzte Pektin resorbierbare und im Stoffwechsel verwertbare Spaltprodukte geliefert hat. Das in Frage kommende primäre Spaltprodukt der Pektine, nämlich Galakturonsäure, scheint als solches beim Wiederkäuer kaum resorbiert, sondern noch vorher weiter umgesetzt zu werden. Jedenfalls wurde selbst bei den höchsten Pektingaben keine signifikante Erhöhung des nur in Spuren vorhandenen Uronsäuregehaltes im Harn festgestellt, der wahrscheinlich von der Ausscheidung von Glucuronsäure herrührt und von der Ernährung unabhängig ist.

Prüfung der Pektinlöslichkeit „in vitro" mittels frischer Pansenflüssigkeit

Bei diesen Untersuchungen wurde die früher mehrfach benutzte Technik des künstlichen Pansens[1] unter Verwendung frisch entnommener Pansenflüssigkeit angewandt, die einmal nach reiner Heufütterung der Versuchstiere und in anderen Ansätzen nach Schluß der Versuche mit der höchsten Zulage an reinem Pektin gewonnen war. Jeweils wurden genau 3 g lufttrockenes Ausgangsmaterial der Einwirkung der Pansenflüssigkeit im Gärgefäß ausgesetzt und danach der Rückstand auf Pektin analysiert. Die Ergebnisse sind in der folgenden Tabelle aufgeführt. Charakteristisch für die benutzten beiden Arten der Pansenflüssigkeiten war es, daß der nach reiner Heufütterung vorhandene Saft (I) eine wesentlich stärkere Fähigkeit hatte, Cellulose aufzulösen, als der nach längerer Fütterung mit höheren Gaben reinen Pektins erhaltene (II). Jede Probe wurde in mindestens

Tabelle 3

Löslichkeit des Pektins verschiedener Materialien in Pansenflüssigkeit nach unterschiedlicher Fütterung

Material	Uronsäuregehalt	Löslichkeit I.	Löslichkeit II.
Trockenpektin	54%	99,6%	97,4%
Trockenschnitzel	23	91,8	83,8
Kleegrasheu	10	69,6	55,0
getrocknete Grassilage	12	87,9	77,9
„ Futterrüben	11	86,5	80,7
„ junges Weidegras	7	86,0	—
„ älteres Weidegras	7	81,2	—
Weizenstroh	6,6	65,7	—
Mischung 90% Heu + 10% Trockenpektin	14	83,6	70,4

[1] Näher beschrieben in der Dissertation von D. HEILMANN, Kiel 1959

zweifacher, meist aber drei- und vierfacher Wiederholung angesetzt und analysiert.

Von Interesse sind bei diesen Feststellungen vor allem zwei Befunde. Die Löslichkeit des Pektins ist sehr stark von der Art des Materials, d. h. seines Vorkommens im pflanzlichen Zellverband, abhängig. Bei den Saftfuttermitteln, zu denen grundsätzlich auch die getrockneten Schnitzel zu zählen sind, überwiegt das Protopektin der primären Zellwand und des Zellsaftes, das wegen seines hohen Veresterungsgrades und seiner nur lockeren Bindung relativ leicht in Lösung gebracht werden kann. Das hauptsächlich in der Zwischenzellwand befindliche Pektin der Rauhfutterstoffe ist dagegen viel weniger löslich, was vor allem auf der festen Bindung an andere Gerüstsubstanzen und der starken intermolekularen Vernetzung der Pektinmoleküle beruhen wird. Bei dem isolierten Pektin der technisch reinen Präparate spielt das alles keine Rolle mehr, sie sind auf jeden Fall leicht und praktisch vollständig löslich. Eine längere Fütterung mit leicht löslichem Pektin verändert die Pansenflora, deren Aufschlußfähigkeit für Cellulose erheblich nachläßt. Daraus ergibt sich die nur scheinbar paradoxe Erscheinung, daß nun auch der Aufschluß der Pektinstoffe im Rauhfutter nicht unbeträchtlich schlechter wird.

Zusammenfassung und Schlußfolgerungen

Es wurden weitere Fütterungsversuche mit pektinhaltigen Futterrationen bei Hühnern und Ratten und neue Versuche bei Hammeln durchgeführt, mit denen jetzt u. a. (bei Verwendung neuer zuverlässiger Methoden) Stoffbilanzen für Galakturonsäure ermittelt wurden.

Bei den Versuchen mit Hühnern ergab sich, daß eine Einbeziehung von 10% Trockenpektin in die Gesamtfutterration noch gut vertragen wurde. Die Pektinzulage wurde jedoch nur ganz unvollkommen aufgeschlossen; etwa die Hälfte des Pektins wurde im Kot wieder ausgeschieden. Die Energiebilanz ergab sogar eine allgemeine Verdauungsdepression; dem entsprach auch die stark zurückbleibende Gewichtsentwicklung der Versuchstiere gegenüber der Normalgruppe. Damit steht endgültig fest, daß Pektinstoffe ebenso wie die anderen pflanzlichen Gerüstsubstanzen vom Huhn nicht verwertet werden können.

Bei den Versuchen mit Ratten wurde zunächst festgestellt, daß reine freie Galakturonsäure schlecht vertragen wird, obwohl sie wegen ihrer leichten Löslichkeit praktisch vollständig verdaulich ist. Gewisse, wenn auch kleine Anteile der Uronsäure fanden sich danach im Harn der Tiere, was bei der Fütterung von Trockenpektin, das ebenfalls hoch verdaulich war, nicht eintrat.

Bei Hammeln wurde die Ausnutzung der Pektinstoffe in Kleegrasheu, in einem handelsüblichen Pektinkonzentrat und in Trockenschnitzeln untersucht. Der etwa 10% ausmachende Pektingehalt von Kleegrasheu wurde zu 66% verdaut. Für eine Zulage von 10% Trockenpektin lag die scheinbare Verdaulichkeit bei 92% und bei einer späteren Zulage von 20% Pektin noch etwas höher, 96%. Der über 20% ausmachende Pektingehalt von Trockenschnitzeln wurde zu

89% verdaut. Bei diesen Versuchen wurde in keinem Fall die Ausscheidung von Galakturonsäure im Harn festgestellt.

Parallel zu diesen Versuchen wurden Löslichkeitsbestimmungen „in vitro" für Pektinstoffe in verschiedenen Materialien mit der Pansenflüssigkeit der Versuchstiere durchgeführt. Dabei ergab sich eine sehr deutliche Abstufung der Pektinlöslichkeit je nach der Art des Ausgangsmaterials und nach der Aktivität des Pansensaftes. Pektinpräparate und Saftfuttermittel enthielten das Pektin in leichtlöslicher Form, während das Pektin von Rauhfuttermitteln infolge seiner festeren Bindung in den Zellwänden viel weniger löslich war. Länger anhaltende Fütterung mit leichtlöslichem Pektin führte wahrscheinlich zu einer erheblichen Veränderung der Pansenflora. Die Aufschlußfähigkeit für Cellulose verminderte sich, was auch zu einer Depression im Lösungsvermögen für das Pektin von Rauhfutterstoffen führte.

Aus allen Versuchen dieser Reihe läßt sich etwa folgendes ableiten. Pektinstoffe sind in den Verdauungssäften relativ leicht löslich und das gelöste Pektin wird offenbar weitgehend zersetzt, was hauptsächlich wohl auf bakterielle Tätigkeit zurückzuführen ist. Pektin ist infolgedessen auch scheinbar leicht verdaulich. Ob damit auch ein Abbau zu resorptionsfähigen Produkten eintritt und ob Spaltprodukte des Pektins im Stoffwechsel höherer Tiere in nennenswertem Umfange verwertet werden, ist zu mindesten bei den Tierarten mit einfachem Magensystem unsicher, tatsächlich wohl direkt zweifelhaft. Anders ist die Situation sicher bei Wiederkäuern. Hier werden die Pektinstoffe wie die übrigen Kohlenhydrate durch die Bakterienflora des Pansens aufgeschlossen und zersetzt, wodurch auch eine Verwertung, ähnlich der der sonstigen körperfremden Substanzen, wie Cellulose und Pentosane indirekt möglich wird. Zur Beurteilung des wirklichen Nährwertes der Pektinstoffe reichen aber die bisherigen qualitativen Befunde und die alleinige Feststellung der nicht wieder ausgeschiedenen Anteile des Futters nicht aus. Die Entscheidung können hier nur direkte Nährwertbestimmungen nach den klassischen Methoden bringen.

Bevor solche Versuche unternommen werden, beabsichtigen wir, das Schicksal der Pektinstoffe im Pansen genauer zu erfassen und zwar mit Hilfe eingehender mikrobiologischer Studien, die inzwischen in Gang gekommen sind.

Im ganzen kann man jetzt als einigermaßen sicher annehmen, daß die Pektinstoffe innerhalb der organischen Substanzen der Futtermittel eine Art intermediäre Stellung zwischen den eigentlichen Gerüstsubstanzen und den regulären Nahrungskohlenhydraten von der Art der Stärke und Zucker einnehmen, wobei sie aber trotz ihrer relativ leichten Löslichkeit den ersteren, also Cellulose, Hemicellulosen und Pentosanen näher stehen. Eine Verwertung im Stoffwechsel ist deshalb am ehesten bei der Ernährung der Wiederkäuer anzunehmen.

Резюме

Куры переносили скармливание 10% сухого пектина в общем кормовом рационе, но половину скармливаемого пектина они выделяли в экскрементах. Балансы энергии показали общую депрессию пищеварения.

Этому соответствовало также плохое состояние веса опытных птиц. — В опытах с крысами было установлено, что чистая галактуроновая кислота вследствие ее растворимости практически полностью переваривается, но плохо переносится животными. В моче животных были найдены небольшие количества уроновой кислоты, чего не наблюдалось при кормлении сухим пектином. — У баранов исследовалось использование пектиновых веществ в клеверно-травяном сене, в пектиновом концентрате и в сухом свекловичном жоме. Пектин клеверно-травяного сена (10%) оказался переваримым до 66%, пектин сухого свекловичного жома (20%) переваривался до 85%. При добавлении 20% пектина он переваривался соответственно обоим случаям до 92 и 96%. Далее ставились опыты в vitro с соком рубца после чистого кормления сеном и после продолжительного скармливания пектина. После чистого кормления сеном сок рубца для всех подвергнутых исследованию кормовых веществ, также и для сухого пектина обнаруживал большую способность растворять пектин, нежели сок после кормления животных пектином. Для оценки подлинной питательной ценности пектиновых веществ имеющиеся до сих пор результаты исследований являются недостаточными, а поэтому необходимо провести дальнейшие исследования.

Summary

Chicks were able to tolerate a 10% proportion of dried pectin contained in their total feed rations. Half of the pectin, however, was excreted in the faeces. The energy balance revealed a generalized digestive impression. This was in agreement with the retarded growth rate observed in the experimental animals. Experiments with rats have shown that due to its solubility galacturonic acid is almost completely digestible though poorly tolerated. Small amounts of galacturonic acid were found in the urine of these animals; an effects which was absent if the animals were fed dried pectin. Sheep were tested for their ability to utilize pectin substances contained in grass-clover hay, a pectin concentrate and in dried sugar beet chips.

The digestibility of pectin contained in grass-clover hay (pectin content: 10%) was 66%, that of pectin contained in dried sugar-beet chips (pectin content: 20%) 85%. 92% to 96% of the pectin added to the diet in an anount of 20% were digested. Additionally, in vitro experiments were carried out studying ruminant juice after purified hay feeding and prolonged feeding of pectin. Both with dried pectin and any other type of food the pectin-dis-olving capacity of ruminant juice obtained after feeding a purified hay diet was higher than that of ruminant juice obtained after prolonged feeding of pectin. However, the data hitherto obtained are still insufficient for evaluating the true nutritive value of pectin substances and further investigations will be carried out.

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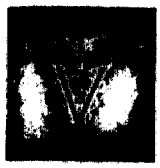

Cholelithiasis in Rabbits: Effects of Several Treatments on Formation and Dissolution of Gallstones

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SUMMARY

Rabbits were fed a gallstone-producing ration for 12 weeks, and formation of gallstones was confirmed by laparotomy. Treatments were administered concurrently with feeding the ration for an additional 12 weeks. Stone dissolution occurred when citrus pectin, cholestyramine, or neomycin was added to the ration. Other materials injected in the rabbits or added to the ration, *i.e.*, atropine, neostigmine, 1-phenyl-1-pentanol, a mixture of terpenes, ergosterol, paraffin oil, or low methoxyl citrus pectin, did not result in stone dissolution.

In the 2nd experiment, rabbits were fed a gallstone-producing ration for 12 weeks with materials added to the ration. Cholestyramine, neomycin, or citrus pectin prevented stone formation and lessened the hepatic fatty degeneration usually found when the gallstone-producing ration was fed. Addition of taurine to the ration did not result in stone dissolution.

Reports of disappearance of gallstones in man have been made for years, thus raising the possibility that the condition might be treated without using surgical techniques. Roentgenographic examinations confirmed the presence of stones, and on later examination they were absent.[1,17] In one instance,[21] more stones were detected by roentgenography than were found by surgical operation 5 days later; this indicated that passage of the stones occurred in the interim.

Gallstones were produced in rabbits by feeding a gallstone-producing ration, and their presence was confirmed by laparotomy. When the ration of these rabbits was changed to a commercial rabbit ration, stone dissolution occurred.[9] Treatment of experimentally induced cholelithiasis in rabbits with individual bile acids or with thyroidal materials was unsuccessful.[11]

The purpose in the present experiments was to determine how gallstone

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formation and dissolution and hepatic fatty degeneration were influenced by several types of treatments. The treatments were designed to alter indirectly the frequency of gallbladder contractions (neostigmine and atropine), bile flow (a mixture of terpenes and 1-phenyl-1-pentanol), the reabsorption of cholesterol and bile acids from the intestinal tract (paraffin oil, pectin, ergosterols, and cholestyramine), and the intestinal microflora (neomycin). Direct observations upon contractions, bile flow, reabsorption, and microflora were not feasible in the present experiments.

Materials and Methods

New Zealand White rabbits weighing 2,000 to 2,500 Gm. each were fed a commercial ration* before the experiment was started. Feed and water were available ad libitum, and the environmental air was maintained at 24 to 26 C. The gallstone-producing ration fed during the experiments contained 40% casein and 15% olive oil.[7,10]

At the end of the experiments, rabbits were killed by exsanguination. The liver of each rabbit was removed and weighed. A portion of the liver was preserved in buffered formaldehyde solution (pH 7.0) for histologic examination. Hepatic tissue sections were stained by hematoxylin-eosin technique. The remainder of the liver was sliced in 5-mm. sections, vacuum dried to a constant weight, and extracted with petroleum ether for 24 hours in Soxhlet's apparatus.

Solid contents of each gallbladder were washed with distilled water and vacuum-dried to a constant weight.[8]

Data were subjected to the analysis of variance technique.[9]

First Experiment.—The gallstone-producing ration was fed for 24 weeks. At the 12th week, extent of hepatic liver degeneration and the amount of gallstones were estimated grossly during laparotomy.[8] Treatments were initiated at this time (12 weeks) and given in addition to the gallstone-producing ration for the second 12-week period. Experimental groups of rabbits were formed, each group containing nearly equal numbers of both sexes.

Groups of rabbits were identified according to treatment, with the treatment agent being injected subcutaneously (amount of agent shown was injected each day per rabbit): group I—control, no treatment; group II—atropine sulfate in physiologic saline solution, 10 mg.; group III—neostigmine bromide in physiologic saline solution, 0.05 mg.; and group IV—1-phenyl-1-pentanol in olive oil, 150 mg. (Table 1). Other groups of rabbits were identified according to treatment, with the treatment agent being added to the ration (amount of agent shown was added per kilogram of ration): group V—mixture of terpenes* consisting of menthol (32%), menthone (6%), pinene (17%), borneol (5%), camphene (5%), cineol (2%), and olive oil (33%), 5 ml.; group VI—ergosterol, 2 Gm.; group VII—paraffin oil, N.F. (white, light, domestic; viscosity 125/135), 100 ml.; group VIII—citrus pectin, N.F.,** 100 Gm.; group IX—citrus pectin, low methoxyl,** 100 Gm.; group X—cholestyramine,† 20 Gm.; and group XI—neomycin sulfate, U.S.P.,‡ 5 Gm. (Table 1).

At the 24th week, rabbits were killed and the liver and gallbladder of each were examined.

Second Experiment.—The gallstone-producing ration was fed and treatment agents were added to the ration for 12 weeks (Table 2). Experimental groups of rabbits were formed with 5 male and 5 female rabbits in each group. Groups of rabbits were identified as follows (amount of treatment agent shown was added per kilogram of ration): group XII—control, no treatment; group XIII—taurine, 20 Gm.; group XIV—citrus pectin, N.F.,** 100 Gm.; group XV—cholestyramine,† 20 Gm., and group XVI—neomycin sulfate, U.S.P.,‡ 5 Gm.

At the 12th week, rabbits were killed and examined as described for the 1st trial.

Results

First Experiment.—General physical condition of the rabbits was good during the first 12-week period, *i.e.*, when the gallstone-producing ration alone was fed.

* Purina Rabbit Checkers without antibiotics, Ralston Purina Company, St. Louis, Mo.

* Rowachol, donated by Rowa Ltd., Bantry, County Cork, Ireland.

** Citrus pectin, N.F., and citrus pectin, low methoxyl, donated by Sunkist Growers, Ontario, Cal.

† Cholestyramine (Questran), donated by Mead Johnson Company, Evansville, Ind.

‡ Neomycin sulfate, donated by Charles Pfizer Company, New York.

TABLE 1—Observations on Gallbladders of Rabbits Fed a Gallstone-Producing Ration (24 weeks) and Given Different Treatments (13th Through 24th Week): First Experiment

Identification			Gallstone formation (No. of rabbits in each classification)**								Dry weight of gallstones at 24 weeks (av. each group, mg.)††
			At end of first 12 weeks of feeding (before treatment)				At end of 24 weeks of feeding and 12 weeks of treatment				
Group No.	No. of rabbits	Treatments*	None	Few	Moderate	Many	None	Few	Moderate	Many	
I	5	None (control)	2	1	1	1	1	0	2	2	114.1
II	4	Atropine sulfate	1	2	1	0	1	0	3	0	36.3
III	4	Neostigmine bromide	0	2	1	1	0	0	1	3	102.6
IV	4	1-Phenyl-1-pentanol	0	2	0	2	0	1	2	1	49.0
V	5	Terpenes	0	0	1	4	0	0	1	4	86.5
VI	5	Ergosterol	1	0	2	2	0	0	1	4	157.4
VII	5	Paraffin oil	1	0	1	3	0	0	0	5	128.7
VIII	5	Citrus pectin, N.F.	0	0	1	3	0	2	0	1	44.7
IX	4	Citrus pectin, low methoxyl	4	0	0	0	1	2	0	1	85.9
X	5	Cholestyramine	1	1	2	2	2	1	1	1	28.9
XI	5	Neomycin sulfate	0	3	0	1	3	1	0	1	88.5

* Compounds given to rabbits of groups II through IV were injected; compounds given to rabbits of groups V through XI were added to the ration. ** Amounts of gallstones were estimated grossly at 12 weeks by laparotomy and at 24 weeks by necropsy. † Statistical differences ($p > 0.05$) were not found for the dry weight of the gallstones.

Body weight gains averaged 36 Gm./rabbit/week during this period.

During the second 12-week period, when treatments were given in addition to the gallstone-producing ration, some rabbits were in fair physical condition and yet failed to gain weight (av. 10 Gm./rabbit/week or less) when given atropine (group II rabbits), neostigmine (group III rabbits), 1-phenyl-1-pentanol (group IV rabbits), ergosterol (group VI rabbits), paraffin oil (group VII rabbits), and neomycin (group XI rabbits). Rabbits in the remaining groups remained in good physical condition and continued to gain weight. It was not possible to correlate weight gains with feed consumption since wastage was high when rabbits were fed a powdered diet.

Extent of hepatic fatty degeneration, as estimated by laparotomy, was usually slight in all groups of rabbits at the 12th week. In the control group (group I rabbits), at the 24th week the extent of degeneration was moderate to severe. Most of the other treatments did not influence the progression of hepatic fatty degeneration during the second 12-week period, as compared with the hepatic changes seen in control rabbits. However, ergosterol (group VI rabbits) and citrus pectin, low methoxyl (group IX rabbits), apparently caused more severe degeneration than in the controls, and cholestyramine (group X rabbits) lessened the extent of degeneration.

Little difference in average weights of liver in rabbits at 24 weeks was found between the control rabbits (group I) and most of the other groups of rabbits. Relationship of liver wet weight to body weight was 2.56% in the control rabbits. Some weight increases occurred in the rabbits given the mixture of terpenes (3.25%; group V rabbits) or paraffin oil (3.75%; group VII rabbits).

In the control rabbits (group I), average liver percentage of dry weight was 32.5%, and average liver ether extract content on a wet weight basis was 7.5%. Percentages for these observations in most other groups of rabbits did not vary appreciably from the data for the control

rabbits, except that the liver ether-extract content in the rabbits given citrus pectin, low methoxyl, was increased (14.5% ether extract; group IX).

Amount of gallstones observed by laparotomy in the control rabbits (group I) increased in the period between 12th and 24th weeks, as did the amount of stones in most other groups of rabbits (Table 1). Apparently, the treatments designed to alter gallbladder contractions (atropine-decrease, group II,[25] and neostigmine-increase, group III,[25]) or increase bile flow (1-phenyl-1-pentanol,[20] group IV, and a mixture of terpenes, group V[27,28]) were without effect upon gallstone dissolution. Some of the treatments designed to alter reabsorption of cholesterol and bile acid from the intestinal tract were also without apparent effect (ergosterol, group VI; paraffin oil, group VII; and citrus pectin, low methoxyl, group IX), whereas other treatments in this category produced some gallstone dissolution (citrus pectin,[16] group VIII; and cholestyramine,[29] group X). The treatment designed to alter the intestinal microflora also caused some gallstone dissolution (neomycin,[24] group XI). Dry weights of gallstones of rabbits at the 24th week varied considerably between rabbits within groups.

Second Experiment.—Rabbits were in good physical condition and gained weight during the 12-week period when fed the gallstone-producing ration without other treatment (control, group XII) or were fed the ration with either added citrus pectin (group XIV) or cholestyramine (group XV). However, rabbits were in fair physical condition and they failed to gain weight or lost weight if they were fed the gallstone-producing ration with either added taurine (group XIII) or neomycin (group XVI).

Hepatic fatty degeneration was more severe in the control rabbits than in rabbits fed added cholestyramine (group XV) or added neomycin (group XVI, Table 2). There were slightly decreased liver weight and liver ether-extract content in groups XV and XVI. Condition of the liver could not be correlated with

TABLE 2—Observations on Livers and Gallbladders of Rabbits Fed a Gallstone-Producing Ration and Given Different Treatments (12 Weeks); Second Experiment

Identification*			Extent of fatty degeneration (No. of rabbits in each classification)**					Analysis of liver (av. each group, %)			Gallstone formation (No. of rabbits in each classification)					Dry weight of gallstones (av. each group, mg.)†
Group No.	Treatment	Sex	Normal	Slight	Moderate	Severe	Wet weight/body weight	Dry weight/wet weight	Ether-extract weight/wet weight	None	Few	Moderate	Many	
XII	None (control)	Male	0	1	3	1	2.43	31.7	4.0	3	2	0	0	8.5
		Female	0	0	4	1	2.61	31.9	6.6	0	2	0	3	61.2
XIII	Taurine	Male	0	0	5	0	2.24	30.2	5.2	3	0	0	2	85.4
		Female	1	1	3	0	2.36	30.4	4.8	4	0	1	1	34.2
XIV	Citrus pectin, N.F.	Male	1	2	2	0	2.75	32.0	5.9	5	0	0	0	1.9
		Female	0	2	2	1	2.43	31.9	6.9	4	1	0	0	7.2
XV	Cholestyramine	Male	3	2	0	0	2.27	29.2	3.3	5	0	0	0	1.1
		Female	2	3	0	0	2.08	29.6	2.6	6	0	0	0	0.8
XVI	Neomycin sulfate	Male	5	0	0	0	2.12	29.9	3.2	5	0	0	0	1.9
		Female	3	2	0	0	2.23	30.8	5.0	5	0	0	0	2.4
L.S.D. ($p < 0.05$)‡			...	...	...	...	0.46	NS	1.7	...	...	...	...	...

* Compounds given to rabbits were added to the rations; there were 5 male and 5 female rabbits in each group. ** Hepatic fatty degeneration was observed grossly and microscopically. † Small amount of solid material in groups XIV, XV, and XVI was usually debris rather than gallstones. ‡ L.S.D. = least significant difference; in the solutions for L.S.D. levels, ( ) indicates levels were not determined or were not applicable, and (NS) indicates no significant differences.

the general physical condition of the rabbits. For example, rabbits fed added neomycin had less hepatic fatty degeneration than control rabbits, but were in poorer physical condition.

Addition to the gallstone-producing ration of materials designed to alter intestinal reabsorption of cholesterol and bile acids markedly decreased or eliminated gallstone formation (citrus pectin,[16] group XIV, and cholestyramine,[29] group XV, Table 2). The treatment designed to alter the intestinal microflora also eliminated gallstone formation (neomycin,[24] group XVI). Addition of taurine (group XIII) to the ration at the 2% level did not result in gallstone dissolution. Taurine was employed in this experiment, since in a previous experiment[11] the addition of taurine to the gallstone-producing diet at the 5% level produced moderate stone dissolution, but the rabbits were in poor physical condition at the termination of the previous experiment.

Discussion

It was not possible to make observations on the effects of the treatments upon gallbladder contractions, bile flow, cholesterol and bile acid reabsorption in the intestinal tract, and intestinal microflora.

Treatments designed to affect gallbladder contractions and bile flow did not effect gallstone dissolution in the present experiment. Gallbladder stasis did not affect gallstone formation in *beta*-cholestanol-induced cholelithiasis in rabbits.[15] The terpene mixture* has been used with some benefit to treat cholelithiasis in man, since symptoms were lessened, and sometimes stones were passed.[12,13,19] The terpene mixture increased bile flow in man[27,28] and in rats.[28] When gallstones were induced in rabbits by *beta*-cholestanol, 1-phenyl-1-pentanol decreased stone formation, presumably by increasing bile flow.[20]

Citrus pectin and cholestyramine given orally prevented gallstone formation and caused gallstone dissolution in the present experiments, presumably by altering the intestinal reabsorption of cholesterol and bile acids. Pectin given orally increased fecal excretion of cholesterol and lowered serum cholesterol in men fed a typical American diet.[16] The oral administration of pectin reduced serum cholesterol in rats.[26] Hamsters fed a gallstone-producing ration had a lower serum concentration of cholesterol and hepatic fatty degeneration when cholestyramine was added to the ration. In these hamsters, gallstones disappeared and gallstone formation was prevented.[2,3,5]

The concentrations of bile acids in the bile of hamsters fed a gallstone-producing diet was changed when cholestyramine was added to the diet; the concentration of chenodeoxycholic acid was decreased and the ratio of bile acids to cholesterol was increased.[6] Cholestyramine given orally increased the conversion of cholesterol to bile acids in man.[29]

In contrast to the results of cholestyramine in the present experiment with rabbits, guinea pigs fed a pelleted ration containing 1% cholestyramine and 3% cellulose preparation formed gallstones.[22] Gallstones were induced in mice by adding cholesterol and cholic acid to the ration; the later addition of cholestyramine to the ration did not result in stone dissolution.[4]

The role of neomycin in preventing gallstone formation and effecting stone dissolution in the present experiment with rabbits is a matter of conjecture. The intestinal microflora metabolize sterols and bile acids and thus may alter bile composition. In *beta*-cholestanol-induced gallstones in rabbits, the stones were composed of allodeoxycholic acid; glycoallodeoxycholic acid was less soluble than glycodeoxycholic acid, and therefore stone formation was probably more likely.[14] Adding neomycin to the drinking water of rabbits fed *beta*-cholestanol in their diet prevented stone formation, and it was theorized that neomycin altered the intestinal microflora and thereby prevented the conversion of *beta*-cholestanol to allodeoxycholic acid.[18]

Alteration of the intestinal microflora

* Rowachol, donated by Rowa Ltd., Bantry, County Cork, Ireland.

in hamsters influenced experimental cholelithiasis in hamsters. Stones composed of cholesterol formed when hamsters were fed a fat-free diet containing considerable dextrose, but few stones were found when rice starch was substituted for dextrose. Feeding rice starch resulted in a predominance of lactic acid bacteria in the intestinal tract.[24] Predominance of lactic acid bacteria may not have been the factor responsible for the lessened gallstone formation, however, since adding various antibiotics and chemotherapeutic agents (oxytetracycline, sulfaguanidine, phthalylsulfathiazole, iodochlorohydroxyquin, neomycin, and nystatin, singly, or in combinations) suppressed the lactic acid bacteria in hamsters fed a fat-free diet containing rice starch, but few stones were formed.[24]

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THE INFLUENCE OF DIFFERENT DIETARY CARBOHYDRATES ON SERUM AND LIVER LIPIDS[a]

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There is little question that in a given population, those with high serum lipids have a high risk of incurring a heart attack. The lipids best correlated are the serum cholesterol and triglycerides, and since these are primarily transported as low density lipoproteins the level of these lipoproteins also correlates with the risk of coronary disease. There are other features as well which are associated with a high coronary risk. A man in his 50's has 4 times the risk of attack as a man in his 30's. An individual with a systolic blood pressure greater than 160 has 4 times the risk of an individual with a systolic pressure of 120. An individual with an electrocardiographic abnormality has 2.5 times the risk of one with a normal electrocardiogram. A cigarette smoker has nearly twice the risk of a non-smoker. Individuals with low vital capacity have approximately twice the risk of those with normal vital capacity. There is little question that the level of serum lipids is important, but it is only one of many statistical parameters associated with this disease. Is it likely that control of the serum lipids will reduce the incidence of coronary disease? Evidence is now slowly accumulating that this is in fact so. But what of all the other parameters? What of age, hypertension, diabetes mellitus, smoking, vital capacity, uric acid level, tension, weight, and abnormality of the electrocardiogram. Will these factors all disappear when our serum lipids are controlled? This, of course, is unknown, but fortunately many of these factors, as well as the serum lipids, can be controlled by modern therapeutics, and hopefully this will also contribute to a reduced incidence of heart disease.

Therapeutically, the management of high serum lipids is not easy. Any insight into the factors controlling the serum

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lipids is of obvious importance. Dr. Hodges has already alluded to the fact that there are many important variables which determine the serum lipid levels, not the least of which is dietary carbohydrates. Many including ourselves have demonstrated that the amount of carbohydrate plays a role in determining the level of certain of the low density lipoproteins which are present in the serum, and that this lipoprotein class is perhaps better correlated with risk of coronary arterial disease than the serum cholesterol or the class of cholesterol rich-low density lipoproteins which is primarily fat-influenced. It was earlier studies, which established that one class of low density lipoproteins may be more important than others that led several workers including ourselves to ask what factors controlled the level of this lipoprotein class. The amount of carbohydrate in the diet appears to be one such factor.

It then became of interest to know if specific carbohydrate varieties were more influential than others in affecting the serum lipoproteins and their lipids and several dietary studies in human volunteers and rats were performed. The following discussion will describe one study made in the rat, and the effects of feeding different carbohydrates on growth, water consumption, stool output, and the serum and tissue lipids.

Nine groups of rats were studied. The rats, nine per group, were 2 months of age at the start. For 18 months all groups received the same rat chow diet. Eight of the nine groups received a carbohydrate supplement mixed with their chow. The supplements were granulated sugar, sucrose (C.P.), fructose, cerelose (glucose), dextrin, galactose, lactose and pectin. The diets thus contained 18% protein, 4.5% fat, and 58.5% of various mixed carbohydrates, and20% of a single added purified carbohydrate. There were several questions to answer. One was, would all of these carbohydrates support growth, as measured by weight, comparably? They did not. The glucose-and fructose-containing carbohydrates sustained growth more efficiently than did the galactose-containing carbohydrates. This is reflected in the calculated protein efficiency ratios as well. As might be expected the pectin-consuming animals grew the least, reflecting the reduced calories consumed.

In observing these animals it was also noted that their water consumption varied inversely with their weight gain, so that those which gained the least drank the most and vice-versa. The water consumption was found to be linearly related to the dried stool residue and animals drank about 15 grams of water for every gram of dried stool output. The weight gain was also found to be inversely related to stool output, so that the glucose-fructose supplemented rats who gained the most had the smallest stool output, and the pectin group which gained the least, the greatest

stool output; the rats consuming galactose-containing carbohydrate types were in between.

Thus, the variety of carbohydrate in the diet can influence the growth rate, the stool output and the water consumption, in the rat.

The carbohydrates fed also helped determine the cholesterol level reached in the blood. The serum cholesterol level was measured in each group of rats after 9, 14 and 18 months of feeding. As the rats mature the serum cholesterol increases. It about triples during this period, despite the low fat content of the diet, which incidently contains 63% mono-, di-and trienes.

If the cerelose group is conpared with the others, at 18 months the cholesterol level in the cerelose group is found to be significantly lower than that found in the granulated sugar-, sucrose-, and lactose-fed animals. Thus, while the serum cholesterol level increases with age, the carbohydrate in the diet is apparently an important determinant of cholesterol level reached.

The same was true of the serum total fatty acids which derive from the serum triglycerides, phospholipids and esterified cholesterol. Between the 9th and 18th months of feeding there was an increase (a doubling) of the serum total fatty acid concentration with age and this age effect could be influenced by the carbohydrate variety in the diet. Again the cerelose supplemented group had serum levels of total fatty acids significantly lower than found in the granulated sugar-, dextrin- and lactose-supplemented groups. It might also be noted that the final concentration of cholesterol and total fatty acids reached was independent of the final weight.

We have seen before that the variety of carbohydrate in the diet can influence the growth rate, water consumption and stool output. It can similarly influence the levels of cholesterol and total fatty acids attained in the serum.

We then asked the question: Do these supplements influence the lipid composition of the internal organs? The composition of the liver was studied. The liver lipid composition is profoundly influenced by the carbohydrate in the diet. The ester cholesterol could be varied from 3% in the rats fed the pectin and cerelose supplements to 10% in the lactose-fed animals. The free cholesterol was as low as 6% in the granulated sugar group and highest in the cerelose and lactose groups, whereas the reverse was true as pertains to the triglycerides, which represented about 12% of the total lipid in the cerelose group and as high as 30% in the fructose-, sucrose- and lactose-fed animals. Finally the phospholipids were as low as 40% in the lactose group, and as high as 60-70% in the granulated sugar, pectin and galactose groups.

Thus the composition of the liver lipids can be profoundly influenced by the carbohydrate variety in the diet.

We then asked the question, could the carbohydrates fed influence the fatty acid composition of these complex lipids in the liver? This was found to be the case. For example, although there was no arachidonic acid in the diet, it may be seen that the type of carbohydrate supplement determined the amount of this fatty acid esterified with cholesterol; as little as 3% in the granulated sugar- and cerelose-fed animals. Many of the animals had no linolenate esterified, others as much as 1.5%. Likewise, type of carbohydrate influenced the linoleate and stearate proportions, but less so, the oleate fraction.

The same was true for only a few of the fatty acids esterified with phospholipid. For example, the C 18:0 fatty acid proportion in the sucrose- and galactose-fed groups were both significantly lower than in the pectin- and granulated sugar-fed rats.

Thus a single carbohydrate representing only 20% of the weight of the diet, chronically fed, can have a wide and diverse impact on the metabolism and probably composition of the whole animal.

All of what has been said must remain for now in the realm of interesting effects of feeding different carbohydrates. Carbohydrates which appear superficially the same, do not always have the same effect. The mechanisms by which many of the effects of different carbohydrates are produced are unknown although interesting speculations are possible.

I should like to leave this final word. Factors associated in a statistical way with any clinical problem, must not be construed as cause. Major changes in our diet to ameliorate the serum lipids may not have the beneficial effects in the long run that we might hope unless we are careful. There remains a need for restraint in our recommendations and a need for not letting our zeal to help interfere with our reason which continues to tell us that a man is not a bile acid cholesterol-consuming rat or a shrimp-eating rabbit. There is need for constantly reminding ourselves of the physician's first therapeutic commandment --"primum non nocere" -- first do no harm.

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The Manifold Effects of Different Dietary Carbohydrates[1]

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The bioconversion of dietary carbohydrate to fatty acids has been known for decades [1]. It has also become well known that the replacement of dietary fat with carbohydrate can influence the variety of fatty acids which accumulate although the mechanism by which this occurs is unclear [2]. Most such studies have been confined to the measurement of tissue fatty acids, although recently attention also has been given to the effect of such substitutions on the fatty acids in the serum lipids [3]. Generally these studies have been short term and have devoted little attention to the effects of feeding different carbohydrates. The primary purpose of the present study was to define the tissue fatty acid and serum lipid changes associated with the feeding of moderate amounts of eight different dietary carbohydrates to rats for most of their lives. In the course of these studies other unexpected and interesting observations were made, and will also be described.

Methods

Seventy-two 2-month-old male, Sprague-Dawley rats were divided into 8 equal groups of comparable average weight. Each group was fed a diet of ground rat chow mixed with 20% by weight of one of the carbohydrates listed in Table I. In addition, one group of 5 rats was fed only a diet of rat chow. These diets were provided for 18

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months. All animals were offered and consumed the same amount of food daily; most of their lives the animals consumed 20 g of diet per day per rat. The animals were housed in an air-conditioned room and their cage positions were changed weekly. They were weighed weekly.

Table I. Varieties of carbohydrate added to rat chow[1] (rat chow, 80%; carbohydrate, 20%)

Name	Source and description
Monosaccharides	
Cerelose	[illegible] hydrate. It is derived from corn sugar, but has not been crystallized. It is said to be 99.3% pure, containing 1 part [illegible] million of maltose, and of a trisaccharide. Its [illegible] content is 0.02%. It is said to be fat free. Corn Products Sales Co., 10 E. 56th St., New York 22, N.Y.
Fructose	d-[illegible]lose, C. P. Nutritional Biochemicals Co., [illegible] Mills Avenue, Cleveland, Ohio.
Galactose d(+)	Anhydrous. Nutritional Biochemicals Co.
Disaccharides	
Sucrose d(+)	Nutritional Biochemicals Co.
Granulated sugar	It is derived from sugar cane and contains no additives. It is reportedly 99.5% pure sucrose. Invert sugar present is 0.0[illegible] – 0.005%, the ash content is 0.007% – 0.002%, and the moisture content is 0.020% – 0.06%. Colonial Sugar Co.
Beta-lactose	Milk sugar; U.S.P.; Hydrate. Nutritional Biochemicals Co.
Oligosaccharide	
Dextrin	It is derived from corn starch, roasted in the presence of HCl and then allowed to repolymerize. The amount of maltose and trisaccharides is said to be small. If 2 g are added to 100 ml water, 65% is soluble and therefore of 'short' chain length. Corn Products Co.
Polysaccharide	
Pectin	Citrus, N.F., M.W. 150,000–300,000; moisture [illegible] 10%. Nutritional Biochemicals Co. Two hundred ml of water were added to the diet containing pectin which made it palatable.

[1] Rat chow, Ralston Purina Company. Analysis: protein 23.0%; fiber 6.0%; ash, 9.0%; carbohydrate, 57.1%; and fat, 4.9%. The diet contained 0.04% Lieberman-Burchard positive sterols; each rat consumed 16 mg of such sterols per day.

Adding 20 parts of carbohydrate to 80 parts of rat chow reduced the protein and fat in the diet, respectively, to 18.4 and 3.6%.

Animals were bled by tail vein, on three occasions, using light ether anesthesia, after 9, 14, and 18 months of feeding. Serum was collected in the usual way and analyzed for total cholesterol [4] and total fatty acids [5].

Two-thirds of the animals survived the duration of the study. They were sacrificed by exanguination. The livers, epididymal fat pads and testes were collected, trimmed and weighed. The livers were homogenized and extracted with $CHCl_3$:CH_3OH (1:1). The extract was washed twice with water, evaporated to dryness *in vacuo*, and stored at −20 C under nitrogen. The major lipid classes were separated by silicic acid chromatography [6]. The cholesterol, cholesterol esters, phospholipid and triglyceride fractions were weighed and the latter three were analyzed for their fatty acid composition, after hydrolysis and esterification [7], on silicic acid chromatography [8]. Each fraction resulting from silicic acid chromatography was monitored by thin layer chromatography [9]. Samples were rechromatographed when necessary. The epididymal fat pad was similarly treated but the column chromatographic step was omitted. Rat chow was also analyzed for its total fat, sterol and fatty acid composition (Table II).

TABLE II. The fatty acid composition of rat chow[1]

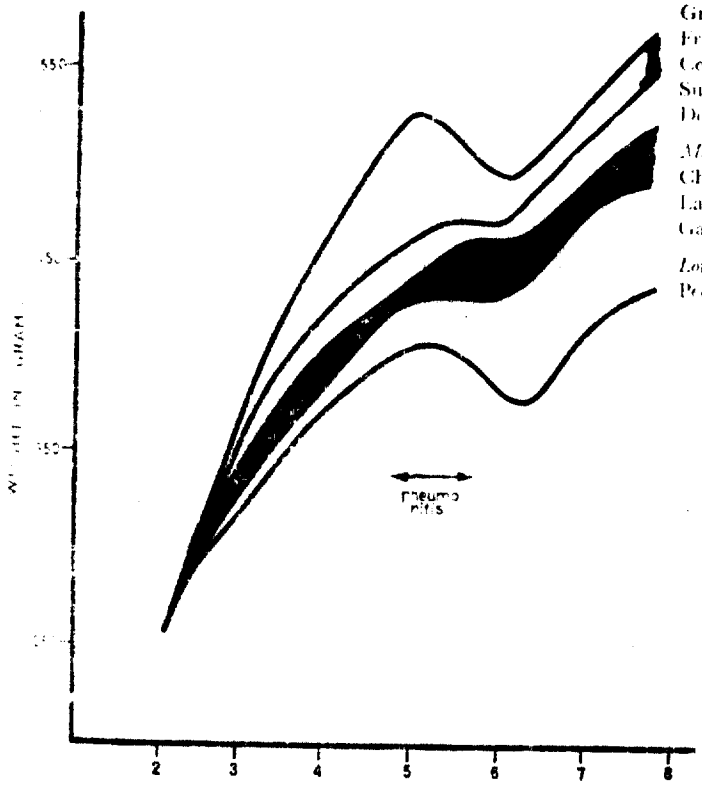
No. of C:No. of D.B.	Percent	No. of C:No. of D.B.	Percent
9:0	0.13	17:0	0.12
10:0	0.39	18	0.27
12:0	0.22	18:0	7.7
14:0	1.5	18:1	33.8
14:1	0.42	18:2	21.8
15:0	0.19	18:3	1.0
16:0	23.18	20:0	0.81
16:1	3.19	20:1	1.10

[1] The rat chow used contained 4.85% total lipids; of this esterified sterol was 1.5%, total sterol 1.77%, triglyceride 25.3%, and the remainder calculated as phospholipid. Each rat consuming 16 g of rat chow consumed 16 mg of Liebermann-Burchard positive sterols per day.

The water consumption and output of stool (dried residue) for each group of rats were also determined. Each was measured daily for four to six days during each of three different seasons. The mean

water consumption and stool output per day per 100 g of rat were then calculated.

The protein efficiency ratio was determined by calculating the ratio of the grams of body weight gain to grams of protein consumed in a six month period.



Weight gain	Mean weight at twenty months	PER[1]
High		
Granulated Sugar	673 g	.391
Fructose	646 g	.368
Cellobiose	637 g	.378
Sucrose	631 g	.355
Dextrin	607 g	.363
Moderate		
Chow	574 g	.199
Lactose	5[illegible]1 g	.319
Galactose	543 g	.280
Low		
Pectin	460 g	.190

Fig. 1. The effect of dietary carbohydrate variety on the weight gain of rats. The weight gain was divided into three groupings labelled high, moderate and low. Only the upper and lower curves for each weight gain group are shown for simplicity. The maximum weights obtained the week before sacrifice are also shown. [1]PER, refers to the protein efficiency ratio, which is the ratio of the body weight gain in grams to grams of protein consumed, during the six month period, between the second and eighth months of the study. The arrows demarcate a period during which the rat colony was affected by pneumonitis.

Results

Growth and protein efficiency ratio (PER). Despite comparable food intake, growth and PER differences were evident and are shown in Fig. 1. Three levels of weight gain, *high*, *moderate* and *low*, and protein efficiency ratios, could be distinguished. Those consuming glucose- or fructose-containing *mono-*, *di-* or *oligo*saccharides enjoyed the greatest weight gain, and had the highest PER. Rats consuming galactose and betalactose had a moderate weight gain and PER; their weight gain was comparable to that of the rat consuming unsupplemented chow. The rats consuming the pectin supplement grew the least, presumably because of the low digestibility and absorption of this carbohydrate [10]. The PER ratios were comparable in the pectin and chow groups, indicating that unsupplemented chow was, in this regard, the least efficient of the diets provided.

None of the rats appeared to have evidence of malnutrition, and all groups of animals but one appeared to age in an expected manner. The one remarkable exception was the group fed pectin. While these animals were smaller by some 200 g, all were obviously healthy and each in fact had the appearance of animals about six months of age; their coats of hair were sleek-white, unlike the bristly, red-pocked coats of the other groups.

Water consumption and stool residue. *Ad libitum* water consumption was found to be inversely proportional to the weight gain; for example, the pectin low-weight gain group drank almost twice the volume of water consumed by the high-weight-gain group, and the moderate weight gain groups were intermediate in this regard. These measurements are summarized at the top of Table III. This relationship held no matter the season.

The output of stool residue was also inversely proportional to the weight gain, in all three seasons. The stool residue in the pectin group was more than twice that of the high weight gain group and half again that produced by the rats of moderate weight gain. These stool weights are summarized at the bottom of Table III.

The relationship between stool residue and water consumption was approximately linear as shown in Fig. 2 which suggests the two are related; this will be discussed later. The volume of water consumed daily was equal to approximately 15 times the dry weight of the stool.

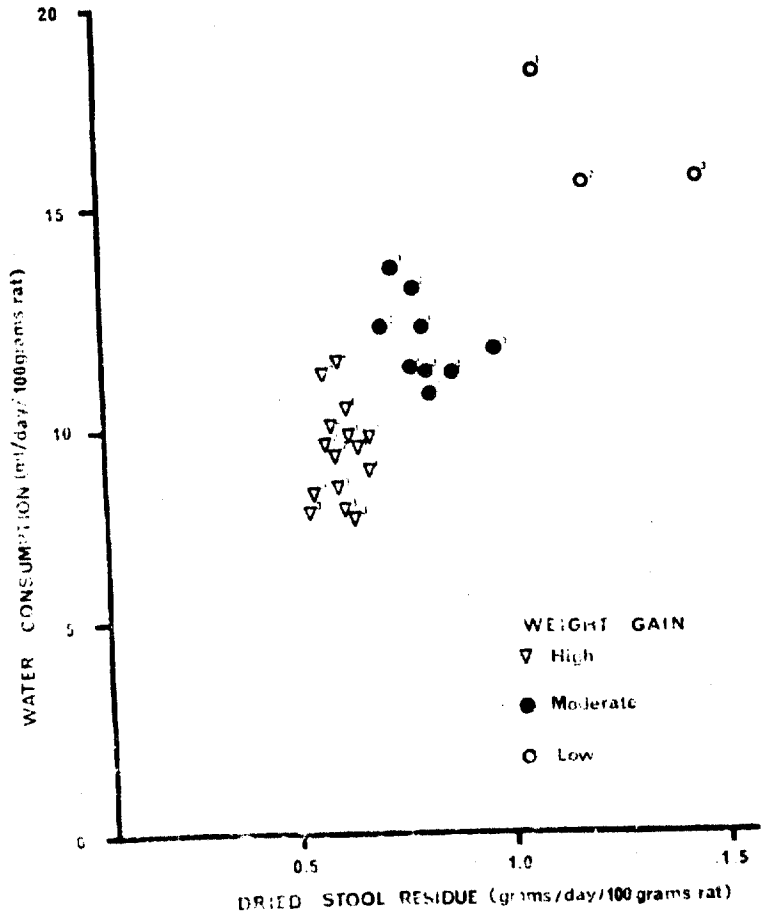


Fig. 3. Interrelationships of water consumption, dried stool residue and weight gain in the rat. The superscripts 1, 2 and 3 refer to the three different periods during which the water consumption and dried stool residue were measured for each group of rats consuming a different carbohydrate. Each symbol represents one of the dietary groups comprising each of the weight gain groups shown in the legend. These data correspond to those listed in Table III.

Since both the water consumption and dried stool weight are related to the amount of weight gain, and the weight gain differences were determined by the carbohydrates added to the diet, it follows that differences in both the water consumption and stool residue were also determined by the carbohydrates added to the diet.

Table III. Effect of various carbohydrate supplements on water consumption and stool dry weight

Weight gain group	High					Moderate			Low
Carbohydrate supplement	Granulated sugar	Sucrose	Fructose	Cerelose	Dextrin	Chow	Galactose	Beta Lactose	Pectin
				Water consumption per day per 100 g of rat[1]					
Winter	9.48	9.33	10.3	[illegible]	9.38	[illegible]	13.6	11.1	18.2
Spring	11.1	9.42	11.4	9.87	9.30	11.3	[illegible]	13.1	15.5
Fall	8.3	7.89	7.66	[illegible]	7.86	11.2	10.7	11.7	15.6
				Dry stool weight per day per 100 g of rat[2]					
Winter	0.62	0.65	0.63	0.63	0.61	0.81	0.74	0.87	1.03
Spring	0.58	0.58	0.64	0.59	0.59	0.78	0.71	0.79	1.19
Fall	0.55	0.58	0.62	0.61	0.62	0.81	0.82	0.97	1.45

[1] milliliters [2] grams

Organ weights. The variety of dietary carbohydrate was also an important determinant of the relative weights reached by the liver and testes. The mean relative liver weight in the galactose-fed rats was significantly greater than in fructose- ($p < 0.05$) and cerelose-fed ($p < 0.02$) animals. Similarly, the dextrin- and pectin-fed animals had relative testicular weights which were significantly greater (all p-values < 0.002) than in the fructose- and sucrose-fed rats. The relative organ weight data are shown in Table IV.

The ratios of the relative liver and testicular weights, shown also in Table IV, and the low correlation coefficient of these relative weights ($r = 0.247$), suggest that in the same animal, the final relative weights of these organs need not necessarily be comparably influenced by the same carbohydrate variety.

Serum lipids. Serum cholesterol determinations were made after 9, 14 and 18 months of feeding; serum total fatty acids were determined at 9 and 18 months; these data are summarized in Table V. The major finding was a steady increase in these lipid levels with increasing age in every group; a virtual doubling of the serum lipids occurred in the last 9 months of their lives. This increase was independent of the carbohydrate provided.

The variety of dietary carbohydrate however did appear to be of some importance in determining the level of the lipid increases. For example, the final serum cholesterol levels in the cerelose and pectin groups were significantly lower than the levels found at the end of the study in the groups fed granulated sugar ($p < 0.02$) and beta-lactose ($p < 0.02$). The serum total fatty acids in the groups fed glucose or pectin were also significantly lower than found in the groups fed dextrin ($p < 0.02$) or beta-lactose ($p < 0.02$).

The serum lipid levels seemed not to be importantly influenced by the level of weight gain. For example, despite the marked difference in weight gain throughout the study, the serum lipid levels in the groups fed cerelose and pectin were comparable.

Composition of liver lipids. The dietary carbohydrate was of some importance also in determining particularly the proportion of triglycerides and free cholesterol found in the liver lipids. The distributions of the major classes of liver lipids for each diet group are shown in Table VI. The triglyceride portion was significantly lower in the cerelose and galactose-fed rats, than in the fructose-, sucrose- and lactose-fed rats ($p < 0.01$ to < 0.03). The cholesterol portion

Table IV. The influence of dietary carbohydrate variety on relative organ weights

Weight gain group	Carbohydrate supplement	Liver weight / Body weight × 100 (Mean ± SD)[2]		Testicle weight / Body weight × 100 (Mean ± SD)		Liver weight (g)[3] / Testicle weight (g) (Mean ± SD)	
		No.[1]		No.		No.	
High	Granulated sugar	4	3.33 ± 0.37	4	0.93 ± 0.05	5	3.55 ± 0.17
	sucrose	6	3.35 ± 0.37	6	0.75 ± 0.02	6	4.57 ± 0.79
	cerelose	5	2.63 ± 0.23	5	0.84 ± 0.13	5	3.60 ± 0.26
	fructose	7	2.90 ± 0.10	7	0.69 ± 0.15	7	4.63 ± 1.04
	dextrin	4	3.32 ± 0.36	5	[illegible] ± 0.19	4	3.13 ± 0.17
Moderate	chow	5	3.09 ± 0.14	5	[illegible]	5	4.16 ± 0.33
	galactose	6	[illegible]	6	0.95 ± [illegible]	6	4.20 ± 0.50
	lactose	6	3.40 ± 0.32	6	0.75 ± [illegible]	6	4.01 ± 0.54
Low	pectin	7	3.1[illegible] ± 0.2[illegible]	6	[illegible]	6	2.85 ± 0.33

[1] number of animals studied [2] standard deviation [3] [illegible]

Table V. **Effect of age and dietary carbohydrate supplement on the serum cholesterol and total fatty acids**

Weight gain group	High						Moderate		Low
Carbohydrate supplement	Granulated sugar	Cerelose	Dextrin	Sucrose	Fructose	Chos	Beta Lactose	Galactose	Pectin
Months of feeding	Cholesterol mg/100 ml (Mean ± SD)								
9	73.1	68.6	72.0	111	95.8	77.4	81.5	93.0	88.3
	(21.3[1])	(13.3)	(17.5)	(35.2)	(20.7)	(13.8)	(16.8)	(18.2)	(16.8)
No.[2]	7	7	6	8	8	8	7	7	9
14	153	97.8	117	168	127	150	138	110	126
	(36.5)	(27.9)	(32.4)	(51.5)	(18.9)	(28.0)	(36.7)	(33.8)	(20.5)
No.	5	6	4	6	7	5	7	6	7
18	170	134	187	239	176	202	190	149	127
	(16.3)	(16.4)	(10.7)	(93.3)	(79.2)	(47.5)	(28.1)	(28.7)	(26.4)
No.	4	4	1	6	7	5	6	6	7
	Total fatty acids meq/liter (Mean ± SD)								
9	6.13	4.51	6.30	8.86	6.27	6.08	6.60	6.04	4.32
	(1.30)	(2.50)	(2.10)	(3.30)	(1.10)	(1.57)	(1.60)	(1.10)	(1.20)
No.[2]	7	8	6	8	8	5	7	7	9
18	14.7	12.6	13.1	19.4	13.6	14.6	18.3	15.2	12.2
	(1.41)	(1.00)	(3.00)	(8.96)	(3.00)	(3.72)	(3.00)	(2.80)	(1.73)
No.	4	4	1	6	5	5	6	6	7

[1] Standard deviation [2] The number of animals assayed

Table VI. [illegible] influence of carbohydrate variety on the distribution of liver lipids

Weight gain group	Carbohydrate [illegible]	N[illegible]	Distribution of liver lipids[1] Cholesterol esters		Triglycerides		Cholesterol		Phospholipids
High	[illegible]		6.30	[illegible]	[illegible]	[illegible]	[illegible]	1.5[illegible]	62.5 ± 13.3
	sucrose	5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	54.9 ± 15.6
	cerelose	4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	8.12	58.9 ± [illegible]
	fructose	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	11.5	4.2[illegible]	54.4 ± 6.24
	dextrin	3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	9.0[illegible]	61.7 ± [illegible]
Moderate	chow		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	54.4 ± 12.[illegible]
	galactose	5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible] ± 3.61
	lactose	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	30.8 ± 17.0
Low	pectin	5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	2.[illegible]	65.6 ± 11.0

[1] mean percent ± standard deviation [illegible] mass studies

of the liver lipids was significantly greater in the cerelose-fed rats than in those consuming galactose or pectin ($p < 0.02$).

Adipose tissue fatty acids. Two important observations were made. Despite the low fat content of the diet five of nine fatty acids in the adipose tissue of all groups were present in proportions comparable to those found in the diet. The four major adipose tissue fatty acids, whose proportions differed from those in the diet, are shown in Fig. 3; two were higher (C 16:1 and C 18:1) and two were lower (C 18:0 and C 18:2). The second observation was that the quality of the dietary carbohydrate appeared to be a determinant of the degree to which the fatty acid distribution in the diet and adipose tissue differed; for example, the fructose-fed rats had a significantly greater proportion of C 16:1 than found in 5 of the other groups fed different carbohydrates ($p < 0.02$) and the C 18:2 proportions found in the groups fed lactose and pectin, though comparable to one another, were significantly greater (all p values < 0.05) than found in all the other groups fed different carbohydrates.

Liver lipid fatty acids. Eight of the ten fatty acids in the *liver triglyceride* fatty acids were comparable to those in the diet. Two differed; the proportions of C 18:2 and C 20:4. These are shown in Fig. 4A. The C 18:2 proportion was lower and the C 20:4 was higher than found in the diet. Three points are worth making: one is that since C 20:4 (arachidonic acid) was absent from the diet it must have been synthesized; presumably the precursor in the diet was C 18:2 (linoleic acid) [11]. It is of interest in this regard that the sum of the mean porportions of these two liver triglyceride fatty acids, in virtually every group, was about equal to the proportion of C 18:2 present in the diet (about 25%). Finally, the C 20:4 proportion that was present in the liver triglycerides appeared to be influenced by the carbohydrate in the diet; for example, the proportion in the pectin-fed rats was twice that present in the rats fed cerelose ($p < 0.05$).

While the *liver cholesterol esters* also had a fatty acid composition not unlike that of the diet there were two exceptions: the proportions of C 18:0 (Stearic) and C 20:4 (arachidonic acids). These are shown in Fig. 4B. The C 18:0 proportion was comparable to that in the diet except in the groups fed sucrose, fructose, and pectin. The proportion of this fatty acid was significantly higher ($p < 0.05$) in these three groups than found in the group fed dextrin; the same was true when pectin and sucrose fed groups were compared with

beta-lactose. Furthermore, despite the great variation in the proportion of arachidonic acid found esterified with cholesterol among the rats within each group (Fig. 4B) the proportion of this fatty acid which became esterified was also very likely influenced by the carbohydrate variety present in the diet.

The liver *phospholipid fatty acid* distribution was quite different from that in the diet. It was comparable to that recorded in the literature for chow fed animals [12]. Despite these shifts from the diet composition, to a fatty acid composition characteristic for phospholipids, one could not help but be struck by the relative lack of influence of carbohydrate variety in influencing the magnitude of the differences between the phospholipid and diet fatty acid composition. Though there were some differences it was not possible to be certain any were significant.

Sucrose and granulated sugar. The [illegible] recently given sucrose as responsible for higher blood lipid levels as well as perhaps being implicated in coronary disease, should not obscure the fact that sucrose and common table sugar though very similar are not identical. It was therefore particularly interesting to contrast the effects of these two almost identical carbohydrates. The adipose tissue fatty acid composition in these two groups was essentially comparable, there was a slightly higher mean level in the C 16:0 fraction, and a lower mean level of the C 20:4 fatty acids in the sucrose-fed rats but these were of doubtful significance. However, a small amount (2.[illegible] ± 0.61%) of C 18:3 was found only in the liver triglycerides of the group fed granulated sugar. Despite considerable variation among rats in each group, the C 18:0 fraction in the liver cholesterol ester was less than half that in the granulated sugar group, but about 50% greater in the C 18:2 fractions; and the mean C 20:4 fraction in the sucrose-fed rats was six times greater than in the rats consuming granulated sugar. The

Fig. 3. Comparisons of the fatty acid composition of the diet and the epididymal fat pads. The four fatty acids in the epididymal fat (open bars) whose proportions differed from that in the diet (hatched bars) are shown. Each open bar represents the mean value for each diet group. The line above or within the bar represents one standard deviation. High, moderate, and low refer to weight gain groups. Non, refers to the number of fat pads analyzed. The groups receiving the different carbohydrates are denoted as follows: *GS* granulated sugar; C cerelose (glucose + D dextrin; *S* sucrose; F fructose; *L* beta-lactose; *G* galactose; P pectin. *Ch* indicates the group fed only rat chow. See the text for additional explanation.

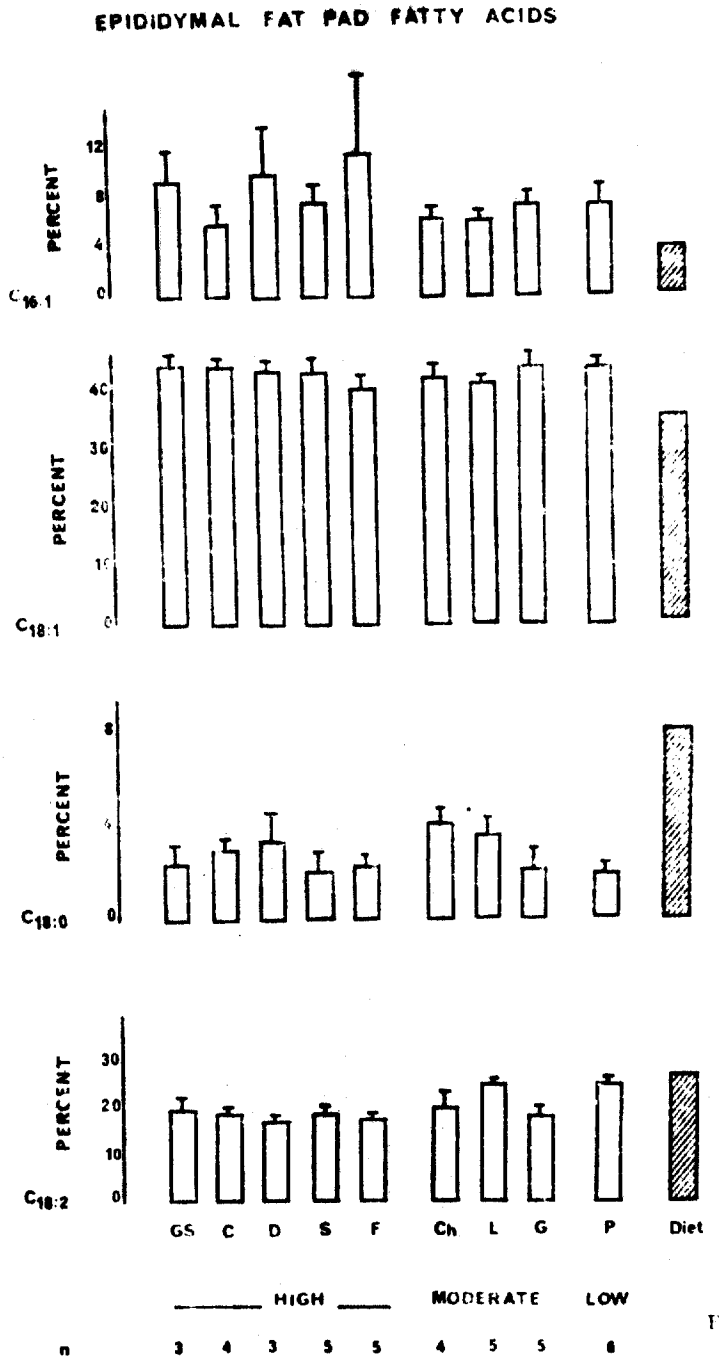

EPIDIDYMAL FAT PAD FATTY ACIDS
PERCENT
$C_{16:1}$
$C_{18:1}$
$C_{18:0}$
$C_{18:2}$
GS C D S F Ch L G P Diet
HIGH
MODERATE
LOW
n 3 4 3 5 5 4 5 5 6

Fig. 1A *A.* Liver triglyceride fatty acids

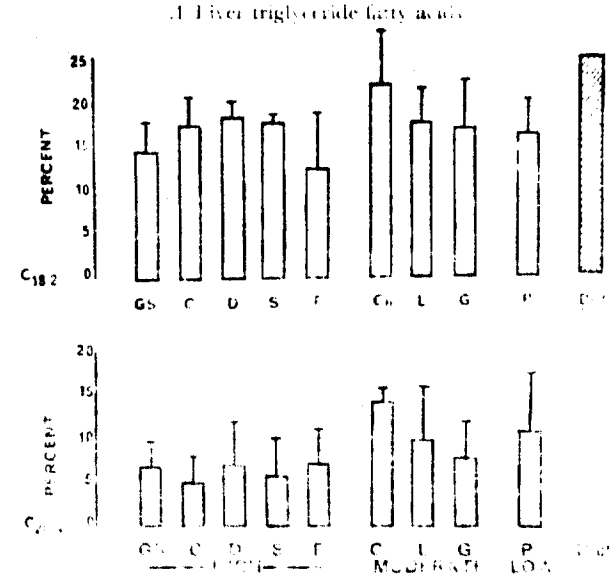


Fig. 1B *B.* Liver cholesterol ester fatty acids

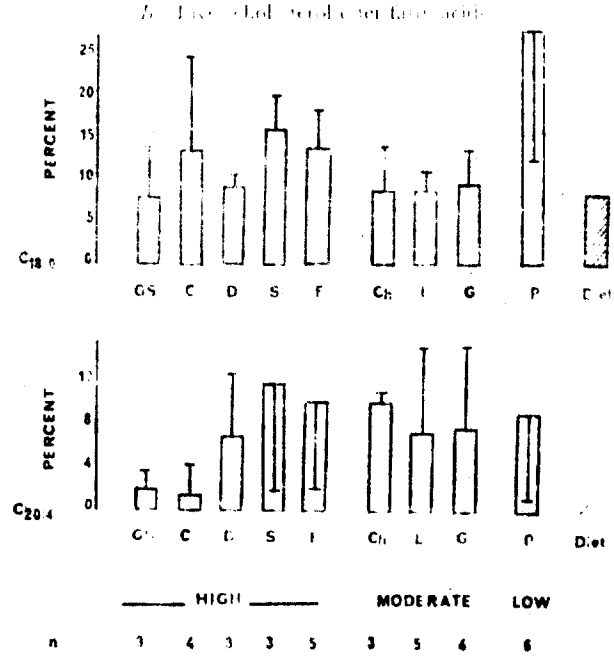


Fig. 1. Comparison of the fatty acid composition of the diet and the liver triglycerides and cholesterol esters. As in Fig. 3 only the fatty acid proportions which differed from those in the diet are shown. See the legend of Fig. 3 for the explanation of abbreviations etc.

appearance of small amounts of behenic acid (1.54 ± 1.10%) in the liver phospholipids was evident only in the granulated sugar group. Finally, although the saturated: unsaturated fatty acid ratios for these two sugars were comparable in the liver triglyceride and cholesterol esters, and differed only slightly in the adipose tissue triglycerides (granulated sugar, 0.322; sucrose, 0.414), this ratio was strikingly different in the liver phospholipids (granulated sugar, 1.22; sucrose, 0.66).

Discussion

Non-lipid studies. This study demonstrates several influences in the rat of chronically consumed dietary carbohydrates including some, to the authors' knowledge, not previously described. It has been demonstrated that the dietary carbohydrate variety plays an important role in determining many characteristics of the rat: the rate at which they grow; their utilization of protein for growth, the final relative size of their livers and testes; their water consumption; and, the non-aqueous residue in their stool. The interrelationships of these gross observations are speculative. It is possible, however, that differences in carbohydrate digestibility and/or an alteration in the intestinal bacterial mass promoted by the carbohydrates may have altered the bulk of the stool. A bulkier stool residue could increase the colonic osmotic pressure, reduce fecal water resorption, and result in increased water consumption. The crude wet stool weights support this interpretation. This would also explain the linear relationship between the stool residue and water consumption noted in Fig. 2.

If the well known protein-sparing effect of dietary carbohydrates is different for different carbohydrates, the differences in the PER could be explained. Such a possibility is also suggested by other studies [14–16]. The nutritional implication of this observation is not minor. Furthermore, different protein-sparing effects of the various carbohydrates used in this study could also result in a significant reduction in the production and possible output of metabolic water, and explain in part the differences in water consumption. GAMBLE was the first to demonstrate the reduced water requirement associated with feeding carbohydrate [17]. Such differences in protein utilization could also contribute to differences in weight gain and organ size.

Lipid studies. The important role of dietary carbohydrate in influencing serum lipoproteins and lipoprotein lipid concentrations is now well known. AHRENS [18] and GORMAN [19] have led in clarifying the important influence of the amount of dietary carbohydrate on the serum lipids and lipoproteins. Others have suggested that the role of not only the amount but also the variety of dietary carbohydrate may be important [20, 21]. The present study indicates that certain dietary carbohydrate varieties may be of influence on the serum lipid levels. The mechanisms of action by which these effects are produced by the different carbohydrates are not known. The studies of KRICHEVSKY *et al.* [22] suggest that the intestinal flora may play an important role, as nonabsorbable antibiotics were able to abolish serum cholesterol differences associated with feeding different carbohydrates for a short period; such a mechanism may be operative when cholesterol is added to the diet which is not the case in the present study.

The present study also underscores the importance of carbohydrate variety in controlling the fatty acid composition of complex lipids in the liver and adipose tissue. The contribution of exogenous fatty acids to the fatty acids found in glycerides and other complex tissue lipids is well known [23]; as shown here the *variety* of carbohydrate is also important. Furthermore, if a single carbohydrate is chronically consumed it need not represent 3/4 of the total calories to exert an influence on the distribution of the saturated and unsaturated fatty acids present in the liver and adipose tissue.

A comparable study of the influence of dietary carbohydrate on the composition of tissue fatty acids was reported by MACDONALD in 1962 [24]. He reported a greater amount of liver lipid accumulation when sucrose rather than maize starch or a starch hydrolysate was fed. He reported further that both the adipose tissue and liver fatty acids varied with the variety of carbohydrate. There were several differences between his and our study; he provided three different carbohydrates to groups of protein-starved rabbits all of whom lost weight and suffered high mortality, and no separation was made of the major lipid classes from the liver prior to fatty acid analysis. Nevertheless, both MACDONALD's and the present studies establish that the lipid response in two different species is related to the type of carbohydrate consumed.

There are many possible mechanisms by which carbohydrates might influence lipid metabolism. Three of these might be men-

tioned here. One possibility, already noted, is an alteration in the intestinal flora. The phenomenon of diet-induced changes in the species comprising the fecal bacterial mass has been known for at least forty years [25, 26], and redemonstrations of this phenomenon with novel experimental diets continue to appear [27]. Alteration of the flora might influence the absorption of the fecal sterols, by virtue of the bacterial conversion of the sterols to non-absorbable varieties [28].

Another possibility is suggested by the studies of VAGELOS and others [29]. Fatty acid (palmitate) biosynthesis has been found to be stimulated by several tricarboxylic acid cycle intermediates, but particularly citrate. Citrate appears to produce this effect by altering certain physical characteristics of an enzyme, acetyl-CoA carboxylase, which is responsible for the initial carboxylation of acetyl-CoA to malonyl CoA; this facilitates the subsequent enzymatic reactions of fatty acid synthesis. It is possible that various dietary carbohydrates might increase the availability of Krebs-cycle or other intermediates potentiating fatty acid synthesis, or that the individual carbohydrates might also influence the enzymes responsible for fatty acid synthesis directly and thereby determine the fatty acid which will be formed. The peculiar localization of carbohydrates such as fructose in the semen and allantoic fluid, glycogen in muscle and liver, and lactose in the lactating breast, may well serve a role in directing the variety of fatty acids which are synthesized in these organs. These data certainly suggest that the study of *in vitro* fatty acid synthesis in the presence of a variety of carbohydrates might be of interest.

A third possibility to explain the appearance of unique fatty acids on carbohydrate feeding is that there are impurities in the refined and chemically pure carbohydrates which are fed. For example, there is evidence from the present study that despite the obvious similarities, recrystallized purified large crystal sucrose produces different effects on lipid metabolism from those produced by the feeding of ordinary commercial granulated sugar available to the general population. This suggests that impurities are present in one or both which account for these differences.

There is one additional point. Lipids comprise a major part of the membranes of the cells, including the endoplasmic reticulum and the many intracytoplasmic organelles. It is likely that the metabolic activity of each membrane is influenced by its structure.

While the knowledge of the structure-function relationships of membranes is still in a primitive state, it is known that the fatty acid composition of the mitochondrion influences its gross appearance and its ability to synthesize ATP [30]. Thus, if the dietary carbohydrate influences the complex lipids comprising the membranes of such organelles, it is likely that the functioning of these organelles will also be changed. This may be a means by which cellular adaptation to nutrients in the diet may occur.

Summary

The influence of the consumption over a period of eighteen months of eight different dietary carbohydrates on the rats' growth rate, water consumption, stool output, organ size, serum lipids, liver lipid distribution, and liver lipid and adipose tissue fatty acid composition was studied. Generally speaking, glucose- and fructose-containing carbohydrates supported weight gain more adequately than galactose-containing carbohydrates which in turn were more efficient than pectin. Weight gain was found to be inversely related to water consumption and the dried stool output. Relative liver and testes weights were not comparably influenced by the different carbohydrates. There was no correlation of serum lipid levels and weight gain, but of those carbohydrates associated with the greatest weight gain, cerelose (glucose) consumption was associated with the lowest serum lipids. The distribution of some of the major lipid classes in the liver was markedly effected by dietary carbohydrate variety, as was the fatty acid composition of adipose tissue and liver.

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Discussion

D. KRITCHEVSKY (Philadelphia, Pa.): It is well documented that sucrose is more cholesteremic than glucose in cholesterol-fed rats, rabbits and chickens. Your data suggest that in long-term feeding, sucrose retains its hypercholesteremic effect even in the absence of dietary cholesterol. We have fed chickens cholesterol plus various combinations of carbohydrates (glucose, fructose, sucrose, lactose, glucose plus fructose and sorbitol). Over a 30-day period of study, sucrose is consistently the most hypercholesteremic carbohydrate. This result has been observed in several different experiments.

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Failure of Intestinal Bacteriostasis Following Administration of Pectin to the Rat

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A NUMBER of reports have appeared concerning the bactericidal properties of pectin preparations (1-6). In general these indicate that pectin *per se* exerts no bactericidal effect, although the pH of some pectin preparations and the acidic by products of pectin digestion by intestinal micro-organisms were responsible in some cases for a decrease in the number of test organisms observed in *in vitro* studies. When experimental solutions were buffered to correspond with conditions present in the intestinal tract, no bactericidal action attributable to pectin was observed. In contrast to these studies nickel pectinate has been shown to possess bactericidal properties both clinically in man (7) and in *in vitro* experiments with intestinal micro-organisms (1,3,4,5).

It has been demonstrated by a number of investigators that although rats will grow and reproduce on purified rations containing six B complex factors

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[illegible] hydrochloride, riboflavin, pyridoxine hydrochloride, calcium pantothenate, nicotinic acid and choline chloride), incorporation of sulfaguanidine or sulfathiazole in such rations results in retardation of growth, alopecia, spectacled eyes, opthalmitis, porphyrin caked whiskers, achromotrichia and other manifestations of dietary inadequacy presumably due to inhibited synthesis of essential factors by intestinal microorganisms (8-11). The development of these symptoms in animals maintained on a similar ration but containing a suspected bacteriocide might accordingly be used as a physiological indicator of intestinal bacteriostasis. In the following experiment pectin and nickel pectinate were incorporated in a purified ration containing the B complex factors listed above and

Table 1

Composition of Experimental Diets and Distribution of Rats.

	A	B	C	D	E	F	G	H
Pectin[1]		2.5	5	10				
Nickel pectinate[2]					2.5	5	10	
Sulfaguanidine								1
Sucrose	73.5	71.0	68.5	63.5	71.0	68.5	63.5	72.5
Vitamin Test Casein[3]	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0
Salt Mixture[4]	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Number of animals in group	6	4	6	6	4	6	6	6

To each kg. of the above mixture were added the following synthetic vitamins: thiamine hydrochloride 20 mg., riboflavin 20 mg., pyridoxine hydrochloride 20 mg., calcium pantothenate 100 mg., nicotinic acid 100 mg., choline chloride 1200 mg. and 5 mg. 2-methyl naythaquinone. In addition each rat received the following daily supplement: corn oil (Mazola) 800 mg., alpha-tocopherol 0.5 mg., and a Vitamin A-D concentrate[5] containing 50 U.S.P. units of vitamin A and 5 U.S.P. units of vitamin D.

1. N.F. Pectin, California Fruit Growers' Exchange, Ontario, California.
2. Pectalin Powder, containing 0.5 per cent nickel as nickel sulphate, Emory W. Thurston Laboratories, Los Angeles, California.
3. S.M.A. Corporation, Chagrin Falls, Ohio.
4. Salt Mixture No. 1 (Sure 1942).
5. Nopco Fish Oil Concentrate, assaying 800,000 U.S.P. units of vitamin A and 80,000 U.S.P. units of vitamin D per gram.

their bacteriostatic properties contrasted with that of a known bacteriocide (sulfaguanidine).

Procedure and Results.

Fifty-two male and female rats* weighing 30 to 35 grams were weaned at 21 to 23 days of age and litter mates divided as far as possible among the eight experimental groups listed in table 1 and a ninth maintained on a natural food ration**. Animals were kept in metal cages with screen bottoms to prevent access to feces and sufficient food was administered to assure ad lib feeding. Feeding was continued for eight weeks.

No significant difference in rate of growth or gross appearance was observed in any of the rats with the exception of those receiving diets G and H. Incorporation of pectin at levels of 2.5 to 10 per cent of the ration or nickel pectinate at 2.5 to 5 per cent failed to exert any grossly visible deleterious effect. Animals receiving diet G (10 per cent nickel pectinate) failed to grow, losing weight steadily until death. The short survival time on this ration (10±3 days) indicates however that death was not due to bacteriostasis with resulting nutritional deficiency***. Animals receiving sulfaguanidine (diet H) grew normally for the first three weeks of feeding at which time they did not differ grossly from litter mates on control rations. Subsequent to this period growth was retarded, and animals developed the typical syndrome of the sulfaguanidine-fed rat (8, 9).

These results indicate that under the conditions of the present experiment and in the dosage employed, pectin and nickel pectinate were ineffective as an intestinal bacteriocide for microorganisms synthesizing essential nutrients for the rat.

Summary

No evidence of bacteriostasis as indicated by retardation of growth or the precipitation of nutritional deficiencies was observed in the rat following incorporation of pectin and nickel pectinate in a purified ration containing six B complex factors (thiamine hydrochloride, riboflavin, pyridoxine hydrochloride, calcium pantothenate, nicotinic acid and choline chloride).

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* From the animal colony, Department of Biochemistry, University of Southern California. Kindly provided by Dr. H. J. Deuel, Jr.

** Purina Dog Chow supplemented once weekly with lettuce.

*** Food and water consumption were voluntarily reduced subsequent to the first day of feeding, animals resembling in gross appearance and length of survival a subsequent group weaned at a [illegible] and weight but starved to death. The possibility of nic[illegible] at this level has not been excluded.

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Effects of Gum Guar, Locust Bean Gum and Carrageenan on Liver Cholesterol of Cholesterol-Fed Rats.* (27585)

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Our previous studies(1) indicate that pectin N.F. when fed at a 5% level in the diet largely counteracted the increment in liver cholesterol and liver total lipids induced by cholesterol feeding in the rat. Other roughage or bulk-forming materials such as cellulose, agar, sodium alginate, protopectin and calcium silicate (Micro-Cel) were ineffective in this regard. Data are presented here indicating that Gum Guar, Locust Bean Gum and carrageenan also have significant activity in counteracting the increment in liver cholesterol and liver total lipids induced by cholesterol feeding in the rat.

Procedure. The basal ration consisted of sucrose, 61%; casein,† 24%; cottonseed oil, 10%; salt mixture,‡ 5%; and the following vitamins per kg of diet: thiamine hydrochloride, 20 mg; riboflavin, 20 mg; pyridoxine hydrochloride, 20 mg; calcium pantothenate, 60 mg; nicotinic acid, 100 mg; ascorbic acid, 200 mg; biotin, 4 mg; folic acid, 10 mg; p-aminobenzoic acid, 400 mg; inositol, 800 mg; 2-methyl-1,4 naphthoquinone, 5 mg; vit B_{12}, 150 μg; choline chloride, 2 g; vit A, 5000 U.S.P. units; vit D_2, 500 U.S.P. units; and alpha-tocopheryl acetate, 100 mg. Tests were conducted with rats fed the basal ration, the basal ration + 1% cholesterol, and the basal ration + 1% cholesterol + the various supplements indicated in Table I. The cholesterol and test supplements were incorporated in the basal ration in place of an equal amount of sucrose. Fifty-six male rats of the Holtzman strain with an average body wt of 43.6 g (range 38 to 50 g) were divided into 7 comparable groups of 8 each, placed in metal cages with raised screen bottoms (2 or 3 rats per cage), and provided the test diets and water *ad libitum*. Animals were fed on alternate days and all food not consumed 48 hours after feeding was discarded. Body weights were recorded weekly. After 28 days of feeding, the rats were anesthetized with sodium pentobarbital, and blood was withdrawn from the aorta into a heparinized syringe. Livers were excised, blotted to remove excess blood, weighed and stored in a freezer until analyzed. Lipid was extracted from the livers by the method of Thompson *et al.*(2), and cholesterol was determined on liver and plasma by the method of Nieft and Deuel(3). Total lipids were determined gravimetrically on an aliquot of the liver extract.

Results. The increment in liver cholesterol and liver total lipid induced by cholesterol feeding in the rat was largely counteracted by the concurrent feeding of Gum Guar, Locust Bean Gum or carrageenan at a 10% level in the diet. The effects of these supplements were similar to, although slightly less marked than, that of a comparable amount of pectin N.F. Cholesterol feeding also induced a slight increment in plasma cholesterol level, which was partially counteracted by concurrent feeding of pectin N.F. and several of the other test supplements. Differences between the various groups however were not stati-

* This investigation was supported in part by a research grant from Nat. Heart Inst., N.I.H., P.H.S.

† Vitamin-Free Test Casein, General Biochemicals, Inc., Chagrin Falls, Ohio.

‡ Hubbell, Mendel and Wakeman Salt Mixture, General Biochemicals, Inc., Chagrin Falls, Ohio.

TABLE I. Effects of Dietary Supplements on Plasma and Liver Cholesterol and Liver Total Lipid of Cholesterol-Fed Rats (8 Animals/Group).*

Supplements fed with basal ration	Body wt at sacrifice, g†	Plasma cholesterol, mg/100 ml†		Liver cholesterol, mg/g†		Liver total lipid, %†
		Free	Total	Free	Total	
None	197.4 ± 4.9	20.1 ± 1.6	85.2 ± 5.8	1.63 ± .09	2.09 ± .09	4.74 ± .3
1% cholesterol	201.3 ± 6.8	21.0 ± 1.1	105.5 ± 5.0	2.72 ± .14	16.17 ± 1.42	9.03 ± .7
Idem + following supplements:						
5% pectin N.F.	185.4 ± 6.1	19.6 ± 1.0	92.8 ± 4.2	2.45 ± .13	5.59 ± .64	6.31 ± .4
10% " "	181.7 ± 5.6	18.2 ± 1.4	95.6 ± 3.3	2.38 ± .17	4.12 ± .42	4.78 ± .3
10% Gum Guar	177.6 ± 5.2	20.8 ± 1.2	91.3 ± 4.3	2.29 ± .12	6.05 ± .79	6.29 ± .5
10% Locust Bean Gum	180.3 ± 5.4	24.0 ± 1.5	103.3 ± 5.0	2.38 ± .08	7.59 ± .73	6.81 ± .4
10% carrageenan	191.9 ± 3.8	20.8 ± 1.6	89.3 ± 5.5	2.22 ± .09	5.52 ± .62	5.86 ± .3

* Test supplements obtained from the following sources: pectin N.F. (citrus), Sunkist Growers, Ontario, Calif.; Gum Guar and Locust Bean Gum, Hathaway Allied Products, Los Angeles, Calif.; carrageenan (Gelcarin MR 100), Marine Colloids, Inc., of America, New York. Test supplements were all natural sources of hydrophyllic complex carbohydrate colloids made up of repeating units of (1) galacturonic acid in the case of pectin, (2) mannose and galactose in Gum Guar and Locust Bean Gum, and (3) sulphated galactose in Gelcarin MR 100. The pectin N.F. employed was a purified material obtained from the dilute acid extract of the inner portion of the rind of citrus fruits. It had a methoxyl value of 10.7% on a moisture-ash-free basis. Gum Guar was the ground endosperm of Guar (known botanically as *Cyamopsis tetragonolaba*) seed with a galactomannan content of approximately 80%. Locust Bean Gum was obtained from the endosperm of the kernels of the Carob tree. It was a hemicellulose product of about 4 parts mannose and 1 part galactose. Gelcarin MR 100, a highly purified product designed for use in milk or milk products, is known chemically as carrageenan. The latter occurs naturally in a number of red seaweeds (class Rhodophyceae) but is obtained principally from the group of seaweeds known as Irish moss.

† Including stand. error of mean.

tically significant.§ Findings are summarized in Table I.

Discussion. A number of studies indicate that, in general, populations habitually subsisting on diets low in fats and animal protein tend to have a low concentration of serum cholesterol and a low incidence of cardiovascular disease(4-7). Such diets contain a number of constituents which are either absent from or present in only minute amounts in the diets of populations with a high incidence of hypercholesterolemia, atherosclerosis and coronary artery disease. Walker and Arvidsson(8) and Higginson and Pepler(9) were among the first to call attention to the high fiber content of the Bantu diet as a possible explanation for the low serum cholesterol level observed in the Bantu population. A similar suggestion was also made by Bersohn *et al.*(10). More recently Keys *et al.* (11) conducted controlled experiments in which groups of physically healthy men subsisted alternately on "American" and "Italian" types of diets, devised to be comparable in calories, proteins and in kind and amounts of fat but differing in the sources of carbohydrates. An abundance of fruits and vegetables in the Italian type diets (which tended to be high in complex carbohydrates such as pectins, hemicelluloses and fiber) replaced equivalent calories in simpler carbohydrates in the American type. Serum cholesterol lev-

§ Food intake was determined for rats in the various groups. Since the weight increment of rats fed the test supplements was less than that of rats fed the basal + cholesterol diet, the question arose whether the reduction in weight increment of rats fed the test supplements may not have been due to a reduction in amount of diet and hence cholesterol ingested and whether the reduced cholesterol intake in turn may not have been the cause of lower liver cholesterol and liver total lipid levels. This, however, does not appear to be the case, for differences in body weight between the various groups were not statistically significant whereas differences in liver cholesterol and liver total lipid were. Furthermore, an even greater reduction in body weight was observed (unpublished findings) in rats fed the basal ration + cholesterol + 20% alfalfa meal without an accompanying reduction in liver cholesterol and liver total lipid values. The possibility that the test supplements inhibited absorption of cholesterol from the gut, however, has not been excluded.

els were significantly lower with the "Italian" type diets. In subsequent studies Keys *et al.* (12) reported that citrus pectin when fed at a level of 15 g per day caused a slight but statistically significant reduction in serum cholesterol levels in physically healthy, middle-aged men; cellulose (fiber) fed under comparable conditions was without significant effect. These studies suggest that cellulose or fiber *per se* was not responsible for the low serum cholesterol levels of the Bantu and comparable groups, but that pectin, another complex bulk-forming carbohydrate, may have had some activity in this regard.‖ An analysis of the diets of native populations with low serum cholesterol levels indicated that in addition to pectin such rations also contain gums and/or other complex carbohydrates such as colloids of marine plants which were found in the present experiment to cause a highly significant reduction in liver cholesterol levels in the cholesterol-fed rat. Further studies are indicated to determine what effect these substances might have, when administered alone or in combination with one another and pectin, in treatment of hypercholesterolemia and atherosclerosis in man.

‖ Unpublished studies from this laboratory indicate that different batches of pectin may vary markedly in anti-cholesterol activity. Pectic preparations with a methoxyl content of 5% or less were without activity in counteracting the increment in plasma and liver cholesterol levels induced by cholesterol feeding in the rat in contrast to the marked activity exhibited by pectin N.F. preparations of relatively high methoxyl content (10.7% on a moisture-ash-free basis).

Summary. The increment in liver cholesterol and liver total lipid induced by cholesterol feeding in the rat was largely counteracted by concurrent feeding of Gum Guar, Locust Bean Gum or carrageenan at a 10% level in the diet. Effects were similar to, although slightly less marked, than that obtained with a comparable amount of pectin N.F.

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Influence of Citrus Pectin Feeding on Lipid Metabolism and Body Composition of Swine

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ABSTRACT Pigs receiving 5% citrus pectin in the ration for 4 weeks had significantly higher serum cholesterol, triglyceride and phospholipid levels, more backfat and higher liver cholesterol levels than pigs receiving the control ration. The addition of 5% fat to the ration resulted in significantly higher serum and hepatic cholesterol and increased backfat. The inclusion of 5% pectin plus 5% added fat in the ration caused a highly significant increase in serum cholesterol, phospholipid and triglycerides, hepatic cholesterol and backfat thickness compared with that of the animals fed either fat or pectin alone. There appeared to be a definite synergistic effect to the addition of both fat and pectin to the ration. The increases in serum and hepatic lipid levels noted as the result of feeding pectin to swine are exactly opposite to the hypocholesterolemic activity of this compound which has been reported in man and the rat.

Citrus pectin has been shown by Keys et al. (1) to possess a slight but statistically significant hypocholesterolemic effect when administered to patients at a level of 15 g/day. Similarly, Wells and Ershoff (2) have reported that citrus pectin fed to male rats at a level of 5% of the diet was effective in counteracting the increased plasma and liver cholesterol levels induced by cholesterol feeding. Further studies by these authors (3) indicated that pectin feeding had essentially no effect on plasma and liver cholesterol levels in the cholesterol-fed rabbit, guinea pig and hamster.

Because of this apparent species difference in the effect of pectin on cholesterol metabolism, it seemed worthwhile to study the influence of this compound on the lipid metabolism of swine since this animal in many ways resembles man in nutrient requirements and digestive physiology. An additional objective of this study was to determine what effect pectin might have on counteracting the anticipated increase in serum and liver cholesterol reported by several workers (4–6) to result from the addition of fat to swine rations.

MATERIALS AND METHODS

Twenty Minnesota no. 3 weanling barrow swine were allotted at random to 4 pens, each with identical feeding and watering facilities. The 4 groups of 5 animals each were fed the rations shown in table 1. Pectin was added to rations C and D during the last 4 weeks of the 12-week feeding period. The computation of net energy values is difficult because it is not known to what extent pectin contributes to the caloric content of the ration. It has been suggested that colon bacteria can hydrolyze the partially methoxylated polygalacturonic acid chain of pectin, forming galacturonic acid and short-chain fatty acids (7). Since it has not been shown that appreciable absorption of these hydrolytic products occurs, no caloric value has been assigned to the 5% pectin added to rations C and D, although it is probable that pectin does make some small contribution to the net energy of the rations.

Body weights and blood samples via heart puncture were obtained initially and at 4, 8, 10 and 12 weeks. At the end of the experiment all animals were processed through the College meats laboratory at which time blood, liver, fat and muscle samples were obtained for lipid analysis, and the following carcass measurements obtained: back fat thickness, *longissimus*

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TABLE 1

Composition of experimental rations

	Control (A)	Control + 5% fat (B)	Control + 5% pectin (C)	Control + 5% fat + 5% pectin (D)
	% of ration	% of ration	% of ration	% of ration
Milo	69.5	62.8	62.8	57.4
Soybean meal	7.0	7.8	7.8	8.0
Cottonseed meal	7.0	7.9	7.9	8.1
Fish meal	2.5	2.5	2.5	2.5
Molasses	5.0	5.0	5.0	5.0
Dehydrated alfalfa meal	5.0	5.0	5.0	5.0
Trace mineralized salt [1]	0.5	0.5	0.5	0.5
Bentonite	2.0	2.0	2.0	2.0
Limestone flour	0.5	0.5	0.5	0.5
Vitamin mix [2]	1.0	1.0	1.0	1.0
Citrus pectin	—	—	5.0 [3]	5.0 [3]
Hydrogenated vegetable oil	—	5.0	—	5.0
	100.0	100.0	100.0	100.0
Proximate analysis of diet				
Crude protein, %	16.1	16.7	16.9	16.6
Ether extract, %	2.8	6.4	2.4	6.7
Estimated net energy/kg feed, kcal	1591	1729	1505	1643

[1] The percentage composition of the trace mineralized salt was: NaCl, 93.7–98.9; and not less than the following amounts of the remaining ingredients were used: manganese, 0.2; iron, 0.16; copper, 0.033; cobalt, 0.01; iodine, 0.007; and zinc, 0.005.

[2] The vitamin mix contained per 454 g: (in milligrams) vitamin B_{12}, 0.8; niacin, 750; riboflavin, 50; pantothenic acid, 250; choline, 5000; chlortetracycline, 1000; butylated hydroxy toluene, 5675; and (in grams) $ZnSO_4$, 136.2; and vitamin A, 150,000 IU, and vitamin D_3, 150,000 IU.

[3] Citrus pectin was added to the ration the last four weeks of the 12-week feeding period. This material was kindly provided by Mr. Grant Palmer, Research Division, Sunkist Growers, Corona, California.

dorsi area, ham weight and percentage of dressing. Serum cholesterol was determined according to the method of Pearson and associates (8), tissue cholesterol by a modification of the technique of Mann (9), triglycerides by the method of Van Handel and Zilversmit (10) and phospholipids according to the procedure of Zilversmit and Davis (11).

RESULTS AND DISCUSSION

The average initial serum cholesterol values between swine in the 4 pens were quite similar; however, by the eighth week the effect of feeding 5% added fat in rations B and D was becoming noticeable in a slight but nonsignificant increase in the serum cholesterol of the pigs receiving these rations (table 2). This observation is in agreement with the work of Barnes et al. (4, 5) and Reiser et al. (6) who have demonstrated an increase in the serum and tissue cholesterol levels of swine following the addition of fat to the ration. At the beginning of the eighth week citrus pectin was added to rations C and D (table 1). Two weeks later the animals receiving 5% pectin in the ration had significantly higher serum cholesterol levels than those pigs fed ration A ($P < 0.01$), with the pigs receiving fat plus pectin showing a significantly higher serum cholesterol level than those pigs fed pectin alone ($P < 0.01$). At the end of the 12-week experimental period, animals receiving the control ration had significantly lower serum cholesterol levels than those in any other treatment group ($P < 0.01$). Feeding pectin had approximately the same effect on serum cholesterol levels as feeding 5% added fat. Feeding 5% added fat plus 5% pectin resulted in a marked increase in serum cholesterol compared with the effect of feeding 5% added fat alone.

Serum triglyceride and phospholipid determinations on blood taken at the time of slaughter indicated an almost identical pattern to that of serum cholesterol levels (table 2). The pigs receiving pectin in

TABLE 2

Influence of feeding citrus pectin and fat on serum and tissue lipid levels of swine

Variable	Ration				SE of mean
	A	B	C	D	
Serum cholesterol, mg/100 ml					
Initial	94	90	91	98	± 6.3
8 weeks	99	108	100	107	± 9.7
10 weeks	103	112	122	141 **	± 5.1
Final	91	109	107	144 **	± 6.6
Serum triglycerides, mg/100 ml	30.5	32.5	41.0	47.3 **	± 3.43
Serum phospholipids, mg/100 ml	76.0	94.0	99.6	113.6 *	± 11.1
Adipose tissue cholesterol, mg/g	0.269	0.260	0.241	0.269	± 0.0150
Muscle cholesterol, mg/g	0.570	0.544	0.577	0.600	± 0.0166
Liver cholesterol, mg/g	3.01	3.29	3.25	3.39 *	± 0.075
Liver triglycerides, mg/g	5.08	4.69	4.50	4.51	± 0.565
Liver phospholipids, mg/g	16.10	20.71	19.52	20.69	± 1.880

* $P < 0.05$.
** $P < 0.01$.

the ration, either alone or with 5% added fat, had significantly elevated serum triglyceride and phospholipid levels compared with those of the control animals or with those of pigs fed 5% added fat in the ration ($P < 0.01$).

The response of tissue lipid levels to the various treatments was less pronounced. Adipose tissue and muscle cholesterol levels were unaffected by the inclusion of fat or pectin in the ration. Phospholipid and triglyceride determinations were not made on these tissues.

The hepatic cholesterol level of the pigs receiving both pectin and fat (ration D) was significantly greater than that of the control animals ($P < 0.05$) but not significantly different from pigs fed 5% added fat or 5% pectin in the ration. No treatment differences were noted in the liver triglyceride or phospholipid levels.

Body composition was altered by the inclusion of fat and pectin into the ration, although body weight gain, feed consumption and feed efficiency for the 4 treatment groups were essentially identical throughout the 12-week feeding period (table 3). Measurements of backfat thickness closely paralleled the serum lipid levels, with the pigs fed ration D having significantly thicker backfat than those in any other treatment group ($P < 0.01$) and the control animals (ration A) having significantly less backfat than those in the other groups ($P < 0.01$). As noted with serum cholesterol the backfat of the pigs fed either 5% added fat or 5% pectin did not differ significantly from each other. Tanner (12) has reported a similar relation between serum cholesterol and physique in young men, finding a statistically significant relationship between choles-

TABLE 3

Influence on feeding citrus pectin and fat on body composition of swine

Variable	Ration				SE of mean
	A	B	C	D	
Body weight, kg					
Initial	23.6	23.6	23.6	23.6	± 0.50
Final	100.2	103.0	99.3	99.3	± 3.36
Average daily gain, kg	0.93	0.94	0.90	0.90	± 0.014
Feed conversion, kg feed/kg gain	3.54	3.46	3.76	3.55	—[1]
Backfat thickness, mm	31.24	34.80	37.08	39.37 **	± 0.940
Longissimus dorsi area, cm^2	23.35	22.45	22.84	20.13	± 1.360
Ham weight, kg	8.2	8.0	8.1	8.0	± 0.24
Dressing, %	72.9	74.3	73.6	74.4	± 0.57

** $P < 0.01$.
[1] Not applicable, pigs fed in groups of 5.

terol level in the blood and subscapular subcutaneous tissue thickness.

The *longissimus dorsi* area followed closely the pattern of backfat thickness, with the leanest animals (ration A) having the largest *longissimus dorsi* area and the fattest pigs (ration D) having the smallest. Although there was a 3.22-cm^2 difference in *longissimus dorsi* area between the control group and the pigs receiving ration D, the difference was not statistically significant.

Pigs receiving fat in the ration (rations B and D) had slightly lighter hams and a slightly higher percentage of dressing.

Taking into consideration the normal biological variation usually noted in a group of 20 animals such as those used in this study, it is unusual that so many parameters of lipid metabolism were so significantly altered by the treatments imposed. This is particularly unexpected when the marked effects of pectin feeding on lipid levels reported here are in a direction exactly opposite to those reported previously in man (1) and rats (2). It appears that although feeding pectin to swine increases the levels of serum and tissue lipids, the feeding of fat plus pectin results in a synergistic response much higher than noted with the feeding of either fat or pectin alone. The possibility that the differing calorie-protein ratios of the rations may have had some influence on the results of the experiment does not appear to be significant, because the 2 rations containing pectin (rations C and D), which were fed for the last 4 weeks of the experiment, actually had a lower energy-to-protein ratio than comparable rations with no added pectin. The pigs receiving these lower energy rations might have been expected to be leaner and have lower serum cholesterol levels than animals fed ration B but the opposite effect was noted. The validity of this explanation, however, rests on the assumption that pectin does not make an appreciable contribution to the energy content of rations C and D.

The results of this experiment indicate that there is a definite species difference in the effect of pectin on lipid metabolism. Wells and Ershoff (3) suggest that the "anticholesterol" effect of pectin may be confined to nonherbivorous species since the hamster, guinea pig and rabbit do not respond to pectin feeding by a lowering of serum or liver cholesterol levels. These workers reported that the cholesterol-fed rabbit actually exhibited slight but nonsignificant elevations in plasma and liver total cholesterol when fed 5% citrus pectin (3). Since the pig is an omnivore, it was thought that the response of lipid levels to the feeding of pectin, in this animal, would be similar to that observed in man; however, the rather unexpected results of this study have shown this theory to be without foundation.

For pectin to exert such a divergent action on lipid metabolism, depending upon the species involved, suggests the need for future investigations on the mechanism of action by which these species differences might be mediated.

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EFFECT OF PECTIN ON ATHEROSCLEROSIS IN THE CHOLESTEROL-FED RABBIT*

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(Revised, received June 15th, 1966)

SUMMARY

Californian male and female rabbits were fed for 11 months a diet containing 1 % cholesterol and 5 % of either pectin or cellulose. The male, pectin-fed rabbits had consistently lower blood cholesterol levels and a lower incidence of thoracic and abdominal atherosclerosis than did either the control animals or the females also receiving pectin. Both male and female rabbits on the pectin-supplemented diet excreted more lipid and sterol in the feces than did the control animals. There were no differences between dietary treatments in the severity of coronary lesions, but the female rabbits had more severe lesions than the male animals. Mortality was high between 6 and 11 months, but was somewhat lower on the pectin than on the control diet.

We have previously reported[1,2] a significant retardation of atherosclerosis by etary pectin in chickens fed either cholesterol-free or cholesterol-supplemented diets. hypocholesterolemic effect from the feeding of pectin has been observed under nditions of cholesterol feeding in the chicken, and in swine[3,4]. KEYS *et al.*[5] observed lecrease from pectin in serum cholesterol of human subjects on an average American et (supplying approximately 600 mg cholesterol per day); FISHER *et al.*[3] found ectin effective in lowering plasma cholesterol of man only when the diet contained 70 mg cholesterol (2 eggs per day). WELLS AND ERSHOFF[6] reported no effect from ectin feeding on plasma and liver cholesterol levels in the cholesterol fed rabbit and ggested that herbivores might not respond to pectin feeding in the same way as nivores. In our laboratory we have observed a significant hypocholesterolemic ect from pectin in cholesterol-fed rabbits[3], and have expanded this experimentation include a study of the effect of pectin on cholesterol-induced atherosclerosis.

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EXPERIMENTAL

Young Californian male and female rabbits, weighing approximately 2 kg each, were housed in individual cages which permitted collection of feces. The rabbits were fed for a period of 11 months on a cholesterol-supplemented (1 %) diet (Table I). One group of animals was given the diet with 5 % citrus pectin added; the control group received the same diet but in place of pectin 5 % cellulose was added. Food and water were available to the animals at all times. At periodic intervals, blood was taken from an ear vein for plasma cholesterol determination by methods previously described[8]. Feces were collected from each rabbit for total lipid and cholesterol determination after 2 months on the experimental diets. The feces were dried to constant weight in a forced-draft oven at 100°C and ground; samples were taken in triplicate for lipid extraction with chloroform–methanol (2 : 1). Lipid was determined gravimetrically by evaporating the solvent from the filtered extract. Sterols giving the ZLYKIS reaction were determined on an aliquot from the lipid extract[9].

At the termination of the experiment the rabbits were killed with chloroform and the heart and aorta removed to the iliac bifurcation. After cleaning the aorta from adhering tissue, it was graded separately for the thoracic and abdominal portions on the basis of scores from 1 to 3 with half-unit intervals. A score of 1 signified an essentially clean vessel, and 3 a severely affected vessel. Following visual grading, the aorta and heart were stored in formalin solution for histological examination. The histologi-

TABLE I

COMPOSITION OF RABBIT CHOW

Ingredient	*Amount (%)*
Ground corn	19.00
Ground oats	10.00
Ground barley	15.00
Wheat middlings	5.00
Soybean meal (50 % protein)	10.00
Corn gluten meal	2.50
Corn gluten feed	2.50
Alfalfa meal	15.00
Dried whey	1.25
Corn distillers solubles	2.50
Linseed meal	7.50
Calcium phosphate	0.75
Calcite flour	1.25
Trace mineral mix	0.10
Sodium chloride	0.40
Vitamin-antibiotic mix	0.25
Pelleting binder (Durabond)	1.00
Pectin[a] or cellulose[b]	5.00
Cholesterol	1.00

[a] Citrus Pectin, N. F. purchased from Sunkist Growers, Inc. Englewood Cliffs, N.J.
[b] Solka floc, Brown Co., New York, N.Y.

cal scoring was carried out according to procedures pre[illegible] grades from 1 to 4 were assigned, respectively, to height of plaque, [illegible] and foam cells, and degree of calcification. The degree of medial [illegible] was scored in the thoracic aorta where it was much more severe than in the abdominal aorta. The individual grades were summed up into a single score for each aorta.

Two cross sections of the heart were made, stained with Verhoeff Van Gieson and examined histologically. One of these sections was taken near the base of the left ventricle just below the insertion of the aortic valves and the other about 1 cm distal and parallel to the first section. All the extra and intramural branches of the coronary artery found in these sections were subjected to careful examination and assessment on an arbitrary scale (1 — 4) which took account of both the magnitude of the lesions and the number and size of vessels affected.

RESULTS

There were no significant differences in the body weights of the rabbits on the two treatments either at 6 months or at the end of the experimental period (Table II). With the exception of the pectin-fed males, the other animals all lost some weight between 6 and 11 months on the study. Up to 6 months mortality was generally low; between this time and the end of the experiment, however, mortality increased greatly with more rabbits succumbing on the control diet (46 %) than on the pectin diet (35 %, Table II).

TABLE II

BODY WEIGHTS AND PLASMA CHOLESTEROL VALUES OF RABBITS FED CHOLESTEROLEMIC DIETS WITH OR WITHOUT 5 % PECTIN

	Cellulose		*Pectin*	
	M	*F*	*M*	*F*
Number of animals				
1st day	11	13	12	8
6 months	9	10	11	8
11 months	6	7	7	6
Body weight (g)				
1st day	2243 ± 107[a]	2273 ± 109	2229 ± 109	2265 ± 151
6 months	3099 ± 100	3176 ± 101	3133 ± 128	3060 ± 35
11 months	2908 ± 150	3019 ± 192	3241 ± 137	2960 ± 103
Plasma cholesterol (mg/100 ml)				
1st day	51 ± 4	73 ± 6	62 ± 4	66 ± 7
3 months	450 ± 56	501 ± 44	250 ± 54	501 ± 62
6 months	891 ± 88	720 ± 73	553 ± 108	876 ± 70
11 months	734 ± 105	691 ± 58	582 ± 74	660 ± 47

[a] Mean value with its SE.

TABLE III

SEVERITY OF ATHEROSCLEROSIS IN RABBITS FED CHOLESTEROLEMIC DIETS WITH OR WITHOUT 5 % PECTIN

	Cellulose		*Pectin*	
	M	*F*	*M*	*F*
Thoracic aorta				
Macroscopic score	3.67 ± 0.15[a]	3.86 ± 0.15	2.43 ± 0.32	3.50 ± 0.18
Histological score	8.17 ± 1.25	7.43 ± 1.18	5.86 ± 1.13	8.17 ± 0.98
Abdominal aorta				
Macroscopic score	2.25 ± 0.25	2.93 ± 0.28	1.57 ± 0.13	2.67 ± 0.24
Histological score	3.67 ± 0.66	4.00 ± 0.65	2.57 ± 0.49	4.17 ± 0.61
Coronary histological score	2.00 ± 0.36	2.57 ± 0.22	2.00 ± 0.30	2.50 ± 0.22

[a] Mean value with its SE for the number of animals indicated at 11 months in Table II.

Plasma cholesterol values were generally significantly lower in the pectin-fed male rabbits compared with the male controls, but there were no essential differences between the control- and pectin-fed females. This sex difference was confirmed (Table III) by lower severity of atherosclerotic lesions in the thoracic and the abdominal aorta of the pectin-fed males, but not in the females, in comparison with the cellulose-fed control rabbits. The atherosclerotic scores for all females were significantly higher than those for the males. No differences due to treatment were observed in the coronary arteries, but the females again had more severe involvement than the males.

Table IV shows the daily food intake, fecal excretions and the composition of the feces from the pectin-fed and the cellulose-fed animals. In this instance the feces of the male and female rabbits on the pectin regimen both showed a reduced percentage

TABLE IV

FECES COMPOSITION FROM RABBITS FED CHOLESTEROLEMIC DIETS WITH OR WITHOUT 5 % PECTIN

	Cellulose		*Pectin*	
	M	*F*	*M*	*F*
Food intake (g/day)	131 ± 11[a]	124 ± 8	128 ± 7	117 ± 8
Fecal excretion (g wet weight/day)	185 ± 23	129 ± 12	178 ± 17	169 ± 8
Moisture (%)	51.1 ± 2.2[a]	50.0 ± 2.0	45.3 ± 2.3	37.9 ± 2.5
Fat (% dry basis)	6.42 ± 0.25	6.90 ± 0.31	8.01 ± 0.30	7.96 ± 0.46
Sterol (% dry basis)	2.30 ± 0.14	2.41 ± 0.07	2.92 ± 0.12	2.86 ± 0.06

[a] Mean value with its SE for the number of animals indicated at 1st day in Table II.

moisture content and an increased percentage lipid and sterol [illegible] basis, the differences in lipid and sterol content are even greater [illegible] basis.

DISCUSSION

This study extends the finding of a beneficial effect from pectin feeding to another species, although the effects appeared less dramatic than for the chicken. The increased fecal excretion of lipids, including sterol, suggests that pectin mediates its beneficial action on atherosclerosis by affecting the absorption of fat and cholesterol in the digestive tract. However, there was a sex difference in the response of California rabbits to pectin supplementation. While the males responded in a manner similar to cockerels in an earlier study[2] by having lower plasma cholesterol levels, less severe atherosclerotic involvement, and an increased fecal excretion of lipid and sterol, there was no such effect in females.

The effects from pectin in the rabbit observed in our laboratory differ from the observations of WELLS AND ERSHOFF[6] who reported no response to pectin feeding. It may well be that genetic differences among breeds of rabbits in susceptibility to atherosclerosis[7] might account for these contrasting results.

It is interesting to compare the patterns of atherosclerosis between rabbit and chicken. While the latter species shows considerable spontaneous atherosclerotic lesions on cholesterol-free diets which are seldom observed in the former species, the rabbit appears to be much more sensitive to cholesterol feeding than the chicken. The severity of lesions observed in the present experiment with 1% dietary cholesterol after 11 months, is not usually observed in chickens even with greater levels of cholesterol and over longer periods.

The high mortality observed between 6 and 11 months (Table II) is almost certainly related to the ingestion of dietary cholesterol. PRIOR *et al.*[11] have carefully studied hypercholesteremia in the rabbit and have concluded that cholesterol feeding beyond 5 months leads to "serious functional derangement", loss of appetite, and body weight. High mortality in rabbits fed cholesterol for prolonged time periods, has also been noted by others[12]. While the pectin-fed rabbits suffered fewer deaths than the controls, pectin clearly is only partially effective in ameliorating the harmful effects of a cholesterol-containing diet.

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THE RETARDATION BY PECTIN OF CHOLESTEROL-INDUCED ATHEROSCLEROSIS IN THE FOWL

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INTRODUCTION

WELLS AND ERSHOFF[1] and KEYS *et al.*[2] recently reviewed statistics obtained in population studies which suggest a possible beneficial effect on plasma cholesterol levels and heart disease in man from the ingestion of certain complex carbohydrates, such as pectin. These workers reported that purified pectin significantly reduced plasma cholesterol in man or rat when the diet of both contained cholesterol. These results might, at least in part, be explained on the basis of an earlier report by LIN *et al.*[3] which showed that dietary pectin increased both endogenous and exogenous cholesterol excretion in rats, thereby reducing its absorption.

More recently we reported[4] significant retardation of the development of aortic lesions following feeding of a diet containing purified citrus pectin without cholesterol supplementation to mature cockerels. We noted a marked reduction in the incidence and severity of spontaneous atherosclerosis but failed to observe a cholesterol-lowering effect by pectin on plasma or liver.

The present paper reports an investigation of the effects of dietary pectin on the induction of atherosclerosis in cockerels given a diet containing whole egg powder (as a source of cholesterol).

MATERIAL AND METHODS

Two groups of 39 Cornell random-bred Leghorn cockerels were raised from hatching on their respective rations for 24 weeks. They were individually weighed periodically, and food consumption was measured continuously on a group basis over the time intervals stated in Table II. Plasma cholesterol was determined at the end of the 24-week experiment for 20 individual birds, randomly selected from each of the two experimental groups, by methods previously described[5].

The basic experimental diet is given in Table I. The atherogenic substance was whole egg powder, which, in the amount provided in the diet, supplied approximately

TABLE I

COMPOSITION OF BASIC DIET

Ingredients	*Amount (%)*
Whole egg powder*	25.0
Soybean meal (50 % protein)	25.0
Fiber**	3.0
Dicalcium phosphate	2.0
Limestone-trace mineral mix	1.0
Sodium chloride	0.5
Choline chloride (70 % concentrate)	0.2
Vitamins***	0.2
Glucose monohydrate	to 100.0

* Protein (N × 6.25), 42 %; fat, 50 %, cholesterol, 2.6 %.
** Solka Floc, Brown Company, New York, N.Y.
*** For composition see SUMMERS AND FISHER[11].

0.5 % cholesterol. The experimental variables were non-nutritive fiber (Solka Floc, Brown and Co., New York) and citrus pectin (N.F. grade, Sunkist Growers, Inc., Englewood Cliffs). These were added to make 5 % of the final diet for the first 7 weeks of the experiment and 3 % thereafter as it was found that the pectin-fed cockerels grew at a much slower rate than the controls.

Twice during the experimental period when the birds were 7 and 24 weeks old, excreta were collected from the two groups for determination of extractable lipid and cholesterol. At the first collection (7 weeks) one pooled sample was analyzed for each experimental group. Lipid was extracted with chloroform methanol (2 : 1); after repeated extraction followed by filtration, lipid was measured as the residue

TABLE II

BODY WEIGHT AND FOOD CONSUMPTION OF CONTROL AND PECTIN-FED COCKERELS

Measurement	*Diet supplement*	
	fiber	*pectin*
Number of birds		
start	39	38
end	34	29
Body weight (g)		
4 weeks	312 ± 9*	190 ± 7
12 weeks	1395 ± 29	1088 ± 31
19 weeks	1910 ± 48	1881 ± 35
24 weeks (final)	1936 ± 45	1939 ± 35
Food consumption (g/bird/day)		
first 4 weeks	19	15
next 8 weeks	67	66
next 9 weeks	84	100
final 3 weeks	92	102

* Mean value with its standard error.

after evaporation of solvent. Cholesterol was determined on a portion of the lipid residue redissolved in chloroform methanol by the method used for the plasma cholesterol determination[5].

Before the end of the experiment the food passage-time was determined in five cocks from each experimental regimen. They were starved overnight, to insure a greater degree of uniformity in the amount of food present in the digestive tract, and then 10 g of feed, in the form of a slurry to which carmine had been added to act as a marker, were introduced into the crop through a funnel. The elapse of time between the administration of feed and the first appearance of carmine in the excreta was considered the food passage-time. First (trial 1), the pectin-fed group received marked pectin diet and the fiber-fed group marked fiber diet. Subsequently about a week later (trial 2), the test diets were reversed for the same birds, that is to say the pectin-fed group received marked fiber diet and the fiber-fed group marked pectin diet.

At the end of the experiment the birds were killed with chloroform and the livers removed, weighed, homogenized, and extracted for lipid. Total lipid and cholesterol were determined as for the excreta. The heart and aorta were also removed. The aorta was macroscopically graded on a blind basis as described before[1]. After the macroscopic grading the aorta and heart were placed in formalin solution for subsequent histological examination by methods also described previously[9]. In describing "spontaneous lesions" we refer primarily to intimal proliferation of the abdominal aorta which tends to encroach upon the lumen of the vessel. This type of proliferation, although fibrotic, contains considerable lipid deposition and is, therefore, "atherosclerotic".

RESULTS

Body weights and feed consumption. The pectin-fed cocks grew at a slower rate than the fiber-fed (control) birds (Table II). The former group, however, reached the same body weight as the controls by the end of the 24-week experimental period. During the second half of the experimental period the pectin-fed cocks consumed more food than the controls. But even during the early weeks of the experiment when the absolute daily food intake was essentially the same for the two groups, when this intake was related to body weight, it was considerably greater for the pectin-fed birds.

Plasma and liver cholesterol. The pectin-fed cocks had significantly lower plasma cholesterol levels than the controls (Table III). The total liver-lipid and liver cholesterol was not significantly different in the two groups.

Lipid and cholesterol excretion. During the first analysis of excreta (at 7 weeks) the pectin-fed birds excreted almost three times as much lipid material and twice as much cholesterol as did the controls (Table IV). In the analyses made towards the end of the experiment (at 24 weeks), significant differences were again noted in both lipid and cholesterol excretion, but the differences were smaller.

Atherosclerosis. Table III summarizes the macroscopic and histological findings on coronary and aortic atherosclerosis. Judged on both macroscopic and histological

TABLE III

INCIDENCE OF ATHEROSCLEROSIS AND PLASMA AND LIVER CHOLESTEROL [illegible] FED COCKERELS

Measurement	*Diet supplement*	
	fiber	*pectin*
Cholesterol		
plasma (mg/100 ml)	220 ± 11	170 ± 11
liver (% of fresh weight)	3.29 ± 0.30	2.75 ± 0.24
Liver lipid (% of fresh weight)	8.93 ± 0.50	8.15 ± 0.32
Abdominal aorta		
macroscopic score (mean)[1]	1.71 ± 0.09	1.36 ± 0.07
frequency of severe scores[2]	13/34	6/29
Histological grading[1]		
diffuse intimal proliferation (mean)	0.53	0.48
localised fibrous plaques (mean)	1.79 ± 0.16	1.52 ± 0.14
frequency of severe scores[2]	19/34	15/29
media (mean)	1.62	0.72
frequency of severe scores[2]	9/34	0/29
Thoracic aorta		
mean histological score[1]	0.65	0.28
frequency of severe histological scores[2]	6/34	1/29
Coronary arteries		
mean histological score[1]	0.62	0.07
frequency of severe histological scores[2]	3/34	0/29

[1] Scoring was based on a 1.0–3.0 scale with 1.0 indicating virtually no lesions and 3.0 very severe ones.
[2] Severe scores were those with grades 2.0 or above.

gradings, abdominal aortic atherosclerosis showed a lower incidence in the pectin fed birds than in the controls. It is noteworthy that the major differences between the

TABLE IV

LIPID AND CHOLESTEROL IN EXCRETA OF CONTROL AND PECTIN-FED COCKERELS

Excreta measurement	*Diet supplement*	
	fiber (% *dry excreta*)	*pectin* (% *dry excreta*)
At 7 weeks[1]		
extractable lipid	4.87[3]	14.52
cholesterol	0.87	1.56
At 24 weeks[2]		
extractable lipid	6.86 ± 0.47[4]	9.05 ± 0.49
cholesterol	0.61 ± 0.04	0.79 ± 0.05

[1] Up to this time the test diet provided 5 % pectin.
[2] From 7 weeks to the end of the experiment the test diet provided 3 % pectin.
[3] Mean value for excreta composited from 10 birds per group.
[4] Mean value with its standard error for 3 separate analyses of excreta composited from 10 birds per group.

two groups occurred in the medial involvement, with little difference in the localized fibrous plaques and diffuse type of intimal proliferation. In the thoracic aorta, lesions, when observed, occurred mainly in the control animals; thoracic lesions are not typical of the spontaneous disease in fowls. Coronary atherosclerosis was not seen in any of the pectin-fed cocks; there was a low incidence among the control birds.

Food passage-time. Table V gives the results of studying the passage of carmine-marked food through the digestive tract. The food passage time was shorter in birds

TABLE V

FOOD PASSAGE TIME THROUGH THE DIGESTIVE TRACT OF BIRDS GIVEN PECTIN OR FIBER SUPPLEMENTED DIETS

Diet supplement during 24 weeks	*Trial 1*		*Trial 2*	
	marked diet	*time (min)*	*marked diet*	*time (min)*
Pectin	Pectin	127 ± 9*	Fiber	125 ± [illegible]
Fiber	Fiber	170 ± 19	Pectin	107 ± 28

* Mean value with its standard error for 5 cocks in each group.

fed continuously on pectin than in those fed the fiber supplemented diets. In the pectin-fed group the passage time was the same, however, when either the fiber or the pectin test diets were given at 24 weeks. The change in diet from fiber to pectin supplementation produced the most marked difference in the food passage time in birds which had been fed the fiber-supplemented diet for 24 weeks.

DISCUSSION

In the present study, day-old cocks were given an atherogenic egg powder diet supplying 0.5 % cholesterol. This experiment leads to similar conclusions of a beneficial effect from pectin as observed in our earlier investigation on the use of pectin in relation to spontaneous avian atherosclerosis[4]. As in the earlier study, food consumption was greater in the pectin-fed birds. When related to body weight, the higher intake by the pectin-fed cocks suggests that pectin acts by decreasing the utilization of food. This suggestion is supported by the data on the amount of lipid and cholesterol excreted as well as by the food passage-times. The latter may be the important factor. The fact that less lipid was excreted after 24 than after 7 weeks is probably due to the pectin-content of the diet having been lowered from 5 % to 3 % after the first 7 weeks of the experiment.

While the present results on cholesterol-induced atherosclerosis suggest that the major benefits of pectin might have been the reduced absorption of lipid and cholesterol, it should be recalled[4] that at least part of the effectiveness of pectin may also be due to a reduction in total calories absorbed. Earlier we showed[10] that

a restricted food-intake significantly retarded avian atherosclerosis [illegible] was low in fat and devoid of cholesterol.

The coronary lesions, as well as those of the thoracic aorta found [illegible] study were typical for cholesterol-induced atherosclerosis and are usually not observed in spontaneous lesions[9]. The small number of thoracic lesions for induced atherosclerosis observed in this study may be typical of the strain of birds used, since we have made similar observations in past studies[6,7]. It is also possible that the relatively high level of protein in the diet was protective in this regard[8]. That no coronary lesions were seen in the pectin-fed birds which also had only a minimal amount of thoracic atherosclerosis, suggests that the greater excretory elimination of cholesterol by the pectin-fed birds played a major role in the retardation or prevention of these lesions. Relatively little diffuse intimal proliferation of the abdominal aorta was found in the present experiment. This was probably because the birds were young, in the present instance, just under six months of age. Considerable diffuse intimal proliferation was previously observed[1] in adult cocks over 3 years of age.

Our results confirm, to a large degree, the report by LIN *et al.*[3] who showed that dietary pectin increased excretion of cholesterol in rats. Both sets of findings with rats and cockerels are at variance with the preliminary report of WELLS AND ERSHOFF[1] who claimed no effect from pectin on cholesterol excretion in cholesterol-fed rats, despite a marked cholesterol-lowering effect by pectin on blood and liver. No obvious resolution of this difference is apparent, except that the rats used by WELLS AND ERSHOFF were considerably younger than those used by LIN *et al.*

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SUMMARY

Day-old Leghorn cockerels were given a diet containing egg powder and supplemented either with pectin or non-nutritive fiber. After 24 weeks on the diets, the pectin-fed birds had significantly lower blood levels of plasma cholesterol, eliminated more lipid and cholesterol in their excreta, and had a significantly lower incidence and severity of atherosclerosis as measured macroscopically and histologically. Using carmine as a marker, food was observed to pass faster through the alimentary tract of the pectin-fed birds. Throughout the study, the pectin-fed birds consumed more feed than the controls, despite the fact that the former grew more slowly during the early stages of development than the latter. The results suggested

that pectin may be effective in reducing the incidence and severity of both spontaneous and cholesterol-induced avian atherosclerosis. The present study suggests that pectin acts by accelerating the transit of food through the alimentary tract resulting in an increased excretion of possible atherogenic substances, as well as of total nutrients (calories).

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Dietary Pectin and Blood Cholesterol [1]

Fausch and Anderson recently suggested species differences in the blood cholesterol response of pectin-fed animals (1). Feeding swine an essentially cholesterol-free diet, these workers observed that pectin produced an elevation in serum cholesterol. These observations seemed inconsistent with the hypocholesterolemic effects of pectin reported for man (2) and for the rat (3). Fausch and Anderson also cite work suggesting that only non-herbivorous animals respond to pectin with lowered blood cholesterol.

Based upon our experience with three species — man, chicken and rabbit — we should like to offer a different interpretation for the observations of Fausch and Anderson: pectin produces a lowering of blood cholesterol only in the presence of dietary cholesterol. The following results are presented in support of this hypothesis:

Species	Dietary cholesterol	Plasma cholesterol	
		Pectin diet	Control diet
		mg/100 ml	*mg/100 ml*
Man	−	165 ± 9	167 ± 6
	+	157 ± 15	191 ± 10
Chicken	−	111 ± 6	96 ± 4
	+	170 ± 10	220 ± 11
Rabbit	+	345 ± 50	478 ± 35

The pectin response of chickens fed a cholesterol-free ration (4) was very similar to that reported by Fausch and Anderson for swine; however cholesterol feeding reversed the response. In our experiments with humans, dietary cholesterol exceeded twice the amount ingested by the subjects studied by Keys et al. (2); concomitantly we observed a correspondingly greater hypocholesterolemic effect from pectin. Finally, we also obtained a lowering of blood cholesterol in an herbivorous species when we fed pectin to rabbits.

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Avian Atherosclerosis: Retardation by Pectin

Abstract. *A highly significant retardation of spontaneous atherosclerosis was observed in 2-year-old cockerels fed on a standard diet supplemented with 5 percent pectin for 18 months. The pectin-fed birds excreted three times as much lipid extract and almost twice as much cholesterol as did the control cockerels fed the standard diet supplemented with 5 percent nonnutritive fiber.*

For a prolonged period animal species (fowl) highly susceptible to spontaneous atherosclerosis was fed with a mixed human-type diet relatively rich in pectin-containing foodstuffs, and a [illegible] ment of the disease was noted [illegible]. Because of the complexity of the diet used previously and the uncertainty as to the nature of the effective substance responsible for the retardation of atherogenesis, we conducted an experiment in which pectin (National Formulary grade) was used as the only variable in the diet.

Two groups of 2-year-old cockerels were used, with [illegible] birds in each group. Both groups were fed on a corn-soybean ration containing 15 percent protein, to which was added 5 percent

Table 1. Effect of long-term pectin consumption on changes in body weight, food intake, cholesterol content of plasma and liver, and severity of atherosclerosis in adult cockerels fed on a diet containing nonnutritive fiber (cellulose) or pectin.

Measurements	Diet supplement	
	Cellulose	Pectin
Body weight (g) at start	2327 ± 49*	2240 ± 58
After 18 months	2511 ± 50	2317 ± 52
Food consumption (g/day)	118 ± 5	124 ± 4
After 18 months		
Plasma cholesterol (mg/100 ml)	96 ± 4	111 ± 6
Liver cholesterol (mg/g wet wt)	5.19 ± 1.3	5.87 ± .20
Abdominal aorta:		
Macroscopic score	2.04 ± .12	1.39 ± .08
Severe scores†	58	20
Weight-area ratio	53.3 ± 1.4	50.7 ± 1.5
Histological grading:		
Intima†	32	21
Localized fibrous plaques†	68	45
Media†	26	8

* Mean ± standard error for 24 and 20 birds surviving on the nonnutritive fiber and pectin supplemented diets, respectively. † Frequency of severe scores (%); severe scores were those with grade 2.0 or above; scoring was based on a 1 to 3 scale with 1 indicating essentially no lesions and 3 very severe plaques.

pectin (first group) or 5 percent nonnutritive fiber (second group). The animals, kept in individual cages, had continuous access to food and water and were maintained on these diets for 18 months. Body weights and food consumption were recorded at monthly intervals. Toward the end of the experimental period, blood was taken from a wing vein of each bird for determination of the cholesterol content of the plasma (2).

At the end of the 18-month period the surviving birds were killed: livers were removed for cholesterol determination, and aortas removed from the heart to the iliac bifurcation. After careful cleaning, each aorta was visually scored on a scale from 1 to 3 with 0.5-unit intervals in which a value of 1 indicated an essentially clean blood vessel, and a value of 3 represented an aorta practically occluded by plaques. After the macroscopic scoring, the aortas were weighed and the area was determined by planimetry to obtain the weight-area ratio (*3*). They were then examined histologically by techniques described previously (*4*), on the basis of (i) diffuse intimal proliferation, (ii) localized fibrous plaque formation, and (iii) medial lesions. Within each of these characterizations, lesions were graded as to severity on a 1 to 3 scale.

The pectin-fed birds had significantly ($p < .001$) fewer atherosclerotic plaques in the abdominal aorta than the control birds, measured both macroscopically and histologically (Table 1). Only the weight-area ratio did not show a significant difference, suggesting that this measurement is more a reflection of the diffuse intimal proliferation than of the localized plaque of which the macroscopic score appears to be more representative. In addition to the aortic measurements given in Table 1, three aortas from control birds only showed calcium deposition in localized fibrous plaques. No hypocholesterolemic action could be ascribed to the pectin feeding, in contrast to reports for the rat and for man (*5*). Indeed, the pectin-fed animals had significantly higher concentrations of cholesterol in the plasma and liver.

The birds fed with pectin consumed slightly more food throughout the experiment than did the control birds fed with cellulose (Table 1). The control birds, however, gained almost three times as much weight as did the pectin-fed birds, indicating that pectin reduced the utilization of nutrients.

Table 2 shows the results of a separate study in which analyses were made of the excreta of 10-week-old chickens fed pectin or cellulose in diets which contained 10 percent fat and 0.5 percent cholesterol. There was no difference in the moisture content of the excreta, although the excreta from the birds fed pectin gave the appearance of being diarrhetic. However, the pectin-fed birds lost three times as much lipid and almost twice as much cholesterol per gram of excreta as did the control birds.

These results confirm our previous suggestion (*1*) that pectin may be efficacious in retarding spontaneous avian atherogenesis. The excreta analyses, in conjunction with the food intake and patterns of body weight, suggest two possible modes of action for pectin: it might accelerate food passage or interfere with the digestive processes so that less nutrients become available for absorption; or it might specifically interfere with the absorption or reabsorption of atherogenic substances (cholesterol). With regard to the first possibility we have recently shown that a restricted pattern of food intake was beneficial in retarding avian atherosclerosis (*6*). The higher plasma and liver cholesterol values of the pectin-fed birds make the second possibility appear unlikely.

Table 2. Moisture, total lipid, and cholesterol content of excreta from chickens fed pectin or nonnutritive fiber (cellulose).

Excreta measurement	Diet supplement	
	Cellulose	Pectin
Moisture (%)	73.1	73.5
Extractable lipid (mg/g dry excreta)	48.7	145.2
Cholesterol (mg/g dry excreta)	8.7	15.6

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DIETARY PECTIN AND PLASMA CHOLESTEROL IN SWINE

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FAUSCH AND ANDERSON[1] have reported that pectin-fed swine had significantly higher blood cholesterol levels than control animals. Since their observations seemed to contradict the hypocholesteremic effects of pectin shown in man[2] and in the rat, these workers suggested a species difference in response to pectin feeding. We have offered[4] an alternative explanation, based on studies with man and chickens, to the effect that "pectin produces a lowering of blood cholesterol only in the presence of dietary cholesterol". The results of the following trials corroborate our suggestion.

MATERIAL AND METHODS

Four groups of either six 8-week old Yorkshire pigs, three of each sex, or four 16-week old male Hampshire pigs were assigned on the basis of body weight to the dietary regimens. The four groups received either 5% pectin or 5% cellulose in the presence or absence of dietary cholesterol (in the form of egg powder). The composition of the basic diet was as follows (in percentages): corn meal, 55.95; soybean meal (50% protein), 10.0 or 30.0; whole egg powder, 0 or 20.0; alfalfa, 3.0, dicalcium phosphate, 2.0; limestone with trace minerals, 1.0; corn distillers solubles, 2.5; sodium chloride, 0.5; vitamin mix, 0.05.

During the 4-week experiment, food intake was measured on a group basis while body weights and plasma cholesterol determinations[5] were recorded on an individual basis. Since the results for both trials were similar, they were pooled for presentation.

RESULTS

Table I shows that pectin did not influence body weight or plasma cholesterol level of swine on the cholesterol-free diet. Cholesterol feeding raised the plasma chol-

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TABLE I

INFLUENCE OF PECTIN FEEDING ON BODY WEIGHT AND PLASMA CHOLESTEROL [illegible]

Diet	*Final body weight (kg)*[1]	*Plasma cholesterol (mg/100 ml)*[2]
Cholesterol-free		
cellulose[3], 5%	49.1	108 ± 4
pectin[4], 5%	48.4	102 ± 5
Cholesterol-containing[5]		
cellulose, 5%	47.8	162 ± 10
pectin, 5%	47.4	133 ± 7

[1] Ten pigs per group.
[2] Mean value ± standard error for 14 determinations per group; in one trial the six pigs were bled once only; in the other, the 4 animals were bled twice.
[3] Solka floc, Brown Co., Berlin, N. H.
[4] Pectin N. F., Sunkist Growers, Inc., Englewood Cliffs, N. J.
[5] Approximately 0.5%.

esterol level and under these conditions pectin exerted a significant ($P < 0.05$) hypocholesteremic action. There was no difference in body weight between the pectin and cellulose-fed animals receiving dietary cholesterol. However, the cholesterol-fed pigs were somewhat lighter in weight than those not given cholesterol, possibly due to the higher calorie:protein ratio resulting from the lipid content (50%) of the whole egg powder.

DISCUSSION

These results support our suggestion[4] that pectin lowers blood cholesterol only when there is an exogenous source of cholesterol. Under such conditions we have shown an increased excretion of cholesterol by the pectin-fed chicken relative to its control[6]. In the absence of dietary cholesterol, pectin has either no effect on plasma cholesterol[4] or may produce an elevation[1,6] as a result of increased mobilization of body lipids in the energy economy of the pectin-fed animal.

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Utilization of the Energy in Pectins by Monogastric Animals

by D. Gädeken

Working in the Institute for Animal Nutrition Research at the University of Kiel I have, over the year just passed, conducted respiration studies with rats in order to determine the net energy yield of pectins.

Pectins are members of the carbohydrate family; according to Woender's studies they belong among the N-free extract substances. They are high molecular compounds, with molecular weights which can exceed 100,000. Their basic building components are d-galacturonic acid monomers which are bound by glycosidic linkages and are partly esterized with methyl alcohol. The various pectins differ from each other in the length of their chains and in degree of esterization.

Almost all plant substance contains pectins, in varying proportions. Chiefly pectins are found in roots, tubers and fruits but they are also present in seeds, stalks and leaves. The highest pectin content -- at 10 - 30 percent of the dry substance -- is found in turnips and turnip products, in apples, and in citrus fruit. Up to 10 percent may be found in the different grasses; the content is 1 percent in grain. Because of the pectin content present in various plant substances the animal, under regulated feeding conditions, can be furnished with a considerable amount of pectin in his diet.

Earlier studies have demonstrated that pectins are broken down up to 90 percent in the digestive tract, a breakdown which we are sure resembles that for cellulose in that it results from the activity of microorganisms in the rumen or in the distal gut. No one has successfully demonstrated pectin-reducing enzymes in the gastric juices of man or animals. The breakdown of pectin apparently occurs through action on the galacturonic acid, which is partly resorbed, partly passed further along in the intestine. It can be demonstrated that the organism is capable of resorbing certain amounts of galacturonic acid -- with the small intestine acting as the chief organ of resorption. Along with the galacturonic acids, other breakdown products such as acetic acid, butyric acid, formic acid, and CO_2 are formed. Since, with monogastric animals, the breakdown of pectins takes place in large degree in the distal gut, the resorption capabilities of such animals is limited. With ruminants the behavior differs. Here a breakdown into products capable of resorption has already taken place in the rumen; propionic acid, acetic acid, and butyric acid can be demonstrated there, along with products of the disintegration of other carbohydrates. A relatively satisfactory utilization of pectins is possible in ruminant digestion.

In connection with my studies of the entire metabolic process I applied a method of differential analysis to an evaluation of the net energy yield -- and therefore the u tilization -- of pure pectins in 8 adult, male, Wistar rats. I further studied whether different proportions of pectins in the ration affected the value of the net energy in any fashion. I added pectins in varying amounts to a constant basic ration, achieving pectin contents of 12 percent and 24 percent of the ration. I conducted respiration studies by using Haldane's gravimetric measurement of gaseous exchange in the resting animal. From these results, the different chemical balances were calculated and energy estimates were made.

As the diet additive I used a highly esterized preparation derived from apple pectin which may be obtained on the market under the trade name "Pectin for the Pharmacy" and which is directly available from the manufacturer Dr. Theodor Schuchardt, Munich.

The following findings cite average values for the 8 individual test animals.

The high digestibility of pectins frequently cited in the literature was also established in these studies. Digestion coefficients of over 90 were found, depending on the respective pectin content of the ration. The apparent digestibility in the 12 percent ration was 97.1 percent and in the 24 pe rcent ration 93.4 percent. Corresponding values for apparent digestible energy were similarly high -- at 95.8 and 92.1 percent respectively. For both rations, two series of studies yielded a 95 percent statistical agreement. Since, in monogastric animals, pectins are broken down by microorganisms in the distal gut, the apparent digestion coefficients do not reveal what proportion of the breakdown products are actually resorbed. The significantly higher values for the pectins in the 12 percent ration permit us only to assume a relatively more intense activity of the microorganisms in the intestine. It is questionable whether this more intense activity enables the animal to make use of a greater proportion of the pectin. There is the possibility that, though the intense bacterial activity may lead to a breakdown of a greater proportion of the pectin, it may also mean that a larger proportion of the breakdown products are so far disintegrated that the animal can make no more use of them.

The use of pure pectin as an additive had the effect of depressing digestion -- a fact frequently ignored in studies which require the feeding of pure foodstuffs. Measurements of the nitrogen balance revealed for all tests a certain retention of nitrogen. This retention was more pronounced with the pectin-augmented rations than it was with rations which were pectin free. To be sure, this heightened retention was significant only with the 24 percent pectin.

Pectin, administered as a pure N-free foodstuff, had the same effect on N and C and on the energy content in the intestine as had other N-free foodstuffs fed in pure form (such as sugar and starch). These other foodstuffs also reduced the separation of N and C and diminished the energy content in the intestine.

Measurements of the carbon balance established that the addition of pectin altered only to a minor degree the relatively meagre separation of C out of the basic diet. With the 12 percent ration the C-separation was diminished slightly. The administration of a 24 percent pectin ration led to a heightened retention of carbon . However, this still amounted to only 7 percent of the amount of carbon served daily to the animals. In comparing values between pectin-augmented rations and pectin-free rations, however, one must consider the greater live weight of the animal when pectin-augmented ration was administered and the consequent increase in subsistence needs. When I adjusted for this factor, a heightened retention was still apparent.

Since only a minor proportion of the carbon was released, the disposition of the remainder of the carbon became a matter of interest. The apparent high digestibility of the pectin and the reduced separations of carbon that resulted in the intestine when pectin-augmented rations were fed leads us to the possible assumption that the diminished release of carbon indicates that the pectin is indeed further broken down in the intestine but that only a few of its breakdown products can be resorbed. The conjecture-that the intestinal flora break the pectins down not only into galacturonic acids or other organic acids capable of resorption but also that, in the greater part, they are completely broken down into CO_2 and water - was confirmed by the measurement of gaseous exchange, for the most substantial portion of the carbon administered in pectin-augmented rations could again be rediscovered in the form of CO_2. In the 12 percent pectin diet this proportion amounted to 86.0 percent. This compared to 74.9 percent in the higher pectin diet. Of course, the reason for the difference cannot be directly determined, but the calculated P-value (at 6 percent) lay just over the 5 percent limit, so that one might well trace the difference in CO_2 production back to the differing content of pectin in the rations.

In monogastric animals, therefore, the bacterial breakdown of pectins does not end with galacturonic acids or other similar products. Rather, the most substantial portion of the pectins are completely broken down into CO_2 and water. The high CO_2 production is good evidence for the viable breakdown of pectins by the intestinal flora. At that level, the breakdown activity is so intense that the animal

organism cannot resorb any large amount of the possible intermediate products of the breakdown. The clear difference in CO_2 production noted for the varying content of pectin in the rations is interesting for, according to the findings, complete pectin breakdown is proportionately smaller the higher the content in pectin. Possibly, when increased amounts of pectin are broken down, the resulting increase in the formation of galacturonic and other organic acids creates a pH shift in the intestine, which in turn inhibits further activity by the microorganisms. Because of this, the animal is able to resorb a greater proportion of the possible intermediate products of the breakdown.

Table 1

Energy Data for the Pectin Augments

Pectin content of the ration per g pure foodstuff		12%	24%
Gross energy	cal	3708	3708
Digestible energy	cal	3553	3415
Metabolizable energy	cal	3603	3479
Net energy	cal	208	751
Utilization coefficient in % of the gross energy		5.6%	20.3%

Energy utilization is shown in Table 1. The digestible energy of the pure pectin amounts per gram to 3553 calories or 95.8 percent of the gross energy for the 12 percent ration, and 3415 calories for the 24 percent ration. Since the addition of pectins to the diet led to a reduced liberation of energy in the intestine, the values for the energy available to metabolism were somewhat higher. All these values must be taken with reservations, however, for the digestibility ascertained by test findings appreciably exceeded the true digestibility -- probably by several fold. That is, values for metabolizable energy obtained according to customary definition contained a certain amount of energy which in reality never passed the intestinal wall. Thus we can make no judgement at all about that portion of the breakdown products of the pectin which were actually resorbed.

When adjustments were made to allow for digestive depressions and for variations in live body weight the result was a net energy yield per g of foodstuff of only 208 calories for the 12 percent pectin ration, a utilization coefficient of only 5.6 percent of the gross energy. With the 24 percent ration a better utilization

was observed. Here, per gram of feed, the net energy amounted to 751 calories, or a utilization coefficient of 20.3 percent of the gross energy.

As the foregoing findings indicate, the values for digestible energy and for metabolizable energy, which normally serve as valid reference measures, are less well suited as reference measures for the pectins.

Substantial individual variations existed among the different test animals; the average error in utilization coefficients and in the values for the various chemical balances support this. However, the differences between animals were demonstrated to be statistically non-significant in both series of tests. Statistical probability measures, as well as evidence from the varying CO_2 productions learned from the evaluation of gaseous exchange, support the influential role in the production of the net energy played by the content of pectin in the ration.

Summary

With utilization coefficients of 20 percent and under, pure pectin is only poorly utilized by monogastric animals. In all probability, this also applies to the easily soluble pectins in natural diets. Thus, uncritical calculations for N-free extract substances could yield spuriously high results for diets high in pectin. To obtain the exact biological value for such feeds, the pectin content should be known, particularly for monogastric animals.

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Über die energetische Verwertung von Pektinstoffen bei Monogastriden

Von D. GÄDEKEN*)

Im Institut für Tierernährungslehre der Universität Kiel habe ich im letzten Jahr im Rahmen von Untersuchungen über den Nährwert der Pektinstoffe Respirationsversuche mit Ratten durchgeführt, um die Nettoenergie der Pektine zu bestimmen.

Die Pektine sind eine zu den Kohlenhydraten gehörende Stoffgruppe, die im WLENDER Analysengang innerhalb der N-freien Extraktstoffe erfaßt werden. Es sind hochpolymere Verbindung mit Molekulargewichten, die 100 000 übersteigen können. Grundbausteine sind D-Galakturonsäurereste, die durch glykosidische Bindung verbunden und teilweise mit Methylalkohol verestert sind. Die verschiedenen Pektinstoffe unterscheiden sich in der Kettenlänge und im Veresterungsgrad.

Fast alle Pflanzenmaterialien enthalten Pektinstoffe in unterschiedlichen Anteilen, und zwar hauptsächlich in Wurzeln, Knollen und Früchten, aber auch in Samen, Stengeln und Blättern. Die höchsten Pektingehalte mit 10--30 % in der Trockensubstanz finden wir in den Rüben und Rübenprodukten, Äpfeln, Apfel- und Citrustrestern. In den verschiedenen Weidegräsern und Kleearten werden teilweise bis zu 10 % Pektin gefunden, während in den Getreidekörnern die Gehalte um 1 % liegen. Durch die unterschiedlichen Pektinanteile in den verschiedenen Pflanzenmaterialien können den Tieren unter gewissen Fütterungsbedingungen teilweise recht beträchtliche Mengen Pektin verabreicht werden.

Aus der Literatur geht hervor, daß die Pektinstoffe im Verdauungstrakt bis zu 96 % abgebaut werden, wobei dieser Abbau ähnlich wie bei der Cellulose mit Sicherheit durch die Tätigkeit der Mikroorganismen im Pansen bzw. im Dickdarm stattfindet, da es nicht gelungen ist, in den Verdauungssäften von Mensch und Tier pektinspaltende Fermente nachzuweisen. Der Pektinabbau verläuft offensichtlich über Galakturonsäure, die teils resorbiert, teils weiter im Darm umgesetzt wird, wobei es erwiesen ist, daß der Organismus befähigt ist, zumindest im Dünndarm als dem Hauptresorptionsorgan gewisse Mengen Galakturonsäure zu resorbieren. Neben der Galakturonsäure werden andere Spaltprodukte wie Essigsäure, Buttersäure, Ameisensäure und CO_2 gebildet! Da bekanntlich der Pektinabbau bei monogastrischen Tieren erst in den distalen Darmabschnitten in großem Umfange einsetzt, kann die Möglichkeit der Galakturonsäureresorption im Dünndarm kaum genutzt werden. Anders liegen die Verhältnisse bei den Wiederkäuern. Bei diesen Tieren findet ein Abbau zu resorptionsfähigen Produkten bereits im Pansen statt, wobei nachweislich auch Essigsäure, Propionsäure und Buttersäure gebildet werden können, also Produkte, die bei der Zersetzung anderer Kohlenhydrate ebenfalls entstehen. Eine relativ günstige Verwertung des Pektins bei der Ernährung der Wiederkäuer ist daher anzunehmen.

Anhand von Gesamtstoffwechseluntersuchungen habe ich nun die Nettoenergie und damit die Verwertung von reinem Pektin mit Hilfe der Methode des Differenzversuches bei 8 ausgewachsenen männlichen Wistar-Ratten ermittelt. Weiter habe ich untersucht, ob unterschiedliche Pektingehalte in der Ration den Nettoenergiewert in irgendeiner Weise beeinflussen. Zu diesem Zweck wurden die Pektinzulagen zu einem gleichbleibenden Grundfutter in unterschiedlichen Mengen gegeben, entsprechend einem Gesamtpektingehalt der Ration von 12 % bzw. 24 %. Die Respirationsversuche wurden nach dem auf der gravimetrischen Messung des Gaswechsels beruhenden Verfahren von HALDANE durchgeführt. Die Ansatzwirkung der Versuchsrationen wurde über die energetisch ausgedrückte stoffliche Bilanz berechnet.

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ausscheidung im Harn dürften unter Berücksichtigung des geringen Kohlenstoffansatzes ein Hinweis dafür sein, daß das Pektin zwar im Darm weitgehend abgebaut wird, daß von den Spaltprodukten jedoch nur wenig resorbiert werden kann. Die Vermutung, daß die Darmflora des Pektins nicht nur zu Galakturonsäure spaltet oder andere resorbierbare organische Säuren bildet, sondern daß größtenteils ein vollständiger Abbau zu Kohlendioxyd und entsprechend auch zu Wasser stattfindet, wurde durch die Messung des Gaswechsels bestätigt, denn der überwiegende Teil des Kohlenstoffs der Pektinzulage konnte in Form von CO_2 wiedergefunden werden. Dieser Anteil betrug bei der 12%igen Pektinzulage 89,9% gegenüber 74,9% bei der höheren Pektinzulage. Diese Differenz ließ sich zwar nicht absichern, aber der errechnete P-Wert liegt mit 6% nur eben über der 5% Grenze, so daß man wohl den Unterschied in der CO_2-Produktion auf den verschiedenen Pektingehalt der Ration zurückführen kann.

Bei Tieren mit einfachem Magensystem wird demnach beim mikrobiellen Abbau aus dem Pektin als letztes Abbauprodukt nicht nur Galakturonsäure oder ähnliche Zersetzungsprodukte gebildet, sondern der überwiegende Anteil des Pektins wird vollständig zu CO_2 und Wasser abgebaut. Die hohe CO_2-Produktion darf man wohl als Beweis für einen regen Pektinabbau durch die Darmflora ansehen. Der Abbau zu CO_2 ist dabei so intensiv, daß der tierische Organismus nicht in der Lage ist, größere Mengen möglicher Zwischenprodukte zu resorbieren. Interessant ist der deutliche Unterschied in der CO_2-Produktion bei den verschiedenen Pektingehalten der Ration, denn nach den Ergebnissen wird der vollständige Pektinabbau mit steigendem Pektingehalt anteilmäßig geringer. Möglicherweise kommt es beim Abbau größerer Pektinmengen infolge vermehrter Bildung von Galakturonsäure und anderen organischen Säuren zu einer pH-Verschiebung im Darm, die dann der weiteren Tätigkeit der Mikroorganismen hemmend entgegenwirkt. Dadurch wird das Tier in die Lage versetzt, einen größeren Anteil der möglichen Spaltprodukte zu resorbieren.

Tab. 1

Energetische Daten der Pektinzulagen

Pektingehalt der Ration		12 %	24 %
je g reinen Nährstoffes			
Bruttoenergie	cal	3708	3708
verdauliche Energie	cal	3553	3415
umsetzbare Energie	cal	3603	3479
Nettoenergie		203	751
Verwertungskoeffizient in % der Bruttoenergie		5,6	20,3

Die energetische Verwertung ist als schlecht zu bezeichnen (Tabelle 1). Die verdauliche Energie des reinen Pektins betrug je g 3553 cal. bzw. 95,8% der Bruttoenergie bei 12% Pektinzulage und 3415 cal. bzw. 92,1% bei der Zulage von 24% Pektin. Da durch die Pektinzulage die Energieausscheidung im Harn verringert wurde, lagen die Werte für die umsetzbare Energie sogar noch etwas höher. Alle diese Werte sind jedoch mit Vorbehalt zu betrachten, denn die ermittelte hohe Verdaulichkeit übersteigt die wahre Verdaulichkeit erheblich, wahrscheinlich um ein Mehrfaches, d. h., daß die in üblicher Definition ermittelte umsetzbare Energie Energiemengen beinhaltet, die in Wirklichkeit die Darmwand nie passiert haben. Alle diese Werte ermöglichen daher keinerlei Rückschlüsse auf den tatsächlichen resorbierten Anteil der Spaltprodukte des Pektins.

Bei der Berechnung des um Verdaulichkeitsdepressionen des Grundfutters und Lebendgewichtsveränderungen korrigierten Ansatzes betrug die Nettoenergie je

Gramm Nährstoff nur 203 cal. bei der 12%igen Pektinzulage. Das entspricht auf den Bruttobrennwert bezogen einer Verwertung von 5,6%. Bei einem Pektingehalt von 24% in der Ration wurde dagegen eine bessere Verwertung gefunden. Hier betrug die Nettoenergie je Gramm Nährstoff 751 cal. entsprechend einer Verwertung von 20,3% der Bruttoenergie.

Die normalerweise als Bezugsbasis dienende verdauliche und umsetzbare Energie sind aus den vorhin genannten Gründen bei den Pektinstoffen als Bezugsgröße weniger geeignet. [redacted]

Der mittlere Fehler der Verwertungskoeffizienten und des Ansatzes ließen erkennen, daß zwischen den einzelnen Tieren große individuelle Unterschiede vorhanden waren. Die Differenzen zwischen den Tieren erwiesen sich jedoch in beiden Versuchsreihen als nicht signifikant. Die Verwertungskoeffizienten bzw. die Nettoenergie der Zulagen der beiden Versuchsserien (mit 12 + 24%) unterschieden sich mit einem P-Wert von 12%, der somit um 7% über der 5% Grenze lag. Obwohl die Differenz statistisch nicht gesichert ist, läßt sich doch ein Einfluß der unterschiedlichen Pektingehaltes der Rationen vermuten, wie dies ja schon aus der verschiedenen CO_2-Produktion bei der Messung des Gaswechsels hervorging.

Zusammenfassung

Reines Pektin wird mit Verwertungskoeffizienten von 20% und darunter von monogastrischen Tierarten nur schlecht verwertet. Dies gilt mit großer Wahrscheinlichkeit auch für die leicht löslichen Pektinstoffe in den Futtermitteln. Diese schlechte Verwertung wird bei der Berechnung des Nährwertes der Futtermittel nicht berücksichtigt, wenn dieser aus der Summe der verdaulichen Nährstoffe errechnet wird, denn die Pektinstoffe werden im Analysengang nicht gesondert, sondern als N-freie Extraktstoffe erfaßt und als solche anderen N-freien Extraktstoffen wie Stärke und Zucker gleichgestellt. Für pektinreiche Futtermittel ergeben sich daraus falsche, nämlich zu hohe Futterwerte. Für solche Futtermittel ist für eine exakte Nährwertermittlung die Kenntnis des Pektingehaltes erforderlich, insbesondere wenn sie an monogastrische Tierarten verfüttert werden.

Dissertation Abstr. 26(8):4209-4210. 1966.

Gilmore-'66
Bib No. 143

EFFECT OF 5 PERCENT PECTIN N. F. OR 5 PERCENT PECTIN L. M. UPON GROWTH, EXCRETION, SERUM PROTEINS, AND MINERAL CONTENTS IN LIVER AND KIDNEY TISSUES OF WEANING MALE RATS OF THE SPRAGUE-DAWLEY STRAIN.

(Order No. 65-14,221)

Norma Magnus Gilmore, Ph.D.
Michigan State University, 1965

Pectic substances are one of the least investigated and understood classes of carbohydrate in the human dietary. They are often classified as part of the non-caloric fiber content of the diet for want of a better place to put them. Investigators have suggested (1) that ingested pectin passes through the animal body until it reaches the large intestine where it is attacked and digested by the flora; or (2) that as pectic substances are water soluble components of plants, they may be digestible carbohydrates and hydrolyzed and utilized in the animal body. Little quantitative evidence is reported in the literature to support one or the other of these assumptions.

As it would be of interest to know whether the feeding of a constant quantity of different pectins could produce differences in growth and specific cellular constituents and functions, pectins similar in molecular weight but significantly different in methoxyl content were fed to 150 weanling male rats of the Sprague-Dawley strain. The basal diet contained 25% casein and in the 2 experimental diets, 5% citrus pectin N. F. (10 to 12% methoxyl) or 5% citrus pectin L. M. (3 to 5% methoxyl) replaced 5% of the sucrose. After 2, 4, and 6 weeks of feeding, animals were randomly selected from each diet for sacrifice, with the final group at 8 weeks. Furfural determinations were done as an indirect measure of possible absorption of pectins.

The pectin N. F. fed animals consumed less diet and gained less weight. The pectin N. F. in the diet appeared to share responsibility for slowing down the rate at which these animals added weight to the body, and it was necessary for animals to consume this diet 5 weeks or longer before this was evident. The feces were the major route of pectin excretion. There was a sharp difference in the quantity of uronic acids as galacturonic acid recovered in the excretory products. Less than 1% of the pectin N. F. consumed could be recovered while 20 to 23% of the pectin L. M. consumed was recovered. Although the furfural yielding substances (FYS) determined in the liver, kidney, and spleen did not suggest that either pectin was deposited in these tissues, there was a small but steady trend in the FYS of the liver that material capable of yielding furfural was increasing in the livers of the pectin N. F. fed animals and decreasing in the livers of pectin L. M. fed animals. The FYS per 100 ml of blood paralleled these patterns, respectively. At 2 weeks, the sera of both pectin fed groups had increased serum albumin (paper electrophoresis) coupled with a decrease in alpha-2 globulin. At 6 weeks, the serum albumin of pectin N. F. fed animals had decreased, alpha-2 and gamma globulins had increased, and there was marked retention of copper (emission spectrograph) by the kidneys. Serum albumin in rats consuming pectin L. M. tended to decrease throughout the study and at 8 weeks the alpha-2 globulin was markedly increased. Magnesium, phosphorus, and zinc in livers of the pectin L. M. fed rats increased during the study's last 4 weeks coupled with marked accumulation of copper in the kidney and later in the liver.

Differences in metabolic response appeared most pronounced for the pectin N. F. fed animals at 6 weeks while those of the pectin L. M. fed animals seemed to evolve more slowly and after this point in time. The differences apparently were related to the methoxyl content of the pectins. There is no strong indication supported by data that pectins were absorbed

and/or hydrolyzed but the metabolic responses observed cannot be easily explained solely by action of the microflora upon pectins. More refined techniques need to be applied to this problem.

Microfilm $3.00; Xerography $7.20. 155 pages.

Hartman-'41
Bib No. 160

Ann. Surg. 114:212-225. 1941.

PECTIN SOLUTION AS A BLOOD SUBSTITUTE

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The present war has reemphasized the urgent need for blood and blood plasma in the fluid or desiccated state, which may be used to combat shock and hemorrhage. In line with other defense work, the collection of desiccated plasma for our own armed forces has been undertaken by the American Red Cross under the supervision of the National Research Council. The present specifications covering the preparation of this material require the desiccation from the frozen state. With the largest units desiccating from the frozen state, such as used by Best at the Banting Institute, only 1,000 units of 250 cc. each can be produced per week. Even with our method[1] of desiccation from cellophane cylinders with a production of 1,200 units of 250 cc. per week, the matter of obtaining such large quantities of human blood remains a great problem. From the standpoint of production alone, to say nothing of cost, transportation, storage, and application, it seems obvious that some other substances which may be used as substitutes or supplements for blood and blood plasma must be found.

The widespread and successful use of blood plasma and blood serum in shock and hemorrhage has demonstrated most convincingly that the primary need in such emergencies is to replenish the volume of circulating fluid with a fluid which has identical or similar physical characteristics rather than new red blood cells. The red blood cells may in fact be distinctly contraindicated in shock without hemorrhage.[2]

In a recent article, Taylor and Waters[3] outline the requirements of a transfusion fluid which will restore and maintain the volume of circulating fluid as follows: "(a) The molecule of the dissolved substance must be of such a size that the fluid will not leave the vessels too freely. (b) The solution must exert an osmotic pressure and possess a viscosity approaching as closely as possible that of whole blood; these qualifications depend upon molecular size and shape. (c) It should be as nearly as possibly isotonic with the contents of erythrocytes. (d) It must, of course, be nonantigenic and innocuous in every respect. In addition, it should be readily available, preferably cheap, and capable of being quickly and easily prepared for intravenous administration."

To meet these requirements, Taylor and Waters propose the use of isinglass (fish gelatin) especially prepared from fish swimming-bladders. Some difficulty in purification has been encountered, but in animal experiments it seems to answer the purpose and only a few reactions were encountered. It

is held superior to ordinary gelatin which does have definite antigenic properties.

The second colloidal material which has been widely used as a blood substitute is gum acacia. Its use was suggested by Bayliss,[4] in 1916 under the title "Methods of Raising a Low Arterial Pressure," and supported by Rous and Wilson,[5] in 1918 under the title "Fluid Substitutes for Transfusion after Hemorrhage." The favorable results reported by Bayliss and Rous and Wilson were confirmed by some investigators and objections were brought out by others. Hanzlik and Karsner,[6] in 1922 in a study of various colloids state regarding acacia: "It injures the circulatory and respiratory systems as indicated by the presence of anaphylactoid symptoms, pulmonary distention, congestion and hemorrhage together with cardiac dilatation. Thrombi in the pulmonary vessels occurred after the injection of acacia in therapeutic doses and concentrations." Maytum and Magath[7] in 1932 confirmed the fact that even the best solutions might be antigenic: "Solution of acacia is a mild antigen . . . there is no danger in the first dose of acacia but subsequent doses should be given cautiously because of the possibility of anaphylactic reactions." Yuile and Knutti[8] in 1939 showed that the livers of dogs injected weekly with acacia may increase five to six times in weight and contain 8 to 10 per cent of acacia by weight. Further it was found that acacia persisted as long as 144 days in the blood and that the plasma proteins were depressed to very low levels, particularly the fibrinogen fraction which remained low for several months.

Andersch and Gibson[9] report that 30 per cent of the injected acacia was found in the livers of dogs and 43 per cent of 46 Gm. injected was found in the liver of a nephrosis patient.

Jackson and Frayser[10] in 1939 found that "the fibrinogen may become very low and cause considerable delay in clotting and prolongation of bleeding if large amounts of acacia are given."

Thus it is apparent that while acacia satisfies the first three requirements, it fails in the fourth since it is antigenic and is stored in the liver, interfering with liver function.

The difficulties encountered in the intravenous use of acacia have been detailed because it is obvious that any new colloidal solution proposed for intravenous use in shock and hemorrhage must eliminate most or all of these disadvantages, if it is to be a truly acceptable substitute for whole blood or blood plasma.

Pectin is a substance very familiar to the profession but its therapeutic application has been confined to the skin and intestinal tract. It may be described, according to Gortner[11] as a colloidal carbohydrate of high molecular weight and rather complex composition. On hydrolysis it is said to produce galacturonic acid, galactose, arabinose, xylose, methanol, and acetic acid. Protopectin is the mother substance of the pectins and both are found in the plant cell walls where they are probably combined with cellulose. Protopectin may be hydrolyzed free from the cellulose and converted into

soluble pectin, usually by treatment with 0.5 per cent ammonium oxalate at 70 to 90° C. or by heating with dilute acids. The pectin resulting from either form of hydrolysis is of high molecular weight, disperses in water to a viscous colloid solution and is readily precipitated from this solution by alcohol.

Myers and Baker[12] believe that lemon pectin is monoarabinomonogalactoliacetylheptamethoxyoctagalacturonic acid, giving a formula of $C_{70}H_{98}O_{38}$ with a molecular weight of 1,866. Bonner[13] suggests a cellulose-like chain formula. Henglein and Schneider[14] nitrated pectin and found the resulting material with the physical characteristics similar to nitrocellulose.

Source and Preparation of Pectin for Intravenous Use.—Pectin in the dry powder form obtained from two manufacturers was used in this work. One brand bore the label "pure grapefruit pectin" and the other "pectin-technical grade." The former, designated as Brand I, is a light brown granular material which is sufficiently soluble in warm, double distilled water to readily make a 1 per cent solution. It is filtered through No. 1 Whatman papers. This solution is slightly opalescent and has a pH of 3.05. Its viscosity is 10.15 at 38° C. and its osmotic pressure is 50 Mm. of mercury. Since the viscosity of whole blood is about six and the osmotic pressure of plasma is 25 to 30 Mm. of mercury, the 1 per cent solution of Brand I is diluted with equal quantities of 1.8 per cent NaCl or double strength Ringer's solution; then phosphate buffers are added in sufficient quantity to bring the pH to 6.5. The pectin obtained from the second manufacturer, Brand II, is a white powder which contains 35 per cent glucose. Because of the difficulty in separating this glucose, the material was used in a 1 per cent solution made up in double distilled water. It is filtered through No. 1 Whatman papers. This solution has a viscosity of 3.5 and an osmotic pressure of 22 Mm. of mercury. Eight-tenths of 1 per cent NaCl and phosphate buffers are added to bring the pH to 6.5. Both solutions are sterilized under steam pressure of 15 pounds for 20 minutes. After sterilization, the solutions are still slightly opalescent and is further buffered to pH 7.2. As with other colloidal solutions, every lot does not turn out alike, therefore it is necessary to test each lot against citrated blood and suspensions of red blood cells, examining for hemolysis, rapid sedimentation, and precipitation of fibrin. If any of these occur the solution must be rejected.

Experimental.—No evidence has been found in the literature to indicate that pectin is an antigenic substance, but since it has not been used intravenously as a therapeutic agent, it seemed desirable to determine whether or not it was antigenic or whether it would produce so-called anaphylactoid symptoms. To this end three guinea-pigs were injected intravenously with 1 cc., 1.5 cc. and 2 cc. of the 1 per cent buffered pectin solution and at the end of [illegible] days with the same amounts intravenously. No reaction occurred.

Twelve rabbits of six and eight pounds each were given 1 per cent buffered pectin solutions intravenously in amounts ranging from 50 to 100 cc. These doses were repeated after one week and after two weeks but no immediate

or delayed reaction occurred. Three dogs, as indicated in Table I, were injected four times over a 26-day period with 1 per cent buffered pectin solution in doses ranging from 300 to 350 cc., each time without anaphylactic symptoms.

TABLE I

LIVER FUNCTION FOLLOWING PECTIN INJECTION

Dog. and Wgt.	Date 1941	Gm. Pectin per Kg.	B.S.P. Liver Function Test		
			5′	10′	20′
P 60					
15.8 Kg.	6/12	0.10	....	....	....
	6/17	0.22	....	....	....
	6/18	....	50%	30%	20%
	6/21	....	25%	15%	neg.
	6/23	0.10	....	....	....
	7/7	0.25	....	....	....
	7/7 (4 hrs.)	....	40%	20%	10%
	7/8 (24 hrs.)	....	40%	20%	7%
	7/11	....	35%	10%	neg.
P 70					
11.3 Kg.	6/12	0.26	....	....	....
	6/17	0.26	....	....	....
	6/18	....	45%	20%	15%
	6/21	....	35%	10%	neg.
	6/23	0.26	....	....	....
	7/7	0.35	....	....	....
	7/7 (4 hrs.)	....	55%	25%	15%
	7/8 (24 hrs.)	....	60%	25%	10%
	7/11	....	35%	5%	neg.
P 80					
17.7 Kg.	6/12	0.17	....	....	....
	6/17	0.17	....	....	....
	6/18	....	45%	15%	5%
	6/21	....	25%	10%	neg.
	6/23	0.17	....	....	....
	7/7	0.23	....	....	....
	7/7 (4 hrs.)	....	50%	35%	20%
	7/8 (24 hrs.)	....	30%	15%	10%
	7/11	....	25%	5%	trace

The disposition of pectin in the body after it is injected intravenously is of primary importance, since one of the principle difficulties with acacia is its retention in the blood and tissues, especially in the liver. Pectin is also taken up by the liver, especially if a single large dose is administered. In six to eight pound rabbits receiving 100 to 150 cc. of the 1 per cent buffered pectin solution at one time and sacrificed three hours later, there was no marked gross enlargement of the liver, but microscopically the hepatic cells appeared swollen and filled with small clear granules. A dog of 16 Kg. receiving 425 cc. of 1 per cent buffered pectin solution, and autopsied 14 hours later, failed to show either gross or microscopic evidence of liver retention. As seen in Table I, the fractional bromsulphthalein test of liver function after the injection of large amounts of pectin solution showed a temporary blocking which

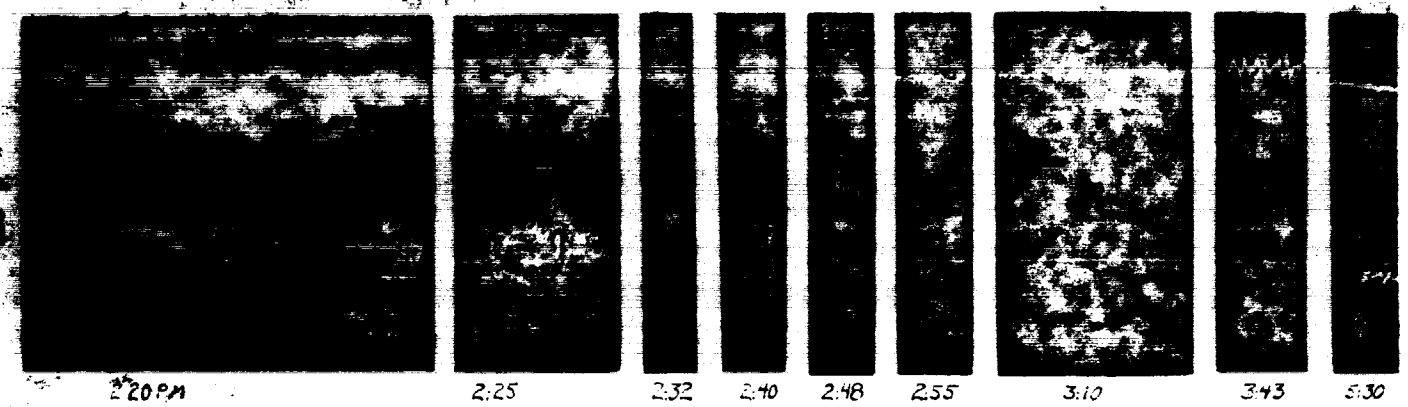

FIG. 1.—Experiment upon dog weighing 11.8 Kg. Effect of intravenous injection of 325 cc. of 1 per cent pectin in shock produced by bleeding of 325 cc. of blood. The final blood pressure = 160 Mm. of mercury.

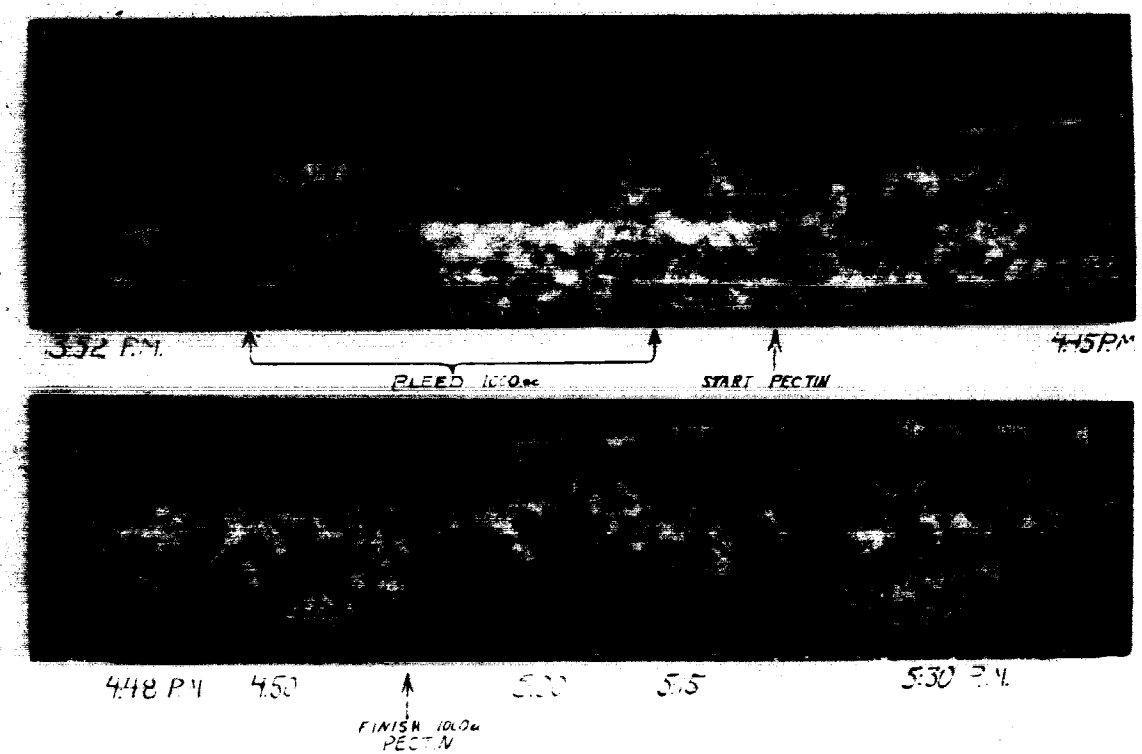

Fig. 2.—Experiment upon dog weighing 23.1 Kg. Effect of intravenous injection of 1,000 cc. of 1 per cent pectin in shock produced by bleeding of 1,000 cc. of blood. The final blood pressure is 160 Mm. of mercury.

cleared rapidly and showed no tendency toward a cumulative storage or blockage even with four large doses in a 26-day period.

The temporary character of the body's retention of pectin is demonstrated further by its excretion in the urine. It is excreted unchanged, as demonstrated by precipitation when equal quantities of urine and absolute alcohol or urine and acetone are mixed. In either case the pectin is thrown down as a white flocculant precipitate. A rough quantitative estimation of the amounts may be made by comparing with a known solution which has been precipitated in the same manner. The excretion of pectin is small for the first eight to 12 hours after the injection but reaches the maximum within 24 hours, and within 48 to 72 hours the major portion of the pectin is eliminated.

Considering the source, method of production, and chemical nature of pectin, there is nothing to suggest that it would be toxic for the body or its component units. Further, pectin has been used extensively in man as a local application to wounds and in the gastro-intestinal tract without toxicity. With solutions prepared and tested as described, there have been no indications of toxicity in either man or animal. Pectin solutions which are not properly adjusted as to the pH and which have a viscosity above five are poorly tolerated in animals and must be guarded against in clinical work.

A series of 12 experiments were performed upon animals in order to assay the value of pectin for blood replacement in shock following (a) hemorrhage; and (b) experimental bile peritonitis, and observe its effect on (c) blood concentration; and (d) as a replacement for plasma in plasmapheresis.

(a) In four animals, shock was produced by *hemorrhage*. As seen from Table II, the amounts of blood removed, while considerable, were not sufficient to alone cause death in all instances, but the effects of pectin in restoring blood pressure are quite striking. As seen from Figures 1 and 2, the restored level of blood pressure was well maintained after the administration of pectin solution intravenously.

TABLE II

THE USE OF PECTIN IN SHOCK FOLLOWING EXPERIMENTAL HEMORRHAGE

[illegible]	Dog Wt. Kg.	Initial Blood Pressure Mm.Hg.	Bled cc.	Blood Pressure After Bleeding Mm.Hg.	Inject cc. 1% Pectin Intravenously	Final Blood Pressure Mm.Hg.	Result
[illegible]	11.[illegible]	[illegible]	225	80	320	155	Died. Lung abscesses found at necropsy
[illegible]	12.2	150	375	50	475*	130	Recovered
[illegible]	13.6	130	500	90	500†	110	Recovered
[illegible]	23.1	180	1,000	80	1,000	100	Recovered

[illegible] 150 cc. whole blood.

[illegible] per cent pectin used in this experiment.

(b) Five animals with *bile peritonitis* were treated with pectin. The bile peritonitis was produced by the intraperitoneal injection of sterile 10 per cent solution of Armour's repurified bile salts. A marked hemoconcentration as

evidenced by a rise in the hematocrit resulted. This rise and the associated marked fall in arterial blood pressure are shown for a typical experiment in Table III. Three animals in this stage were then treated with intravenous

TABLE III

THE USE OF PECTIN IN SHOCK FROM BILE PERITONITIS

Typical Experiment. (In the other two experiments the animals lived ten plus and 22 hour after injection the bile salts intraperitoneally.)

Dog weight—11.8 Kg.
Initial readings: Blood pressure—140 Mm.Hg.; hematocrit—46%.
Inject 89 cc. 10% bile salt solution intraperitoneally.
Time interval—3¾ hours.
Subsequent readings: Blood pressure—60 Mm.Hg.; hematocrit—69%.
Inject 230 cc. 10% pectin intravenously.
Later readings: Blood pressure—128 Mm.Hg.; hematocrit—58%.
Death six hours after intraperitoneal bile injection.

pectin injections as shown in Figures 3 and 4, and a temporary improvement in hematocrit reading and blood pressure level occurred. These experiments were made to observe this effect rather than to attempt a permanent cure since it is well known that in instances of bile peritonitis of this severity even plasma will not prevent death.[15]

TABLE IV

PECTIN PLASMAPHERESIS EXPERIMENT, JULY 5, 1941

Dog Weight 11.8 Kg., Nembutal Anesthesia

	Observation				Remove Cc.			Administer Cc.	
		Gm./100 Cc.							
Time P.M.	Hematocrit	Albumin	Globulin	Total	Whole Blood	Cells	Plasma	Cells	1% Pectin
4:12	41	3.39	1.52	4.91	...	...	..	...	...
4:18	..	....	..	....	200	105	95	...	..
4:45-5:00	..	....	....	....	...	..	..	105	130
5:02	45	2.09	1.73	3.82	...	...	..	...	...
5:05	..	....	....	....	175	95	80	...	..
5:11-5:31	..	....	....	....	...	...	..	95	130
5:35	45	1.76	1.84	3.60	...	...	..	.	...
5:37	..	....	....	....	175	105	70	...	..
5:38-6:03	..	....	....	....	...	...	..	105	100
6:07	50	2.09	1.09	3.18	...	...	..	...	...
6:08	..	....	....	....	175	105	70	..	..
6:09-6:31	..	....	....	....	...	...	..	105	100
6:32	52	1.97	0.87	2.84	...	...	..	...	...
				Totals	725	410	325	410	460

Two other animals were given pectin before the production of bile peritonitis with no demonstrable prolongation of life over that observed in similar control experiments.

(c) In one experiment, pectin solution was rapidly injected to see if it could cause a blood *concentration* or dilution. A rapid dilution was observed, unlike the paradoxic blood concentration following concentrated plasma injections observed by Harkins, Boals and Brush.[16] In this same experiment, frequent determinations of the blood clotting time were made with no demonstrable prolongation being observed.

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4:18	..	....	..	....	200	105	95	...	
4:45-5:00	..	....	..	...	...	..	..	105	130
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5:05	..	....	....	..	175	95	80	...	..
5:11-5:31	..	....	..	..	...	...	..	95	130
5:35	45	1.76	1.84	3.60	...	...	..	.	...
5:37	..	....	.	....	175	105	70	...	...
5:38-6:03	..	....	....	..	...	...	..	105	100
6:07	50	2.09	1.09	3.18	...	...	..	..	.
6:08	..	....	....	....	175	105	70	...	..
6:09-6:31	..	....	....	....	...	...	..	105	100
6:32	52	1.97	0.87	2.84	...	...	..	...	...
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FIG. 3.—Effect of intravenous injection of 255 cc. of 1 per cent pectin in shock produced by the intraperitoneal injection of 60 cc. of 10 per cent sterile bile salt solution. The hematocrit rose from 39 to 59 before pectin was given and then fell to 56. It later rose to 62. The last blood pressure shown on the chart, 45 minutes after pectin administration, is 136 Mm. of mercury. The animal died 22 hours after production of the peritonitis.

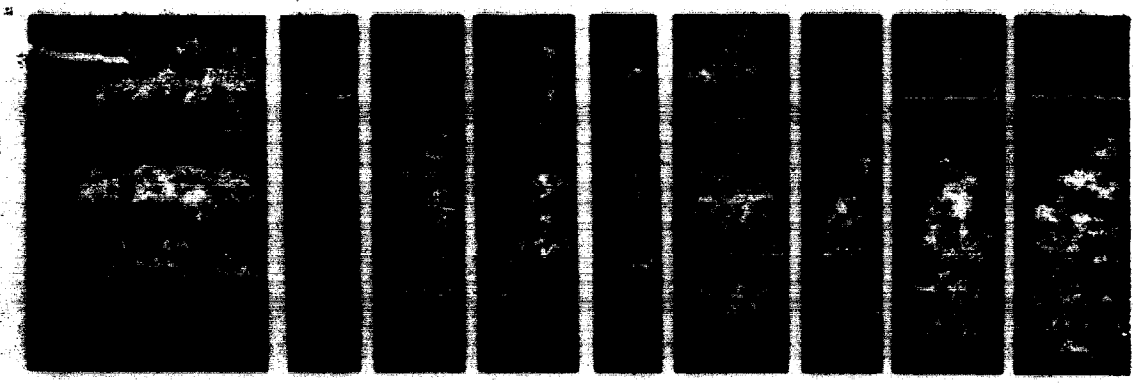

FIG. 4.—Effect of intravenous injection of 230 cc. of 1 per cent pectin in shock produced by the intraperitoneal injection of 80 cc. of 10 per cent sterile bile salt solution. Accompanying hematocrit readings are shown in Table II. The last blood pressure shown on the chart, one hour after pectin administration, is 162 Mm. of mercury. The animal died six hours after production of the peritonitis.

(d) In two dogs, *plasmapheresis* was done. After centrifuging withdrawn samples of blood, the plasma was discarded and replaced by an equal or slightly greater amount of 1 per cent pectin solution. The cell-pectin mixture

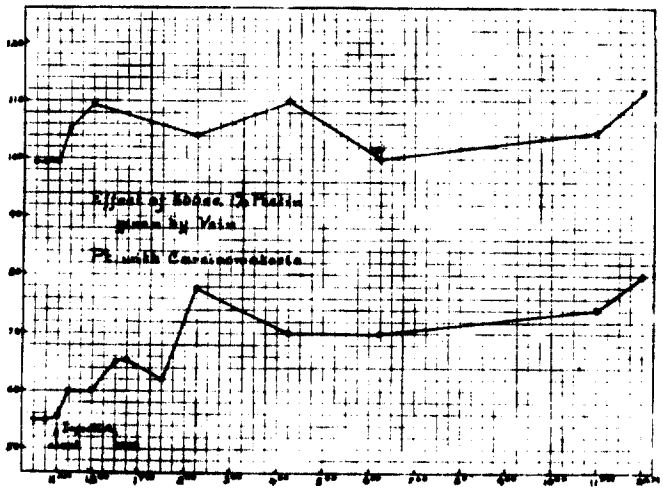

FIG. 5. Case 1: Effect of intravenous injection of 600 cc. of 1 per cent pectin in patient B. M.

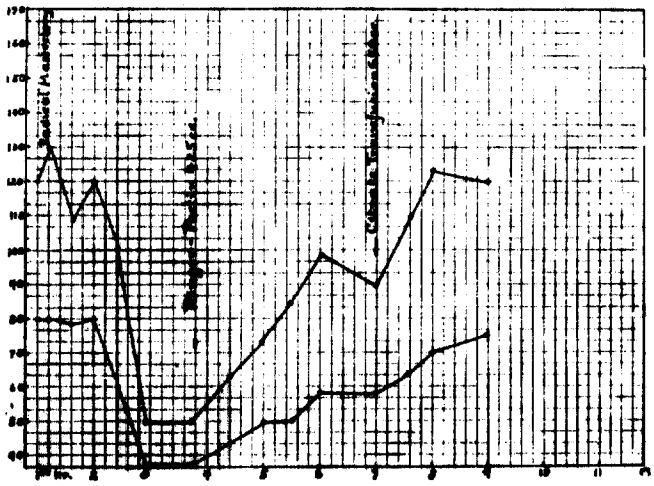

FIG. 6.—Case 2: M. W. No. 16863. A female, white, age 42. Weight 130 lbs. Lump in right breast for three months. Admission blood pressure 140/85. Operation July 1, 1941—Radical mastectomy with skin graft from right thigh. (Preoperative hemoglobin 12.5 Gms.) Anesthetic ethylene. Received 450 cc. of 1 per cent pectin solution intravenously after returning from the operating room in shock. Condition improved and the systolic blood pressure rose from 50 to 98.

[illegible] immediately reinjected in the animal. This procedure was repeated [illegible] four times in one experiment and five times in the other, with[illegible] samples of about 200 cc. of blood each time. In one experiment, [illegible] did not vary at all, while in the other, it increased somewhat [illegible] Table IV. The marked decrease in total plasma proteins following [illegible] replacement in this animal is noteworthy.

CLINICAL OBSERVATION

Case 1.—Pectin Injection in Patient Not in Shock: B. W., a female, age 54, [illegible] a palliative operation for carcinoma of the right breast, October 8, 1940. She [illegible] to the hospital, June 30, 1941, with multiple metastases. An intravenous injection of 600 cc. of 1 per cent pectin was given, July 3, 1941. The chief effects were a slight feeling of well-being and an elevation of blood pressure from a mild hypotensive to a more normal level as shown in Figure 5. A slight chill with a transient temperature elevation to 101.4°F. might well be attributed to a coincident bromsulphthalein test. Furthermore, the patient had a daily diurnal rise to 99.0° F. each day before the pectin administration. Liver function and renal function tests were performed before and after the giving of pectin. On July 2, 1941, the bromsulphthalein retention was ten minutes—20 per cent, 20 minutes—0, and 30 minutes—0, and on July 4, the day after pectin injection, the test was exactly the same. A phenolsulphonphthalein test on July 2 showed [illegible] per cent excretion in one hour and 15 per cent in the second hour for a total of 80 per cent. On July 4, the day after pectin injection, the readings were identical. Hematocrit readings were constant (decrease from 38 to 37) and the clotting time also showed no appreciable change. Blood protein analyses, in grams per cent, were as follows:

	Albumin	Globulin	Fibrinogen	Total
July 2, 1941 (Before pectin)	4.04	2.27	0.40	6.71
July 4, 1941 (Just after pectin)	2.98	4.17	0.43	6.58

The clinical use of pectin in shock is illustrated in Figures 6, 7 and 8.

Discussion.—The use of pectin as the colloid base for a blood substitute is logical because it is one of the most hydrophilic colloids, only 0.5 Gm. of pure material per 100 cc. of solution being required to make a solution with a viscosity and osmotic pressure near that of whole blood. Thus, 14 times more acacia, gelatin or isinglass, gram for gram, is required to make a suitable [illegible] solution, than is required with pectin.

[illegible] source—citrus fruits—and the method of production, chemical and [illegible]ic extraction, give an abundant supply, free of gross bacteriologic [illegible]ation, at nominal cost.

[illegible] preparation and sterilization of the solution is readily accomplished, but [illegible] essentials must be observed, i.e., neutralization; addition of electrolytes; [illegible]ting for (a) viscosity; (b) hemolysis of red blood cells; (c) sedimen[illegible] of red blood cells; and (d) precipitation of fibrin.

[illegible] preliminary experimental work indicates that pectin is nonantigenic [illegible] nontoxic. It is retained in the body for a short period and then eliminated rapidly. There appears to be no cumulative effect in using the solutions described. Liver function as measured by the fractional bromsulphthalein test, and fibrinogen shows slight depression only with massive doses. In the 12 experiments on shock and hemorrhage, prompt and adequate response as

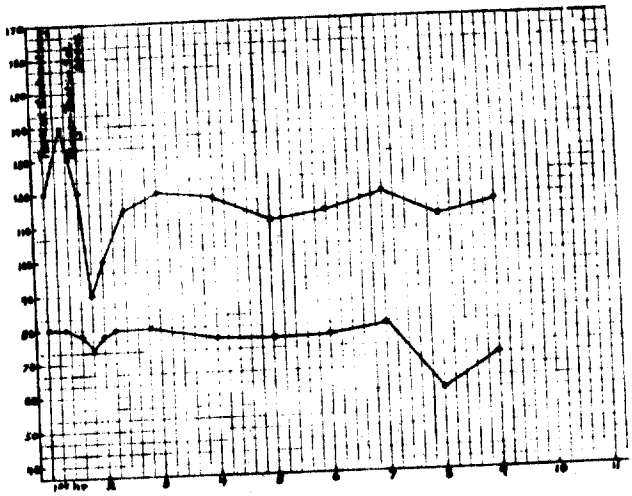

FIG. 7.—Case 3: C. V. No. 271727. White, female, age 56. Gastric ulcer with continuous symptoms for five years. Height 5'2", weight 134½ lbs. Operation July 3, 1941. Partial gastric resection. Preoperative blood pressure 88/64, hemoglobin 14.5 Gms. Received 950 cc. of 1% per cent pectin during the operation rather than the customary transfusion of whole blood. The blood pressure was maintained well above the preoperative level. Postoperative course was uneventful.

FIG. 8.—Case 4: J. M. No. 186791. White, male, age 66. Duodenal ulcer for past 15 years. 5'6" tall, weight 140 lbs. Present studies show marked gastric retention and gallstones. On 7/3/41 had cholecystectomy, appendicectomy and posterior gastro-enterostomy. Admission blood pressure 120/70. Postoperative course uneventful. Anesthetic cyclopropane and ethylene (preoperative Hb. 15 Gm.). Received 900 cc. of 1% pectin during the course of the operation instead of the usual blood transfusion. Blood pressure levels were sustained at satisfactory levels.

[illegible] blood pressure graphs and the general condition of the animals [illegible].

The preliminary clinical application shows the patient (Case 1, Fig. 5) [illegible], with a moderate elevation of blood pressure after 600 cc. of 1 per cent buffered pectin solution. The bleeding time, coagulation time, and liver function were not altered. The mastectomy patient (Case 2, Fig. 6) in shock received a small injection of 450 cc. of the 1 per cent solution but the condition improved steadily over a three-hour period. The two patients receiving injections instead of the usual transfusions during partial gastrectomy operations (Cases 3 and 4, Figs. 7 and 8) maintained satisfactory blood pressure levels throughout. Three additional operative cases and one normal individual have received pectin intravenously without reaction or untoward results. In the operative cases, especial attention was paid to the bleeding time in the tissues of the operative wound but no variation from normal could be observed.

SUMMARY

(1) The intravenous use of pectin solution as a blood substitute in shock is proposed.

(2) One-half per cent of pure pectin solution has about the same viscosity and osmotic pressure as whole blood.

(3) Pectin has a high molecular weight, is nonantigenic and nontoxic.

(4) The source, method of manufacture, and the ease of preparing and sterilizing stable buffered solutions make pectin readily available.

(5) Preliminary experimental and clinical application indicate that pectin solutions are valuable in the management of shock.

(6) There is temporary retention of pectin in the blood and in the liver [illegible] of the unchanged material in the urine is rapid.

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EXPERIMENTAL STUDIES IN CARDIOVASCULAR PATHOLOGY

VI. PECTIN ATHEROMATOSIS AND THESAUROSIS IN RABBITS AND IN DOGS

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Previous investigations had shown that a causal relationship exists between macromolecular colloidal disturbances of the blood plasma and the development of atheromatous and sclerotic lesions of the arteries (Hueper[1]). The evidence advanced indicated that the mechanism operative in the production of these vascular changes consists in the formation on the intima by the pathogenic colloids of films or precipitates which interfere with the proper oxygenation and nutrition of the walls of the vessels. It was demonstrated that proteinic colloids (gelatin, azoproteins) give rise to sclerosing lesions, while lipoidal and carbohydratic, emulsion-forming colloids produce vascular atheromatosis in addition to storage phenomena in various organs (cholesterol, polyvinyl alcohol, methyl cellulose, acacia).

For further confirmation and elaboration of this general principle, another macromolecular substance, pectin, was studied. Colloidal solutions of this macromolecular carbohydrate were injected intravenously into rabbits and dogs, and the effects of this treatment on the blood and on the internal organs were determined. The following report presents the results of these experiments.

EXPERIMENTAL OBSERVATIONS

Pectinum (pure citrus pectin of an average molecular weight of 200,000), which was obtained through the courtesy of Dr. G. Joseph, of the California Fruit Growers Exchange, was used. Citrus pectin belongs to the group of carbohydrate-like macromolecular substances possessing a long-chained molecule which consists of polymerized galacturonic acid molecules with various additional groups, such as galactose, araban, acetic acid and methyl alcohol. The purified product employed in these studies is a pale greenish yellow fine powder that dissolves readily in water when wetted previously with 95 per cent alcohol, giving a clear, slightly yellowish tinted viscous colloidal solution with an acid reaction

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1. Hueper, W. C.: Arch. Path. 28:510, 1939; 31:11, 1941; Pharm. Arch. 12:49, 1941; Medicine 20:397, 1941; Arch. Path. 33:1 and 267, 1942; Am. J. Path. 18:895, 1942.

(1.2 per cent solution—p_H 3.2). This solution, which is an excellent emulsifying agent for gases or liquids, gels on the addition of alcohol but is not stable when subjected to prolonged heating or when brought to a neutral or an alkaline reaction. A brownish precipitate is formed under such conditions, and the solution loses greatly in viscosity because of progressive depolymerization of the pectin molecule (Dehn [2]; Myers and Baker [3]; Hinton [4]; Joseph [5]). There exists a linear relation between the degree of viscosity and the concentration of pectin, as a 1 per cent solution has at 19 to 20 C. a viscosity of 16.08, a 0.5 per cent solution one of 8.44, a 0.25 per cent solution one of 3.22 and a 0.15 per cent solution one of 2.0 to 2.1, or about that of normal plasma. The osmotic pressure of a freshly prepared 1 per cent solution of this pectin is 84 cm. when tested against water.

For the purpose of the experiment, the p_H of the pectin solution was adjusted to about the neutral point (p_H 6.5 to 7.0) either by the addition of lime water

TABLE 1.—*Hematic Reactions in Dog 797, Weighing 6.3 Kg., After a Single Intravenous Administration of 20 cc. of a 1 per Cent Pectin Solution*

	Erythrocytes per Cu. Mm.	Sedimentation Rate, Mm.	Coagulation Time, Min.	Leukocytes per Cu. Mm.	Polymorphonuclears, per Cent	Lymphocytes, per Cent	Viscosity: Whole Blood	Viscosity: Plasma
Start	6,230,000	2.0	10½	13,000	90	8	7.36 @ 18.3 C.	2.09 @ 22.5 C.
5 minutes	6,920,000	39.0	6½	1,800	58	40	7.28 @ 19.0 C.	2.40 @ 22.6 C.
15 minutes	7,280,000	41.0	2½	3,000	92	7	7.40 @ 19.2 C.	2.25 @ 22.7 C.
45 minutes	6,360,000	46.0	6	3,600	94	6	6.04 @ 19.8 C.	2.23 @ 22.7 C.
2 hours	6,140,000	42.0	7	5,800	96	2	6.04 @ 20.0 C.	2.19 @ 22.8 C.
4 hours	5,890,000	44.0	6	9,200	98	2	6.38 @ 20.2 C.	2.15 @ 22.8 C.
6 hours	6,180,000	16.0	7	10,200	93	2	5.86 @ 20.4 C.	2.13 @ 22.8 C.
8 hours	5,610,000	2.0	6½	15,300	94	5	5.88 @ 20.6 C.	2.13 @ 22.8 C.
24 hours	6,180,000	32.0	4	8,400	82	16	5.40 @ 20.8 C.	2.10 @ 22.8 C.
48 hours	5,870,000	38.0	9	18,200	92	5	4.38 @ 21.0 C.	1.85 @ 22.9 C.
72 hours	6,200,000	24.0	4	16,100	83	15	4.76 @ 21.2 C.	1.87 @ 23.0 C.
7 days	6,860,000	21.0	10	22,200	95	3	4.60 @ 23.0 C.	2.04 @ 21.2 C.
9 days	6,670,000	19.0	10	23,900	95	3	4.90 @ 23.2 C.	2.09 @ 21.3 C.
14 days	6,050,000	1.0	12	12,800	98	2	4.94 @ 21.4 C.	1.90 @ 22.3 C.

(0.75 cc. of lime water to 5 cc. of a 1.2 per cent solution of pectin in physiologic solution of sodium chloride) or phosphate buffer solution.

Hematic Reactions.—Single or repeated injections of freshly prepared 1 per cent or 2 per cent pectin solutions were made into the jugular veins of dogs for the study of the hematic effects. In the single injection series 3 dogs weighing about 7 Kg. each were given intravenously 10 cc., 20 cc. and 33 cc., respectively, of a 1 per cent neutralized pectin solution. The procedure followed and the results obtained are presented in table 1, containing the data on the dog which received the medium dose and showed moderately marked hematic effects.

2. Dehn, W. M., cited by Elwell.[7]

3. Myers, P. B., and Baker, G. L.: Fruit Jellies: VIII. The Role of Pectin: 4. The Physico-Chemical Properties of Pectin, Bulletin 187, Technical no. 15, University of Delaware Agricultural Experiment Station, Newark, Delaware, 1934.

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5. Joseph, G. H.: Bull. Nat. Formulary Committee 9:2 and 18, 1940.

Immediately following the injection of the pectin solution there occurred a brief transitory increase in the number of erythrocytes per cubic millimeter followed by a drop to somewhat below the original level. This movement was reversed within the first twenty-four hours for the 2 dogs which received the small and the medium-sized dose, but was progressive for the dog which was given 35 cc. of a 1 per cent pectin solution, so that at the end of the observation period of fourteen days the initial number of 4,300,000 was reduced to 1,700,000. The hemoglobin values and the amounts of packed erythrocytes followed in general the fluctuations of the erythrocyte counts. The sedimentation rate of the erythrocytes was markedly increased after the injection. The rate returned to normal or almost normal at about eight hours after the treatment to reach again high values within the next twelve hours and to remain high for another six to eight days. There was gradual slowing of the erythrocytic sedimentation afterward, with return to normal conditions at the termination of the fourteen day period. While the prothrombin time remained stationary, the coagulation time was in general moderately shortened immediately after the injection. This reaction lasted for several days, after which a return to normalcy was observed.

There developed a progressive decrease in the viscosity of the whole blood, although no such alteration was demonstrable concerning the viscosity of the plasma. This movement was still active at the end of the observation period. The leukocytes dropped sharply and markedly after the injection of the pectin solution in the 2 dogs which received 20 and 35 cc. of this material, while no change occurred in the dog which received only 10 cc. The leukopenic reaction lasted for about six hours. During this phase there was marked diminution in the percentage of the polymorphonuclear leukocytes with a relative increase of the lymphocytes.

Of the 3 dogs used for the study of the effects of repeated injections of pectin solution, 2 belonged to the former series. They received daily five times per week 20 cc. of a 1 per cent solution. After six weeks the daily dose was increased to 30 cc., and after eight weeks the concentration was increased to 2 per cent. During the thirteenth week the daily dose was raised to 40 cc., and during the following and last week of the experiment, to 55 cc. A total of 25 Gm. of pectin was injected. Five days after the last injection the animals were killed. The hematic effects observed are presented in table 2, which contains the data on one of the dogs. These data are characteristic for the entire series.

During the first two weeks of the experiment there was in 1 animal a moderate increase in the number of erythrocytes, while the other 2 dogs showed a moderate drop followed by a brief rise. A mild to moderate reduction in the number of red cells occurred toward the end of the experimental period. The hemoglobin content and the volume of packed erythrocytes followed the trend exhibited by the number of red cells. The sedimentation rate was consistently increased. The prothrombin time fluctuated within normal limits. The coagulation time exhibited at times relatively low values. The number of leukocytes as well as their ratio did not undergo any appreciable changes. Mononuclear leukocytes with basket weave vacuolated cytoplasm were found in small number. The viscosity of the whole blood followed in general the fluctuations in the number of erythrocytes, while the viscosity of the plasma remained within normal limits.

It appeared to be desirable to investigate also the hematic reactions elicited by pectin solutions that had been subjected to autoclaving, as pectin solutions thus treated had been proposed as blood substitutes (Hartman and co-workers[6]).

6. Hartman, F. W.; Schelling, V.; Harkins, H. N., and Brush, B.: Ann. Surg. **114**:212, 1941.

TABLE 2.—*Hematic Reactions in Dog 797, Weighing 6.3 Kg., After Intravenous Administration of, First, 710 Cc. of a 1 per Cent Pectin Solution and, Second, 875 Cc. of a 2 per Cent Pectin Solution*

	Amount of Pectin, Cc.	Erythrocytes per Cu. Mm.	Sedimentation Rate, Mm.	Coagulation Time, Min.	Leukocytes, per Cu. Mm.	Polymorphonuclears, per Cent	Lymphocytes, per Cent	Viscosity	
								Whole Blood	Plasma
Start	.....	6,050,000	1.0	12	12,800	98	2	4.94 @ 21.4 C.	1.90 @ 22.3 C.
9 days	120 × 1%	7,210,000	21.0	7	17,400	84	15	5.12 @ 20.4 C.	1.79 @ 18.2 C.
14 days	100 × 1%	7,650,000	3.0	12	14,400	82	16	7.60 @ 20.6 C.	2.06 @ 22.6 C.
21 days	100 × 1%	6,730,000	13.0	6½	11,500	80	19	6.18 @ 19.5 C.	1.92 @ 23.2 C.
28 days	100 × 1%	6,680,000	11.0	4	9,500	78	11	6.40 @ 21.0 C.	2.06 @ 19.5 C.
35 days	100 × 1%	5,770,000	44.0	13	14,200	84	12	4.66 @ 20.8 C.	1.88 @ 18.2 C.
56 days	190 × 1%	6,200,000	31.0	17	10,200	86	8	6.32 @ 18.8 C.	2.36 @ 18.7 C.
	190 × 2%								
77 days	685 × 2%	5,900,000	40.0	11	20,000	84	10	5.80 @ 18.2 C.	2.66 @ 20.7 C.

Previous investigators (Myers and Baker[3]; Elwell[7]; Bryant[8]; Joseph[5]) had shown that exposure of pectin solutions to heat causes relatively rapid reduction in viscosity and in the size of the pectin molecule (depolymerization), resulting in the formation of a brown flocculent precipitate. Bryant[8] reported that the autoclaving of a pectin solution, consisting of molecules having a molecular weight of approximately 225,000, causes a reduction of the molecular weight after fifteen minutes to 75,000, after thirty minutes to 30,000 and after two hours to 3,000.

Similar observations were made when the pectin solutions used in the experiments reported here were adjusted by the addition of lime water or of phosphate buffer solution to the neutral point and then autoclaved for ten or twenty minutes at 8 or 15 pounds' (3.5 or 7 Kg.) pressure. The viscosity dropped from the original value of 4.96 at 21 C. to 1.2, while a flocculent precipitate was formed in the dark amber liquid having a caramel-like odor. The formation of a precipitate caused a lowering of the osmotic pressure (23 cm. of water). It was demonstrated that there was a similar marked drop in osmotic pressure when flocculation developed in a freshly prepared, nonautoclaved pectin solution left for twenty-four hours in a collodion bag during osmotic pressure experiments. Heated pectin solutions therefore have physicochemical properties differing markedly from those of fresh, unheated ones.

The heated pectin solutions used in the experiment were obtained as follows: The 1.2 per cent pectin solution prepared as described in an earlier paragraph was autoclaved at 15 pounds for twenty minutes. After cooling it was filtered through a Büchner filter and Whatman no. 1 filter paper for the removal of the precipitate formed. It was then resterilized under the same conditions as before. A buffer salt solution (9 Gm. of sodium chloride, 1 Gm. of potassium dihydrogen phosphate, 6 Gm. of disodium acid phosphate and 250 cc. of water) was sterilized separately. For injection purposes three parts of the pectin solution were mixed with one part of the buffer salt solution, giving a mixture of an almost neutral reaction (p_H 6.5). For the preparation of a neutralized 2 per cent pectin solution the amount of sodium phosphate was doubled (12 Gm.). The sterilized, nonneutralized pectin solution is unstable and must be prepared freshly, as on incubation in the ice box it forms a slight fine grayish white precipitate. The amount of this precipitate is moderately increased when neutralized, sterilized pectin solutions are kept in the ice box.

In the hematologic studies with the heated pectin solution 3 dogs were used, which were treated similarly to those in the previous experiment. Table 3 lists the hematic reactions observed in 1 dog after a single intravenous injection of 27 cc. of a sterilized 1 per cent pectin solution. These reactions were characteristic of the entire series.

The results are similar to those obtained with the unsterilized pectin solution. The effect on the number of erythrocytes during the first seventy-two hours is striking. There is also definite and progressive lowering of the viscosity of the whole blood.

Table 4 presents the data obtained after repeated intravenous injections of 1 per cent and 2 per cent sterilized pectin solution.

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7. Elwell, W. E.: Pectin: Its Manufacture, Properties and Uses, Seattle, University of Washington, State W. P. A. Project, 1939.

8. Bryant, E. F.; Palmer, G. H., and Joseph, G. H.: Proc. Soc. Exper. Biol. & Med. 49:279, 1942.

It is evident that this treatment exerts mainly only a hastening effect on the sedimentation rate, leaving the number of erythrocytes, the amount of hemoglobin, the volume of packed red cells, the number and the ratio of leukocytes and the viscosity of the whole blood and of the plasma without appreciable changes.

Organic Reactions.—The changes effected in the various tissues and organs by the repeated intravenous injection of autoclaved and nonautoclaved pectin solution were studied in 7 dogs and 12 rabbits. Four dogs and 6 rabbits were given nonautoclaved pectin solution, and 3 dogs and 6 rabbits were treated with autoclaved pectin solution.

Of the 4 dogs given injections of nonautoclaved pectin solution, 3 were used in the hematic study. The weights of these animals at the start and at the end of the experiment, the total amount of pectin solution administered and the duration of the experiment are given in table 5.

TABLE 3.—*Hematic Reactions in Dog 805, Weighing 6.4 Kg., After a Single Intravenous Administration of 27 Cc. of a 1 per Cent Buffered Sterilized Pectin Solution*

	Erythrocytes per Cu. Mm.	Sedimentation Rate, Mm.	Coagulation Time, Min.	Leukocytes per Cu. Mm.	Polymorphonuclears, per Cent	Lymphocytes, per Cent	Viscosity	
							Whole Blood	Plasma
Start	6,010,000	14	11	10,700	88	9	6.06 @ 16.0 C.	2.00 @ 21.5 C.
5 minutes	6,040,000	46	18	4,600	80	17	5.92 @ 16.5 C.	2.00 @ 21.6 C.
15 minutes	6,000,000	37	9½	8,800	81	14	6.08 @ 17.0 C.	1.98 @ 21.8 C.
45 minutes	5,890,000	41	8	10,900	78	11		
2 hours	5,460,000	28	6	11,100	89	8		
4 hours	5,560,000	36	6	13,700	81	8	5.36 @ 17.4 C.	1.90 @ 21.7 C.
6 hours	5,080,000	4	12½	12,800	80	10		
8 hours	5,020,000	2	13	12,900	80	13		
24 hours	5,050,000	2	4	9,900	87	10	4.92 @ 18.0 C.	1.80 @ 21.4 C.
48 hours	5,180,000	33	7	8,900	71	18	4.32 @ 18.2 C.	1.81 @ 21.4 C.
72 hours	5,080,000	18	5	10,900	84	8		
7 days	5,760,000	20	11	20,900	79	18		
10 days	5,520,000	39	9	21,600	87	10	4.84 @ 21.4 C.	1.86 @ 19.7 C.
14 days	5,410,000	15	.....	17,700	72	23	6.06 @ 19.0 C.	2.13 @ 22.2 C.

The dogs were killed at the end of the experimental period.

The 6 rabbits of this experiment received in the beginning 10 cc. of a 1 per cent solution five times a week. This dose was gradually increased to 30 cc. daily, and the concentration was doubled. Four of the rabbits died after the thirteenth, fourteenth, fifteenth and sixteenth injection, respectively, while the surviving 2 rabbits were put to death after forty-nine injections. The total amount given varied for the individual animal from a minimum of 330 cc. to a maximum of 1,380 cc. of pectin solution, delivered within three to twelve weeks.

The injections were tolerated by the dogs and rabbits without any immediate untoward effects. It was noted in the rabbits, however, that the aural veins which were used for injection became gradually obliterated and that because of this complication 4 of the rabbits had drooping, swollen, purplish red ears.

At postmortem examination the spleens of the 4 rabbits which died early during the experiment were about four times normal size, pale grayish red and relatively soft. The livers were enlarged and had dark brown-red surfaces showing light grayish spotting or were grayish brown. Multiple dark red indurations were present in the lungs.

TABLE 4.—*Hematic Reactions in Dog 805, Weighing 6.4 Kg., After Repeated Intravenous Administration of 550 Cc. of 1 per Cent and 875 Cc. of 2 per Cent Buffered Sterilized Pectin Solution*

	Amount of Pectin, Cc.	Erythrocytes per Cu. Mm.	Sedimentation Rate, Mm.	Coagulation Time, Min.	Leukocytes, per Cu. Mm.	Polymorphonuclears, per Cent	Lymphocytes, per Cent	Viscosity	
								Whole Blood	Plasma
Start	.....	5,410,000	15	.....	17,700	72	23	6.06 @ 19.0 C.	2.13 @ 22.2 C.
3 days	60 × 1%	6,040,000	4	9	17,300	76	17	6.00 @ 20.0 C.	2.12 @ 22.6 C.
10 days	100 × 1%	5,630,000	29	4½ sec.	20,100	91	4	5.50 @ 18.3 C.	1.95 @ 22.8 C.
17 days	100 × 1%	5,200,000	3	13	16,900	76	16	5.38 @ 20.2 C.	1.94 @ 18.8 C.
24 days	100 × 1%	5,680,000	19	9½	14,100	77	16	4.85 @ 20.2 C.	1.76 @ 17.5 C.
45 days	190 × 1% 190 × 2%	5,660,000	46	7½	11,700	71	23	5.65 @ 18.5 C.	2.00 @ 18.4 C.
58 days	685 × 2%	6,300,000	7	11½	14,400	67	26	6.08 @ 17.5 C.	1.64 @ 20.1 C.

In the 2 rabbits and 4 dogs which were killed at the end of the experimental period the spleens were moderately enlarged. The livers were normal in appearance and color. The lymph nodes were somewhat enlarged. All other organs were normal in size, consistency and color.

The following observations were made on microscopic examination:

A few to numerous pulmonary precapillary and capillary vessels were filled by a stringy refringent homogeneous unstained or faintly bluish-stained material, which was surrounded by proliferated endothelial cells and multinucleated giant cells or by a multinucleated syncytial mass. Large polypous accumulations of giant cells or polypous granulomatous lesions consisting of giant cells and foam cells in a mucinous matrix projected into the lumens of several small or medium-sized vessels (fig. 1 *A*). Some of the precapillary vessels had markedly thickened hyaline walls, while others were infiltrated and surrounded by leukocytes and lymphocytes. Small giant cell nodules enclosing globules of homogeneous matter were found in the perivascular tissue. The endothelial lining of the pulmonary artery of 1 dog showed foam-cellular transformation and cushion-like thickenings.

In some animals the myocardium had a loose granular cytoplasmic texture. The myocardial arterioles often had thickened hyaline walls, while larger arteries

TABLE 5.—*Data on Dogs Given Injections of Nonautoclaved Pectin Solution*

Dog	Weight, Kg. At Start	Weight, Kg. At End	Amount of Pectin Solution, Cc.	Duration, Weeks
779	7.1	7.5	1,635	12
797	6.3	6.0	1,605	12
821	9.0	8.8	1,505	10
823	7.8	5.9	400	4

showed hyaline swelling of the media, in which swollen, irregularly arranged and distorted nuclei were embedded (fig. 1 *B*). In several animals there were foam-cellular transformation and thickening of the epicardial and myocardial coronary vessels, occasionally associated with foam cellular infiltration of the media. In others the endothelial lining exhibited edematous swelling, resulting in a scalloped appearance of the intima.

In 2 rabbits the endothelial lining of the ascending portion of the aorta consisted of a single layer of cuboidal foam cells. This was associated in places with diffuse "mucinous" imbibition of the inner media. The muscle cells in such areas were swollen and had loose, almost unstained cytoplasm. In all the dogs the aorta exhibited smaller or larger foci of endothelial and often foam-cellular proliferation. The smallest lesions consisted of groups of three or four crowded endothelial cells, possessing in places foamy, swollen cytoplasm. Somewhat larger ones formed small shallow elevations consisting of densely packed cells with small oval nuclei (fig. 2 *A* and *B*). There were large foam-cellular cushion-like intimal thickenings in other places (fig. 2 *C*). The foam-cellular accumulations were occasionally eroded on the surface and infiltrated with dark oval-shaped cells. In larger lesions both types of cells freely invaded the edematous subintimal media and were found in and around the vasa vasorum. A narrow hyaline necrotic zone was occasionally found in the subintima underneath some of the foam-cellular accumulations. Older lesions showed more or less complete hyalinization of the intimal thickenings, which in a few instances also contained larger areas of calcifi-

Fig. 1.—*A*, pulmonary vessel with polypous foam-cellular and giant-cellular granuloma of the intima; × 150. *B*, myocardial arteriole with swollen and partly hyalinized media containing irregularly distributed round nuclei; × 200.

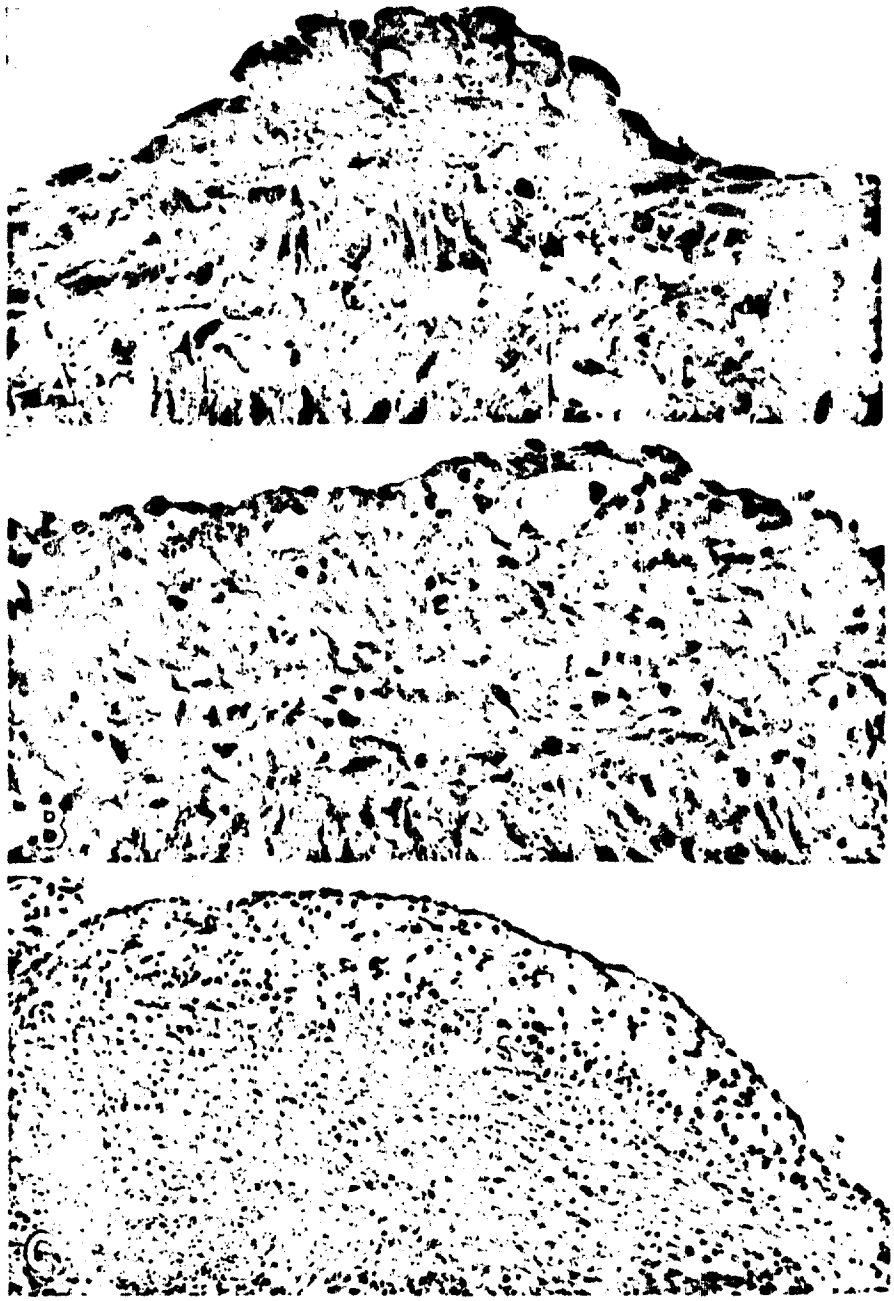

Fig. 2.—*A*, aorta with a small cone-shaped proliferation of the endothelial cells; × 700. *B*, aorta with multiple foci of endothelial proliferation and transformation of endothelial cells into foam cells; × 200. *C*, cushion-like foam-cellular thickening of the intima with beginning invasion of the media; × 150.

cation and of fibrous transformation. Larger areas of hyaline degeneration of the deeper parts of the media were found in several instances (fig. 3 *A*).

Similar lesions were present, though less frequently and extensively, in the arteries branching from the aorta.

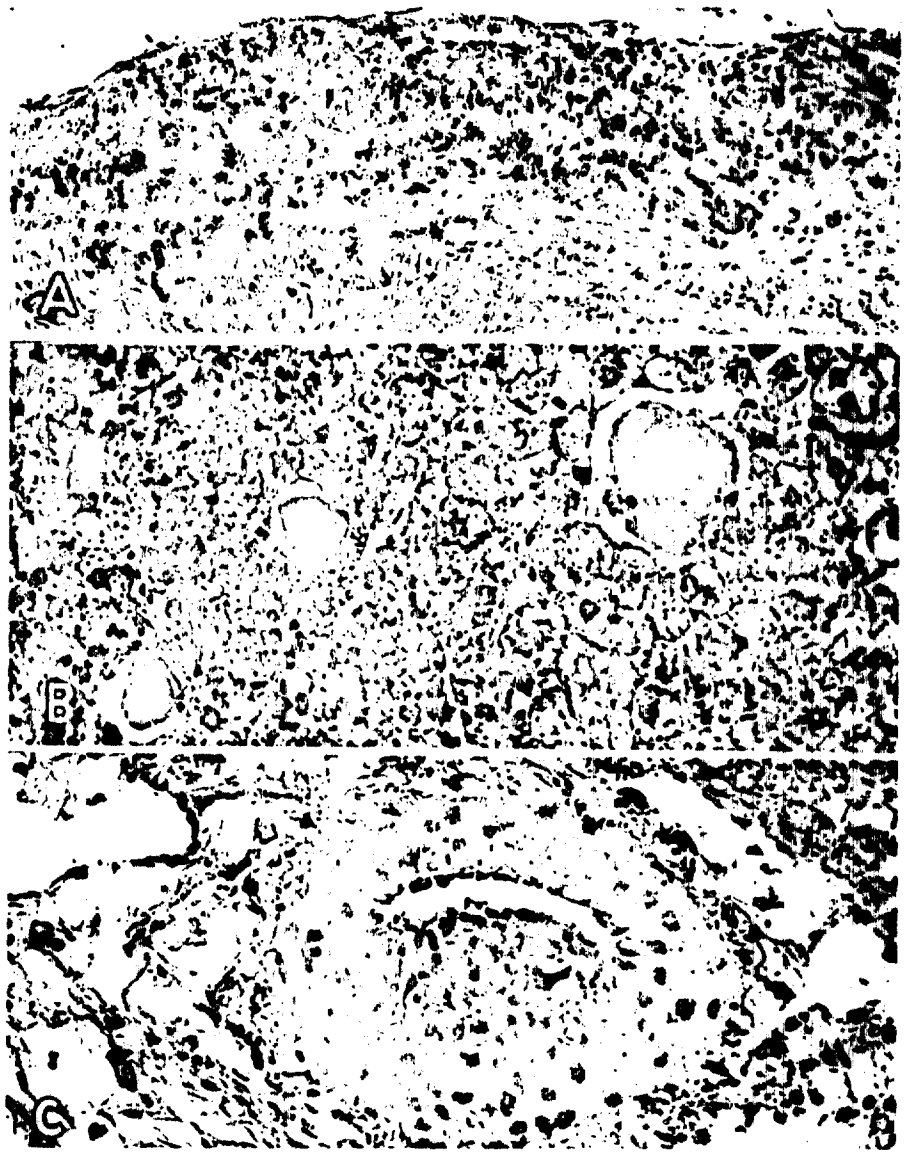

Fig. 3.—*A*, hyaline thickening of the aortic intima with diffuse calcium incrustations; × 150. *B*, liver with foam type liver cells and focal accumulations of Kupffer cells, some of which have the character of multinucleated giant cells; × 350. *C*, small cushion-like thickening of the intima of a hepatic artery; × 180.

Figure 4

(See legend on opposite page)

The thyroid and parathyroid glands were normal.

The hepatic cells in the livers of the different animals showed varying degrees of foam-cellular transformation (fig. 3 *B*). This change was particularly marked in the rabbits, while in the dogs the cytoplasm of these cells had a flaky appearance. Similar differences occurred in the participation of the Kupffer cells in the storage process. These cells in the rabbits were in general swollen, proliferated and not infrequently changed into multinucleated giant cells, which occurred occasionally also in the periportal connective tissue. Giant type and foam type Kupffer cells, on the other hand, were infrequent in the livers of the dogs. Branches of the hepatic artery occasionally contained small cushion-like proliferations of endothelial cells, which had partly a foamy cytoplasm (fig. 3 *C*).

In the 4 rabbits which died after thirteen to sixteen injections the pulp of the spleen was replaced by large conglomerations of huge multinucleated giant cells and foam cells embedded in a homogeneous and stringy material (fig. 4 *A*). Atrophic lymph follicles were surrounded by zones of hyaline necrosis, in the periphery of which were found ill defined syncytial masses with their nuclei arranged in a semicircle. Similar but less extensive lesions were present in 1 dog. In the 2 rabbits which were killed at the end of the experiment as well as in 3 dogs the spleen was hyperplastic and contained a varying amount of brown pigment and sometimes a scattering of foam cells.

Apart from general moderate hyperplasia, there was no pathologic change in the lymph nodes.

The stomach and intestine were normal in all animals except 1 rabbit, in which the small intestine contained several mucosal hemorrhages associated with greenish gray-tinted foam cell infiltrations in the submucosa.

The pancreas showed no abnormalities.

In several animals the reticulum cells of the adrenal medulla were enlarged and possessed foamy cytoplasm. However, this change remained always within moderate limits.

In the kidneys of the rabbits and of 2 of the dogs the glomeruli possessed in varying degrees a grayish purple homogeneous matrix and showed swollen, foam type endothelial cells and multinucleated giant cells (fig. 4 *B*). Thickening of Bowman's capsule, which was occasionally adherent to the grapelike transformed glomerulus, was seen in places. Erythrocytes and hyaline material were found in the capsular spaces. The interstitial tissue of the cortex was diffusely thickened in several animals and was infiltrated with round cells and foam cells. The tubules were in general normal. The arterioles of the canine kidneys often showed endothelial proliferation and hyalinization in the form of shallow or polypous bulges projecting into the lumens. Other arterioles exhibited medial hypertrophy with focal hyalinization. The main renal artery of 1 dog showed marked hyaline intimal thickening with calcium incrustation (fig. 4 *C*).

EXPLANATION OF FIGURE 4

A, spleen with marked atrophy of the lymph follicle, perifollicular hyalinization and numerous multinucleated giant cells and foam cells in the pulp; × 150. *B*, glomeruli with an edematous interstitial tissue, foam type endothelial cells and multinucleated giant cells; × 275. *C*, renal artery with hyaline and calcified thickening of the intima; × 275. *D*, foam-cellular transformation and proliferation of the reticulum cells of the bone marrow; × 250.

The bone marrow of the sternum in the rabbits was a loose structure which was permeated by strands and clusters of large foam cells of reticulum cell origin (fig. 4 *D*). These changes were absent in 3 dogs and were only mild in the fourth. The myeloid elements were mainly of the immature type.

No lesions were found in the cerebral parenchyma, the vascular system of the brain or the choroid plexus.

The anterior portion of the hypophysis contained numerous eosinophilic cells. Some of the pale grayish blue-stained cells were swollen and ballon-like and had foamy cytoplasm.

Many of the sections were stained with ruthenium red for the histochemical demonstration of pectin (Tobler [9]). Large amounts of pectin were visualized by this method as cherry red-stained material in the distended pulmonary capillaries. Reddish granules were found in the foam-cellular intima of the aorta as well as in the foam type liver cells and reticulum cells of the adrenal. The pectin retained in the Kupffer cells was globular or represented a larger solid mass. The giant cells of the spleen were filled with similar masses from which a stringy red-stained network radiated into the sinusoidal spaces. The glomerular capillaries and endothelial cells were filled with reddish globules. Solid casts of

TABLE 6.—*Data on Dogs Given Repeated Injections of an Autoclaved Pectin Solution*

Dog	Weight, Kg.		Amount of Pectin Solution, Cc.	Duration, Weeks
	At Start	At End		
796	7.5	10.75	1,475	11
805	6.4	4.5	1,450	9
845	6.0	4.5	430	4

deep red-stained matter were present in the tubular lumens. The foam type reticulum cells of the bone marrow gave a striking reaction for red-stained globular matter.

Sections of the various organs, especially the liver, the spleen and the aorta, stained for fat substances with sudan III, were practically free from such substances. Only a few orange red granules were visible in a few of the splenic giant cells.

A similar pathologic study was made of 3 dogs and 6 rabbits which were given five injections a week of an autoclaved pectin solution, prepared as described in an earlier paragraph. The 6 rabbits, weighing about 3 Kg. each, received daily 35 cc. of a 2 per cent autoclaved pectin solution for a period of fifteen weeks (74 injections with a total of 2,520 cc. of injected solution). The injections were tolerated well. The rabbits were killed at the end of the experimental period. The autopsy showed that 3 had markedly enlarged spleens. All other organs were grossly normal.

Two of the dogs used in this investigation were those used for the hematic studies. Table 6 gives the experimental data of this series.

As dog 845 became rapidly anemic under the treatment and lost considerable weight (1.5 Kg. in four weeks), it was killed after four weeks of treatment. The autopsy showed a highly edematous pancreas and atelectatic areas in the lungs. The postmortem observations on the other two dogs of this series were normal.

9. Tobler, F.: Ztschr. f. wissensch. Mikr. 23:182, 1906.

The histologic examination of the tissues of the animals revealed the following lesions:

One of the 6 rabbits exhibited the fully developed picture of atheromatosis of the aorta and intimal thickenings with medial degeneration of the renal and pulmonary arteries and capillaries as well as the thesaurosis of the internal organs (giant cell granulomatous lesions in the splenic pulp, foam-cellular transformation of liver cells and reticulum cells of bone marrow). In the other 5 rabbits the reactions elicited were limited to accumulations of foam cells in the bone marrow and to minor foci of intimal thickening and medial hyaline degeneration of the pulmonary and renal arteries and of the aorta.

In the 3 dogs only mild edematous changes in the aortic wall were observed. The intima had in places a garland-like outline caused by the presence of small accumulations of round or cuboidal cells underneath balloon-like swollen endothelial cells. Similar edematous vascular reactions occurred occasionally in smaller vessels of the elastic type. There was no evidence of a thesaurosis in any of the organs studied.

COMMENT

The data presented indicate that the injection of nonautoclaved, freshly prepared solutions of the hydrophilic colloid pectin elicits reactions in the blood and in the tissues of the internal organs which are similar to or identical with those caused by other macromolecular colloidal agents (colloidoclastic leukopenia, accelerated erythrocytic sedimention, reduction of erythrocytes, atheromatosis, thesaurosis). The shortening of the coagulation time noted in these animals, which is in contrast to observations with other macromolecular agents (polyvinyl alcohol, methyl cellulose), represents apparently a specific action of the acid groups contained in the pectin molecule and confirms the statements made by previous investigators concerning the hemostatic effect of pectin (Violle and Saint-Rat [10]; Riesser and Nagel [11]; Sack [12]; Gohrbandt [13]; Derouaux [14]; Riesser [15]; Baumann [16]; Feissly [17]; Ziegelmayer [18]).

The lack of prolonged clotting time and of increased plasmatic viscosity even after prolonged treatment with highly viscous pectin solutions is an indication of the relative instability of this substance in the blood.

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10. Violle, H., and de Saint-Rat, L.: Compt. rend. Acad. d. sc. **180**:603, 1925; Compt. rend. Soc. de biol. **92**:1097, 1924.
11. Riesser, O., and Nagel, A.: Arch. f. exper. Path. u. Pharmakol. **179**:748, 1935.
12. Sack, G.: Klin. Wchnschr. **14**:1536, 1935.
13. Gohrbandt, E.: Deutsche med. Wchnschr. **62**:1625, 1936.
14. Derouaux, G.: Arch. internat. de pharmacodyn. et de thérap. **62**:100, 1939.
15. Riesser, O.: Klin. Wchnschr. **14**:958, 1935.
16. Baumann, E.: Beitr. z. klin. Chir. **166**:298, 1937.
17. Feissly, R.: Compt. rend. Soc. de biol. **92**:317, 1925.
18. Ziegelmayer, W.: Kolloid-Ztschr. **71**:214, 1935.

This fact apparently also accounts at least in part for the absence of any appreciable and persistent myeloid reactions in the blood of the dogs treated by repeated injection of pectin solutions, such as those seen after the repeated intravenous introduction of polyvinyl alcohol and methyl cellulose. The retention of pectin in the reticulum cells of the bone marrow may be active in the same direction, by interfering with the proliferative activity of the bone marrow.

The storage phenomena in the liver, spleen, kidney and bone marrow are, on the other hand, evidence that that part of the pectin which is taken up within cells is of higher stability or is protected by the conditions prevailing in the cytoplasm against the rapidly disintegrative influences operative in the blood. The morphologic manifestations characterizing these thesaurotic reactions bear a marked similarity to certain lipoidoses. Thus, the foam-cellular pectinic transformations of the reticulum cells of the bone marrow resemble similar lipoidal changes associated with Schüller-Christian disease. In this respect it is remarkable that the rabbits showed the various storage phenomena much more highly developed than the dogs. Differences in relative dose of pectin introduced may account in part for these discrepancies.

Mention must be made, however, of the fact, that the thesaurosis was most marked in the 4 rabbits which died early in the experiment, while it was less pronounced in the 2 rabbits which survived longest and therefore received the largest total dose. Similar observations were made in connection with experiments on methyl cellulose thesaurosis, in which the dogs which died spontaneously after a relatively short time in the experiment revealed the most extensive splenic necrosis with numerous giant cell granulomatous lesions identical with those seen in the 4 pectin-treated rabbits. Species-specific and individual metabolic differences therefore may contribute to the variations in the degree of thesaurosis elicited by various colloidal storage substances.

The observations concerning the different developmental stages of pectin atheromatosis leave no doubt that the first sign of the formation of atheroma is a proliferation and swelling of a few endothelial cells. With further development of the multiple small seedlike lesions there occurs progressive proliferation and foam-cellular transformation of the endothelial cells, producing smaller and larger intimal thickenings. Foam cells from these foci may later invade the edematous inner media, while at the same time endothelial cells of the vasa vasorum may take up pectin and be thereby transformed into foam cells. There may occur subsequently a breakdown of the atheromatous tissues, with the development of erosions and the appearance of small round or oval cells with dark-stained round nuclei within the atheromatous lesions and the surrounding media. In other instances, the necrosis of the foam cells

is followed by calcification. Hyaline transformation of the intimal thickenings with or without larger calcium incrustations may follow ultimately. During these intimal developments, the superficial media may undergo degenerative changes which assume the shape of a hyaline band underneath the atheromatous lesions. Larger areas of hyaline degeneration and calcification are found later on in the middle zone of the media.

In addition to these focal alterations, the arterial intima sometimes exhibits more diffuse foam-cellular transformation. Although similar intimal lesions are observed occasionally in the pulmonary vessels, changes typical of this organ are giant cell granulomatous lesions of the intima, such as those previously described in animals given injections of polyvinyl alcohol, methyl cellulose or emulsions of cholesterol. The renal, myocardial and hepatic arterioles, on the other hand, show proliferative and degenerative intimal and medial lesions which are similar to those seen in the large elastic arteries.

While blood smears show the presence of vacuolated mononuclear cells in the circulating blood, there is no evidence that these participate significantly in the production of the atheroma.

There seems to be little doubt that the atheromatous formations produced by the retention of pectin in the endothelial cells of the arterial intima are of less permanence than those elicited by polyvinyl alcohol, methyl cellulose or cholesterol. The data available indicate that the foam-cellular intimal proliferations lose relatively rapidly their pectin content and undergo either hyaline necrosis or fibroblastic organization. Thus, primary atheromatous lesions are transformed into secondary sclerotic lesions. While observations made in animals given injections of polyvinyl alcohol or methyl cellulose (unpublished experiments) suggested the occurrence of such transitions, which are known in connection with cholesterol atheromatosis, the data presented in regard to pectin atheromatosis show that the speed of occurrence of such secondary changes depends on the metabolic stability of the causal atheromatogenic substance.

The capillaries of the renal glomeruli exhibit lesions such as are found in connection with the intravenous introduction of polyvinyl alcohol, methyl cellulose, acacia, ovalbumin-azoprotein, ovalbumin and gelatin, and resemble those seen in certain types of human glomerulitis. They apparently represent reactions to disturbances in glomerular filtration and are elicited by the retention of pathologic macromolecules circulating in the plasma in the glomerular capillaries.

The validity of these conclusions is supported by the observations made in the animals given injections of the autoclaved pectin solution as well as those reported previously by others (Myers and Baker [3];

Joseph [5]; Hinton [4]; Elwell [7]; Bryant, Palmer and Joseph [8]; Bonner [19]). As it was shown that heating pectin solution causes hydrolysis of the pectin molecule and thus its depolymerization, it is significant that the animals treated with the autoclaved pectin solution showed, with the exception of 1 rabbit, an almost complete lack of storage phenomena in the internal organs and the presence of only minor vascular degenerative changes, among which typical foam-cellular lesions were strikingly absent. These findings indicate that the marked reduction in the molecular weight and size of the pectin molecule produced by exposure to prolonged heat resulted in increased and relatively rapid excretion of the injected macromolecular material or in ready progressive metabolic degradation to still smaller molecular units penetrating easily the filtration membranes. It is thus demonstrated that the production of atheromatosis and thesaurosis by the injection of pathogenic macromolecular substances depends on the size of the molecules and on the metabolic stability of these substances.

Inasmuch as autoclaved pectin solutions have been proposed as nonhematogenous blood substitutes in the treatment of shock (Hartman and co-workers [6]; Bryant and co-workers [8]), these observations deserve special attention. It is obvious that both groups of investigators, particularly Hartman and co-workers, employed a solution that contained essentially degradation products of pectin which possessed a molecular weight definitely below that of the smallest plasma proteins. It stands to reason that molecules of this type will penetrate the injured and highly permeable filtration membranes of shocked persons almost as readily as the molecules of crystalloid solutions, and are presumably just as effective as these. Attention must be called to the fact that the concentration of pectin in autoclaved solutions is markedly lower than that in the original solution before sterilization, as a considerable amount of precipitate must be removed from an autoclaved solution before it becomes suitable for intravenous medication. If one considers that pectin solutions are relatively acid (*p*H 3.2) and that the difficulties connected with their preparation are accentuated when such solutions are neutralized, it becomes evident that it is practically impossible to prepare a sterilized and standardized product of neutral reaction which consists of pectin molecules of sufficient size and which does not deteriorate rapidly into a solution of low molecular degradation products under the formation of a precipitate.

It may be pointed out in this connection that the effectiveness of colloid therapy for shock, regardless of the type of colloid used, does not depend primarily on the osmotic pressure exerted by a particular colloidal solution, as any effects depending on such action are more

19. Bonner, J.: Bot. Rev. 2:475, 1936.

readily obtainable by the introduction of crystalloid solutions, but on the hydrophilic properties of the colloid and its retention in the circulating blood even under the conditions of increased vascular permeability prevailing in shock. Any osmotic effect which a colloid may exert, either directly or following its formation of salts, is of value only as long as the aforementioned prerequisites are fulfilled.

SUMMARY

Pectin solutions either freshly prepared and neutralized with phosphate buffer solution or autoclaved and neutralized were injected into dogs and rabbits.

The immediate effects produced on the blood by either of the two solutions are shown in colloidoclastic leukopenia, acceleration of erythrocytic sedimentation and moderate shortening of clotting time.

Repeated injections of pectin solutions do not cause any increase in the viscosity of the plasma or any changes in the number and the ratio of leukocytes.

In dogs and rabbits given injections of the freshly prepared pectin solution, marked foam-cellular storage phenomena develop in the spleen, liver, kidney and marrow in addition to foam-cellular atheromatosis of the various arteries. Older vascular lesions of this origin are characterized by hyaline intimal thickening with or without calcification and by hyaline necrosis and calcification in the media underneath.

Dogs and rabbits given injections of the autoclaved pectin solution showed, on the other hand, with the exception of 1 rabbit, only minor storage phenomena in the bone marrow and foci of hyaline degeneration and thickening of the arteries.

These differences in degree and type of reaction are due to the fact that pectin solutions are markedly depolymerized when exposed to heat and lose thereby much of their macromolecular characteristics and their original physicochemical properties.

These observations confirm the concept of the presence of a fundamental causal relation between physicochemical colloidal disturbances of the plasma and vascular atheromatosis and organic thesaurosis.

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Effect of Hypocholesterolemic Agents on Intestinal Cholesterol Absorption.* (28087)

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Recently, several compounds, administered by the oral route, have been reported to be effective in lowering blood cholesterol levels in man and experimental animals. Stern and Treadwell† observed that cholesterol trimethylacetate, a branched-chain fatty acid ester of cholesterol, inhibited hydrolysis of cholesterol butyrate by pancreatic cholesterol esterase *in vitro*. It was also found that this branched-chain ester reduced blood cholesterol levels in rats when added to a diet containing cholesterol butyrate. According to Vahouny and Treadwell(1), cholesterol trimethylacetate itself is not absorbed in lymph-fistula rats and is not hydrolyzed by intraluminar cholesterol esterase.

Tennant *et al.*(2,3) have reported that MK-135 (cholestyramine), a bile acid-binding polymer, causes a marked reduction of blood cholesterol levels and a lower incidence of atheromatosis in cockerels. No toxic effects, either local or systemic, were detected in dogs fed MK-135 for as long as one year. This compound was also found to be effective in reducing blood cholesterol levels in hypercholesteremic patients(4).

Keys *et al.*(5,6) reported that a diet containing undigestible, or difficultly-digestible polysaccharides, such as cellulose, hemicellulose, or pectin, reduced blood cholesterol levels in humans. In rats, addition of pectin to a cholesterol diet largely counteracted any increase in plasma and liver cholesterol due to cholesterol feeding(7).

Nicotinic acid has been shown to be effective in lowering blood cholesterol levels in rabbits and humans; however, nicotinamide did not have a comparable effect(8,9). A study of the effect of nicotinic acid on hepatic synthesis of cholesterol showed a reduc-

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† Unpublished observations

TABLE I. Composition of Intragastric Test Emulsions.

Emulsion No.	mg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Albumin	50	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oleic acid	292	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sodium taurocholate	288	+	+	+	+	+	+		+	+	+	+	+	+	+	+
Cholesterol-4-C^{14}	50		+	+		+		+	+		+		+		+	
Cholesterol-trimethylacetate	61			+	+											
MK-135	50					+	+									
"	238								+	+						
Pectin	250										+	+				
Nicotinuric acid	100												+	+		
Pyridine-3-acetic acid	100														+	+

All emulsions except emulsions 10 and 11 were made to 3 ml with 0.9% saline.
Emulsions 10 and 11 contained all components and one-half the pectin in 3 ml, and the remaining pectin in a second 3 ml of saline.

tion in incorporation of labeled acetate into cholesterol(10,11). However, the actual mechanism by which nicotinic acid exerts its entire observed cholesterol-lowering effect has not been clarified. Parson *et al*(12), and Miller *et al.*(13) have suggested that nicotinuric acid, a metabolite of nicotinic acid, might play a role in cholesterol metabolism. In fact, addition of nicotinuric acid to the diet did cause a reduction of blood cholesterol levels in humans(14). Also pyridine-3-acetic acid which is structurally related to nicotinic acid was reported to have a blood cholesterol-lowering effect in humans (15). When this substance was administered orally, there was a striking and consistent reduction of total blood cholesterol levels.

Although several mechanisms have been suggested as explanations for the effects of these various compounds on blood cholesterol levels, little definitive data have been presented. In some cases, as with nicotinic acid, the test substance may reduce blood cholesterol, at least in part, by interference with hepatic sterol synthesis. However, it is also possible that interference with dietary cholesterol absorption may be a factor in the observed depression of blood cholesterol levels.

Essentially all absorbed cholesterol enters the blood stream *via* the thoracic lymph duct (16,17). Thus by analysis of the thoracic duct lymph of rats, it is possible to quantitatively determine the direct effect of each of the mentioned compounds on intestinal absorption of cholesterol-4-C^{14} from aqueous homogenates.

Experimental. Adult male rats of the Carworth Farms Wistar strain were maintained on standard laboratory chow‡ and water *ad libitum* prior to operation. The thoracic duct was cannulated by a modification of the procedure of Bollman *et al.*(18), and the animals were subsequently fasted for 18-24 hr with free access to 0.9% saline.

The aqueous test emulsions shown in Table I were prepared immediately before use, as described by Vahouny *et al.*(19). The amounts of oleic acid (292 mg), sodium taurocholate (288 mg), and cholesterol-4-C^{14} (50 mg) used were in an optimum molar ratio of 8:4:1, respectively(20). Albumin was included in the preparation of all test emulsions.

The rats in all groups except those receiving pectin (Groups 10 and 11) were given 3 ml of the appropriate test emulsion intragastrically using light vinyl ether anesthesia. Due to the insolubility of pectin, the rats in Groups 10 and 11 were given 3 ml of the test emulsion containing all the components and only half the pectin, and one hour later were given a second 3 ml feeding containing the remainder of the pectin.

Throughout this study, Group 1 which received only albumin, oleic acid and taurocholate was used as the control group for endogenous sterol absorption. The absorption of fed cholesterol-4-C^{14} under optimal conditions was determined in Group 2. The effect of each test substance on endogenous cholesterol absorption was measured by inclusion of the test compound in the control

‡ Purina Laboratory Chow, Ralston Purina Co., St. Louis, Mo.

TABLE II. Effects of Test Compounds on Cholesterol Absorption from Intestine in Lymph-Fistula Rats.

Number of rats	Lymph cholesterol/24 hr Free	Total	Ester	Cholesterol-4-C^{14} absorbed	"Extra" cholesterol esterified
	(mg)	(mg)	(%)	(%)	(%)
Group 1—Control*					
7	5.0 ± .1†	14.9 ± .8	66.1 ± 1.1		
Group 2—Cholesterol-4-C^{14}					
7	6.8 ± .3	30.8 ± 1.3	77.7 ± .8	37.7 ± 1.8	89.4 ± 1.8
Group 3—Cholesterol-4-C^{14}, cholesterol trimethylacetate					
5	6.8 ± .4	31.5 ± 2.8	77.9 ± 1.7	32.5 ± 4.9	90.2 ± 2.6
Group 4—Cholesterol trimethylacetate					
5	5.3 ± .5	15.4 ± 1.9	64.4 ± 1.6		
Group 5—Cholesterol-4-C^{14}, MK-135 (50 mg)					
4	5.8 ± .4	25.7 ± 1.9	77.5 ± .2	27.7 ± 4.0	94.0 ± 1.8
Group 6—MK-135 (50 mg)					
4	5.1 ± .2	13.3 ± .3	61.6 ± 1.7		
Group 7—Cholesterol-4-C^{14}‡					
6	6.0 ± .3	21.4 ± 1.6	71.8 ± 1.5	16.6 ± 1.9	
Group 8—Cholesterol-4-C^{14}, MK-135 (238 mg)					
6	6.0 ± .3	15.0 ± 1.7	71.0 ± .1	11.0 ± 2.0	87.7 ± 4.1
Group 9—MK-135 (238 mg)					
6	3.5 ± .4	8.7 ± 1.0	58.9 ± 1.8		
Group 10—Cholesterol-4-C^{14}, Pectin					
6	5.9 ± .2	26.1 ± .7	77.3 ± .6	30.7 ± 2.4	92.4 ± 1.4
Group 11—Pectin					
6	5.1 ± .4	14.9 ± 1.1	66.1 ± 1.1		
Group 12—Cholesterol-4-C^{14}, nicotinuric acid					
4	6.5 ± .5	32.4 ± 2.3	79.9 ± .6	32.4 ± 4.4	88.1 ± 1.2
Group 13—Nicotinuric acid					
4	4.1 ± .5	12.7 ± 1.2	67.9 ± 1.3		
Group 14—Cholesterol-4-C^{14}, pyridine-3-acetic acid					
4	6.6 ± .4	34.4 ± 1.0	80.9 ± .7	36.3 ± 2.4	89.4 ± 1.4
Group 15—Pyridine-3-acetic acid					
4	4.2 ± .5	12.1 ± 1.3	65.3 ± 1.0		

* All groups received saline, albumin, oleic acid and sodium taurocholate, as described in Table I, in addition to the indicated supplements.

† Values represent mean ± S.E. S.E. = $\sqrt{(x-\bar{x})^2/n(n-1)}$

‡ This group did not receive sodium taurocholate.

emulsion lacking cholesterol-4-C^{14} (Groups 4, 6, 9, 11, and 15). After administration of the test emulsion, lymph was collected for 24 hr in vessels containing 0.5 ml heparin (1:1000), and was subsequently frozen till analysis.

Free and esterified cholesterol were determined on acetone-ethanol (1:1) extracts of the lymph samples by the procedure of Sperry and Webb(21).. Free and total cholesterol-4-C^{14} were determined as the digitonides, as described by Swell *et al.*(22).

The experimental data are summarized in Table II, and are expressed as mg free or esterified cholesterol per 24 hr lymph collection, and as percentage of the total lymph cholesterol. "Extra" cholesterol represents the chemical difference in total lymph cholesterol between the corresponding control and experimental groups. The percentage absorption of cholesterol was calculated from the total cpm administered and the cpm in total lymph cholesterol per 24 hr.

All differences were analyzed for significance by the "t" test(23); probability values of $>.05$ were considered significant, and $>.01$ as highly significant.

Results and discussion. Experiment 1. Cholesterol trimethylacetate. This experiment was designed to study the specific effect of cholesterol trimethylacetate on endogenous and exogenous absorption of cholesterol. According to Stern and Treadwell(1),† the addition of cholesterol trimethylacetate to a diet containing cholesterol butyrate produced a decrease in the proportion of the esterified fraction in the total blood cholesterol within 1 week. However, by the end of the third

week of feeding, the proportion of esterified cholesterol in the experimental animals had increased and was the same as in the control group. Furthermore, cholesterol trimethylacetate itself was not absorbed in the rat(1). Thus, it appears that this branched-chain ester may inhibit the hydrolysis of cholesterol butyrate by competition for cholesterol esterase in the intestinal lumen. Addition of 61 mg of cholesterol trimethylacetate (which is equivalent to 50 mg of free cholesterol) to the test emulsion containing free cholesterol-4-C^{14} (Group 3) did not alter the percentage absorption of exogenous cholesterol ($p > .05$) (Table II). There was also no significant change in total lymph cholesterol levels (compare Groups 2 and 3) during the 24 hr period after administration of the test emulsion. The data from these groups indicate that cholesterol trimethylacetate does not inhibit absorption of exogenous free cholesterol from the intestine. As is shown in Group 4, which did not receive cholesterol-4-C^{14}, this ester also did not affect the endogenous lymph cholesterol levels or the lymph cholesterol partition.

Experiment 2. MK-135§ or cholestyramine, has been shown to be effective in binding bile acids *in vitro*(3). According to Tennant *et al.*(2), feeding MK-135 to rats does not lower blood cholesterol levels as it does in other species. This species difference may be due to a greater ability of rats to synthesize hepatic cholesterol(24). However, the results of the present study show that this bile acid-binding polymer markedly inhibits absorption of fed cholesterol from the intestine.

Two levels of MK-135 were used in this experiment. In Groups 5 and 6, 50 mg of the polymer were included in the emulsion, an amount equal to the cholesterol fed. In Groups 8 and 9, 238 mg of MK-135 were added to the emulsions. This amount is 2 molar equivalents with respect to the bile salt administered.

As is shown in Table II, addition of 50 mg of MK-135 to the test emulsion containing cholesterol-4-C^{14} decreased absorption from 38% (control level in Group 2) to 28%; furthermore, administration of 238 mg of the polymer with cholesterol (Group 8) further reduced absorption to 11%. The chemical data show that total lymph cholesterol of rats in Group 8 was essentially the same as in Group 1, which received no exogenous cholesterol in the emulsion.

It is apparent from the data obtained in Group 9 (received 238 mg MK-135 but no cholesterol-4-C^{14}) that the large dose of MK-135 also markedly lowered total lymph cholesterol derived from endogenous sources (compare Groups 9 and 1). This depression was probably due in large part to an inhibition of endogenous sterol absorption by effective removal of circulating bile salts.

Evidence that MK-135 is effective in the binding of endogenous bile salts is shown by comparison of cholesterol absorption in Groups 7 and 8 with Group 2. Group 7 received no exogenous bile salt and absorption of the administered cholesterol-4-C^{14} was 16.6%, in comparison with 37.7% in Group 2. In Group 8 addition of MK-135 to the complete emulsion, which contained 288 mg sodium taurocholate, absorption of labeled cholesterol was 11%. This decrease from 16.6 to 11% absorption suggests that the test substance not only effectively eliminated the stimulation of cholesterol absorption by the exogenous bile salt, but also interfered with the effect of endogenous bile salt.

The results of this study suggest that the blood cholesterol-lowering effect of MK-135 is due in part, if not completely, to impaired cholesterol absorption from the intestine, and that the effect is due to binding of bile salts in the intestinal lumen by the resin.

Experiment 3. Pectin‖ feeding at a level of 5% of the diet has been reported to counteract the increase in plasma and liver cholesterol due to cholesterol feeding in rats(7). In the present study, pectin was included in preparation of the emulsions at a level of 250 mg, or 5 times the amount of cholesterol-4-C^{14} used. However, due to the bulk of this compound, it was necessary to feed 125 mg

§ MK-135 was kindly supplied by Dr. Jesse W. Huff, Merck Inst. for Therapeutic Research.

‖ Pectin (polygalacturonic acid, methyl ester) was obtained from Sunkist Growers, Inc., Ontario, Calif.

of the substance with the other components of the test emulsion, and to administer the remainder of the pectin (125 mg) one hour later. Administration of this amount of pectin in an emulsion lacking cholesterol (Group 11) had no effect on total lymph cholesterol or on the cholesterol partition (compare with Group 1) (Table II). However, when pectin was given in a complete emulsion containing cholesterol-4-C^{14} (Group 10), absorption of labeled cholesterol was depressed from 38% in Group 2, to 31% ($p<.01$). Chemical data also showed a significantly lower total lymph cholesterol level, due primarily to a decrease in the esterified cholesterol fraction.

The actual mechanism by which pectin exerts its blood cholesterol-lowering effect is not well defined. Data from the present study indicate that the observed effect of pectin on blood cholesterol is due in part to an inhibition of intestinal absorption of dietary cholesterol.

In Experiment 4, 100 mg of either nicotinuric acid¶ (Groups 12 and 13), or pyridine-3-acetic acid (Groups 14 and 15) were employed. These amounts were twice that of the cholesterol-4-C^{14} administered.

It is apparent from the data summarized in Table II that there is no change in total lymph cholesterol levels due to administration of nicotinuric acid or pyridine-3-acetic acid, either in control groups lacking dietary cholesterol (compare Groups 13 and 15 with Group 1), or in the cholesterol-fed groups (compare Groups 12 and 14 with Group 2). Also, analysis of lymph cholesterol-4-C^{14} showed no significant depression of labeled cholesterol absorption due to administration of either test substance. It was observed that soon after administration of the test compounds, severe diarrhea developed; in addition, survival of the test animals in these groups was only 50% compared to almost 90% in all other groups in the present study.

The results of this experiment suggest that the site of action of nicotinuric acid and pyridine-3-acetic acid in lowering blood cholesterol levels is not on intestinal cholesterol absorption.

Throughout all groups in the present study in which a net increase in lymph cholesterol occurred, the percentage esterification of the "extra" or absorbed cholesterol ranged between 88% and 93%. As has been previously discussed(25), it is apparent that esterification of cholesterol during absorption is independent of amount of cholesterol absorbed, and these data support the importance of esterification of natural sterols in the absorptive mechanism.

Summary. 1. The direct effect of several blood cholesterol-lowering agents on intestinal absorption of cholesterol-4-C^{14} was studied in lymph-fistula rats. 2. MK-135 (cholestyramine) and pectin caused significant reductions in lymph total cholesterol and absorption of cholesterol-4-C^{14}. 3. With MK-135, the larger of 2 doses studied also reduced absorption of endogenous cholesterol from the intestine. 4. Cholesterol trimethylacetate, nicotinuric acid and pyridine-3-acetic acid had no effect on cholesterol absorption or lymph cholesterol levels. 5. In all groups where significant cholesterol absorption occurred, the percentage esterification of absorbed cholesterol was constant (88-93%), irrespective of the extent of absorption.

¶ Nicotinuric acid and pyridine-3-acetic acid were purchased from California Corp. for Biochemical Research.

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CONTRIBUTIONS TO THE STUDY OF TOXICITY AND HEMOSTATIC ACTION OF PECTIN EXTRACTED FROM QUINCES

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Pectins are widely spread in nature, in fruits and some plants with roots; they are found free or as insoluble compounds named protopectins.

From the chemical point of view, pectins are methoxilate polysaccharides with a very large molecule. By acid hydrolysis they yield arabin, methylic alcohol and pectinic acid and by further hydrolysis we get arabinose, galactose and galacturonic acid.

Pectins are soluble in water with which they form very viscous colloidal solutions.

Literature mentions some therapeutic properties of these substances. Thus, studies have been published stressing the antibacterial properties of pectins, fact that lead to their clinical use in intestinal infections or other local infections. Other works state the hemostatic action of pectins.

With pectins obtained according to a method we will describe ulteriorly, we undertook a series of research experiments with the following objectives:

1. determination of toxicity of prepared pectins.

2. experimental and clinical research of their hemostatical activity.

3. study of its antibacterial activity in induced infections and determination of the value of this activity in various diseases as: skin infections (pyodermia), burns and surgical wounds.

4. cautious hydrolysis of pectins in view of reducing their molecular weight and diminish their hemostatical action to enable their utilization as subtitutes for plasma.

In the present article we will present the results obtained when checking the pectin toxicity on mice and their hemostatical action on rabbits.

In our toxicity research experiments we used white mice, weighing 20 g. each. The product was intravenously injected as a 4% solution in an isotonic sodium chloride solution. The mice had been divided in six lots of five mice each; one lot received o.5 ml solution, namely 1 g pectin/kilo-body, while the other lots received 0.6 , 0.7 , 0.8, 0.9 , 1 ml, respectively 1.2 , 1.4 , 1.6 , 1.8 , 2.0g pectin/kilo-body. The animals which received 0.5 , 0.6 , 0.7 , and 0.8 ml tolerated well the product, without showing any eventual external symptom which would have revealed a reaction to the injected substance. Of the lot which had been injected with 0.9 ml, three animals died, while all the animals which had received 1 ml injections, died,only after a few minutes. Death occured after the animals fell in lateral decubitus , followed by spasms, tachycardia and tachypnea.

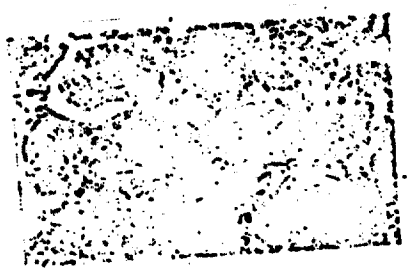


Fig.1.- Kidney section. Hemorrhage in glomeruli and tubules. Agglutination of erythrocytes in vessels. Hematoxylin-eosin coloring.

Fig.2.- Pancreas section. Fibrino-hematic thrombi in the large vessels. Hematoxylin-eosin coloring.

The histopathological examination for which hematoxylin-eosin coloring was used, showed on the dead animals, visceral circulatory trouble, more visible in the kidney, liver and lungs, with capillary stasis and visceral multiple thrombosis as follows:

Kidneys: Hyperemia at all capillary level and especially in the cortex; hemorrhage in glomeruli and tubules,with abundant quantity of ferric pigment; agglutination of erythrocytes in the vessels (fig.1). Aspect of recently formed hematic thrombi.

Pancreas: Hyperemia of capillaries with erythrocyte conglutination and formation

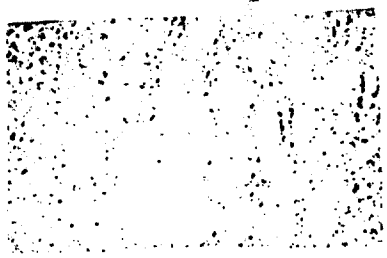


Fig.3.- Liver section Incipient thrombosis in centrolobular vein . Hematoxylin-eosin coloring.

of fine nets of fibrin in the larger vessels. Fibrino-hematic thrombi (fig.2).

Liver. Accentuated hyperemia and lysis of erythrocytes in all the vessels of the liver. Incipient thrombosis aspect(fig.3).

Lungs. Hyperemia of capillaries in alveolar ducts; some capillaries are thrombosed. The thrombi have the aspect either of a hematic thrombus or of a hyalin one. (fig.4).

Spleen. Hyperemia in sinuses and multiple hemorrhage (fig. 5).

Striated muscles. Troubled intumescence of some muscular fibers.

Intestines. Do not present apparent modifications.

Brain. Diffuse hyperemia of capillaries without any other apparent modifications.

Due to the apparition of numerous fibrino-hematic thrombi in the vessels, the histopathological examination results suggest that some intense modifications occured in the coagulability of the blood, provoking the death of the animals.

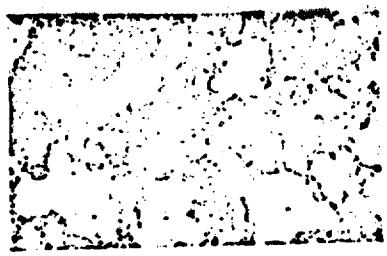


Fig.4.- Lung section. Hyperemia of capillaries in alveolar ducts. Hematoxylin-eosin coloring.

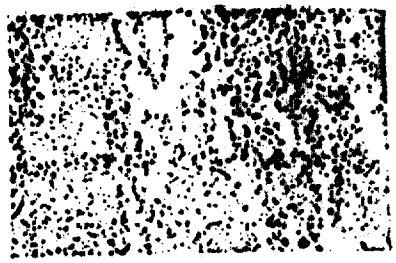


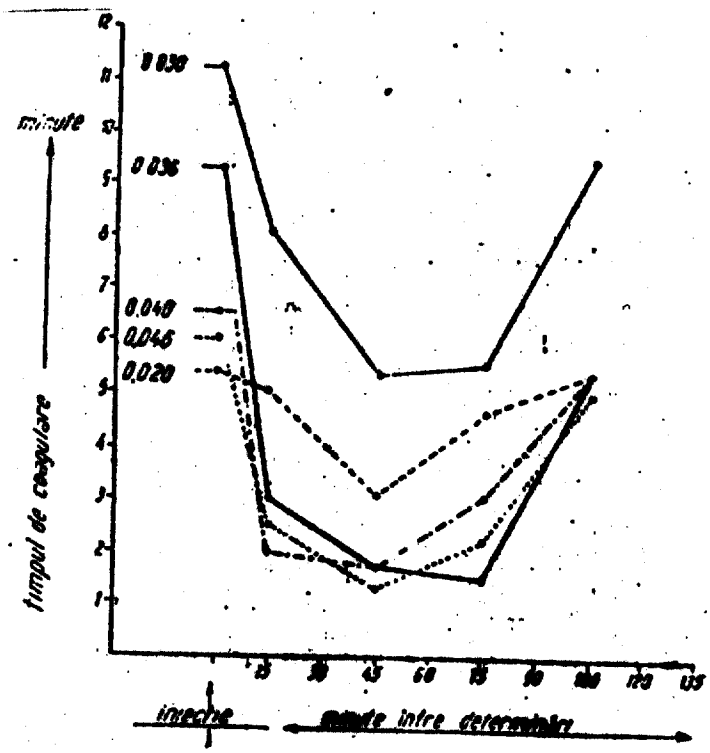
Fig.5.- Spleen section. Hyperemia and hemorrhage in sinuses. Hematoxylin-eosin coloring.

The hemostatical activity has been checked on normal rabbits which received intravenous injections of 4% pectin solutions in isotonic sodium chloride solution. The coagulation time was determined for each animal before the injection and then the determination was repeated after 15, 45, 75, and 105 minutes. These determinations have been made under identical conditions in all cases (humid atmosphere, Petri dishes) using the method of the drop on a slide.

Male rabbits have been used for the experiments; they weighed 1500-2900 g. and were divided in lots of 2 animals each. Each lotreceived injections of different quantities of pectin, as follows:

Lot I -0.046 g pectin/kilo-body
Lot II -0.040 g pectin/kilo-body
Lot III-0.036 g pectin/kilo-body
Lot IV -0.030 g pectin/kilo-body
Lot V -0.020 g pectin/kilo-body

The following graphical representation gives us the variations of the coagulation time for these lots.



Key: a= minutes, b= coagulation time, c= injection, d= minutes between determinations.

This graphical representation indicates the following :

1. Pectins,intravenously administered, have important hemostatical properties, confirmed by the decrease of the coagulation time.

2. The maximum hemostatical activity is established after 30 minutes, continues 45-75 minutes, and returns to normal gradually after 105 minutes.

3. The maximum coagulant action occured when the amounts administered were 0.046-0.036 g/kilo-body,

while the 0.030-0.020 g/kilo-body doses influenced in a smaller degree the coagulation time.

Following through the leukocytosis evolution, we observed that for most of the animals submitted to the experiment, the number of leukocytes increased 2 hours after the administration of pectins, and that this increase continued 48 hours. The hyperleukocytosis returns to normal after 72 hours for most of the animals.

We can draw from our experiments the following conclusions:

1. In case of intravenous administration, the toxic dose for mice was established at 1.80 g pectin/kilo-body. The smaller doses are well tolerated by the animals, which shows that animals can tolerate quite large amounts of pectin.

2 2. An intense hemostatic activity of the pectins has been determined by histopathological examination and by experiments; this activity varies in proportion with the administered doses.

3. The experimental research on rabbits showed us that the maximum hemostatic action occurs when doses of 0.036-0.046 g pectin/kilo-body are intravenously administered.

4. The amounts 0.036-0.046 g pectin/kilo-body reduce the coagulation time with 70-80% of its initial value, while a quantity of 0.030 g pectin/kilo-body reduces it with more than 50%, and one of 0.020 g kilo-body reduces it with more than 30%.

5. The histopathological examination confirms the hemostatic value of the pectins because all the deceased animals had vascular multiple thrombosis.

The results we obtained with the pectin extracted from quinces correspond to the results obtained by A. I. Polejaeva.

The pectin extracted from quinces could be used on some future day to prepare a hemostatic which would enlarge the number of medicaments of the Romanian industry.

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Contribuţii la studiul toxicităţii şi al acţiunii hemostatice a pectinelor izolate din gutui

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Pectinele sînt foarte răspîndite în natură, în fructe şi în unele plante cu rădăcini, în care se găsesc, atît libere, cît şi sub formă de combinaţii insolubile, numite protopectine.

Din punct de vedere chimic, pectinele sînt polizaharide metoxilate, cu molecula foarte mare. Prin hidroliza lor acidă se obţine arabina, alcool metilic şi acid pectinic, iar dacă hidroliza este mai avansată, rezultă arabinoza, galactoză, acid galacturonic.

Pectinele se dizolvă în apă, formînd soluţii coloidale foarte vîscoase.

În literatură se citează unele proprietăţi terapeutice ale acestor substanţe. Astfel, au fost publicate studii care au scos în evidenţă acţiunea antibacteriană a pectinelor, ceea ce a determinat aplicarea lor clinică în infecţii intestinale sau unele infecţii locale. În alte lucrări a fost expusă acţiunea hemostatică a pectinelor.

Preparînd pectinele după un procedeu pe care-l vom comunica ulterior, am întreprins, cu produsele obţinute, o serie de cercetări avînd următoarele obiective:

1. stabilirea toxicităţii pectinelor preparate;
2. cercetarea pe cale experimentală şi clinică a acţiunii lor hemostatice;
3. cercetarea activităţii antibacteriene în infecţii experimentale şi stabilirea valorii ei în diferite boli, ca: infecţii ale pielii (pioderminte), arsuri, plăgi chirurgicale;
4. hidrolizarea menajată a pectinelor, pentru a reduce greutatea lor moleculară şi a diminua acţiunea hemostatică, în vederea folosirii lor ca substituent al plasmei.

În acest articol vom prezenta rezultatele obţinute în legătură cu toxicitatea pectinelor pe şoarece şi activitatea hemostatică pe iepuri.

Pentru cercetarea toxicităţii am folosit şoareci albi în greutate de 20 g. Preparatul a fost administrat pe cale intravenoasă, sub formă de soluţie 4% în soluţie izotonică de clorura de sodiu. Şoarecii au fost împărţiţi în grupe de câte cinci: unui lot i s-au injectat [illegible]

soluție, respectiv 1 g pectină/kilocorp, iar loturilor următoare li s-au injectat cîte 0,6; 0,7; 0,8; 0,9; 1 ml, respectiv 1,2; 1,4; 1,6; 1,8; 2,0 g pectină/kilocorp. Animalele cărora li s-au injectat 0,5; 0,6; 0,7 și 0,8 ml au tolerat bine preparatul, fără să se observe vreun semn exterior care să trădeze eventuale reacții față de substanța injectată. Din lotul căruia i s-au injectat 0,9 ml au murit trei animale, iar din lotul căruia i s-a injectat 1 ml, după cîteva minute de la administrare au sucombat toate animalele. Moartea s-a produs prin cădere în decubit lateral, urmată de spasme și tahicardie cu tahipnee.

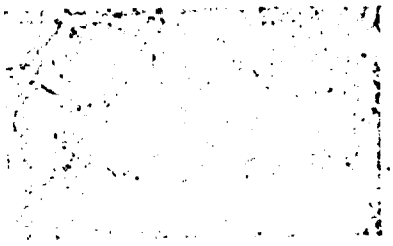


Fig. 1. — Secțiune prin rinichi. Se observă hemoragii în glomeruli și în tubi, precum și aglutinarea eritrocitelor în vase. Colorația hematoxilină-eozină.

Fig. 2. — Secțiune prin pancreas. Trombi fibrino-hematici în vasele mari. Colorație hematoxilină-eozină.

Examenul histopatologic la care a fost folosit colorantul hematoxilina-eozină arată, la animalele succombate, tulburări circulatorie viscerale, mai pronunțate în rinichi, ficat și plămîni, cu stază capilară și tromboze multiple viscerale, după cum urmează:

Rinichi. Hiperemie la nivelul tuturor capilarelor și mai ales în substanța corticală; hemoragii în glomeruli și în tubi, cu formare de pigment feric în cantitate abundentă; aglutinarea eritrocitelor în vase (fig. 1). Aspect de trombi hematici recent formați.

Pancreas. Hiperemia capilarelor, cu conglutinarea eritrocitelor și formarea unor rețele fine de fibrină în vasele mai mari. Trombi fibrinohematici (fig. 2).

Ficat. Hiperemie accentuată și aglutinarea eritrocitelor în toate venele ficatului. Aspect de tromboză incipientă (fig. 3).

Fig. 3. — Secțiune prin ficat. Tromboză incipientă în venă centrolobulară. Colorație hematoxilină-eozină.

Plămîni. Hiperemia capilarelor din pereții alveolari; unele capilare sînt trombozate. Trombul are, fie aspectul unui tromb hematic, fie al unui tromb hialin (fig. 4).

Splina. Hiperemie în sinusuri și hemoragii multiple (fig. 5).

Mușchi striați. Intumescență tulbure a unora dintre fibrele musculare.

Intestin. Nu prezintă modificări aparente.

Creier. Hiperemia difuză a capilarelor, fără alte modificări aparente.

Datorită apariţiei a numeroşi trombi fibrino-hematici în vase, examenul histopatologic pledează în favoarea unor accentuate modificări în coagulabilitatea sîngelui, care au provocat moartea animalelor.

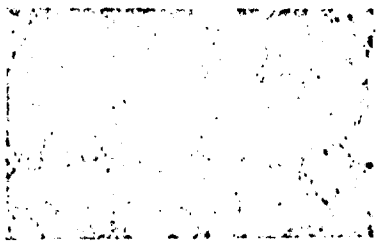


Fig. 4. — Secţiune prin plămîn. Hiperemia capilarelor din pereţii alveolari. Coloraţia hematoxilină-eozină.

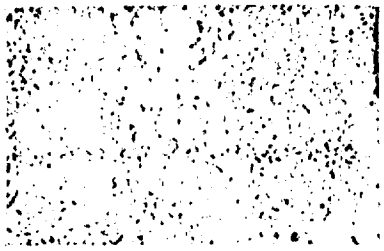


Fig. 5. — Secţiune prin splină. Hiperemie şi hemoragii în sinusuri. Coloraţia hematoxilină-eozină.

Activitatea hemostatică a fost încercată pe iepuri normali, cărora pectinele le-au fost administrate intravenos, sub formă de soluţie 4% în soluţie izotonică de clorură de sodiu. Timpul de coagulare a fost determinat la fiecare animal înainte de administrarea preparatului, apoi determinarea a fost repetată la 15, 45, 75 şi 105 minute. În acest scop, lucrînd în condiţii identice pentru toate probele (în atmosferă umedă, în cutii Petri), a fost folosită metoda picăturii pe lamă.

Pentru cercetări au fost folosiţi iepuri masculi, în greutate de cîte 1 500—2 900 g, împărţiţi în loturi a cîte două exemplare. Fiecărui lot i s-au injectat cantităţi diferite de pectine, după cum urmează:

Lotul I—0,046 g pectină/kilocorp
Lotul II—0,040 g pectină/kilocorp
Lotul III—0,036 g pectină/kilocorp
Lotul IV—0,030 g pectina/kilocorp
Lotul V—0,020 g pectină/kilocorp

Graficul de la pag. 419 reprezintă variaţiile timpului de coagulare la aceste loturi.

Din examinarea acestui grafic rezultă următoarele:

1. Pectinele, administrate intravenos, au dovedit importante calităţi hemostatice, verificate prin scăderea timpului de coagulare.

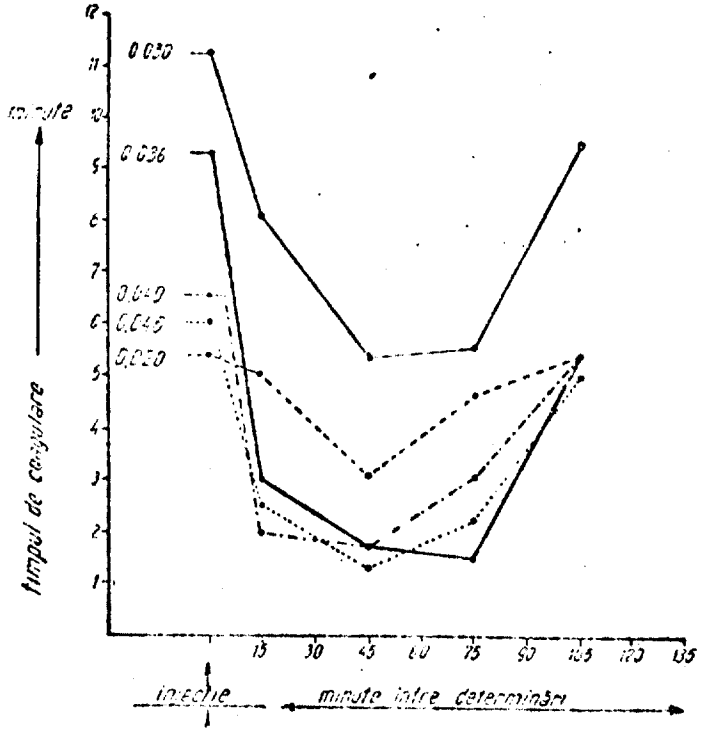
2. Acţiunea hemostatică maximă se instalează după 20 de minute şi se prelungeşte la 45—75 de minute, revenind treptat la normal după 105 minute.

3. Acţiunea coagulantă maximă a avut loc la administrarea a 0,046—0,036 g/kilocorp, în schimb dozele de 0,030—0,020 g/kilocorp au influenţat mai puţin timpul de coagulare.

Urmărind evoluţia leucocitozei, la majoritatea animalelor supuse experimentării s-a constatat o creştere a numărului de leucocite după 5 ore de la administrarea pectinelor, aceasta creştere menţinîndu-se timp de 48 de ore. La cele mai multe dintre animale, hiperleucocitoza revine la normal după 72 de ore.

Din cercetările întreprinse am putut trage următoarele concluzii:

1. La administrare intravenoasă, doza toxică pentru şoareci a fost stabilită la 1,80 g pectină/kilocorp. Dozele mai reduse sînt bine tolerate de animale, de unde rezultă că ele suportă cantităţi destul de mari de pectine.



2. Verificată experimental şi prin examen histopatologic, se constată o însemnată acţiune hemostatică a pectinelor, acţiune care variază în raport cu cantitatea administrată.

3. Cercetarea experimentală pe iepuri arată că acţiunea hemostatică maximă are loc la administrarea intravenoasă a 0,036—0,046 g pectină/kilocorp.

4. Cantitatea de 0,036—0,046 g/kilocorp reduce timpul de coagulare cu 70—80% din valoarea lui iniţială, în timp ce cantitatea de 0,030 g pectină/kilocorp îl reduce cu peste 50%, iar 0,020 g/kilocorp. cu peste 30%.

5. Examenul histopatologic confirmă valoarea hemostatică a pectinelor, animalele sacrificate prezentînd multiple tromboze vasculare.

Rezultatele realizate cu pectina preparată de noi din gutui concordă cu rezultatele obţinute de A. I. Polejaeva.

Pectina obținută din gutui va putea servi la prepararea unui hemostatic care va îmbogăți asortimentul de medicamente al industriei indigene.

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PECTIC ENZYMES
V. THE FATE OF PECTINS IN THE ANIMAL BODY [1]

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ONE FIGURE

(Received for publication May 20, 1940)

For several reasons the fate of pectins in the animal body is of interest. The pectins of various fruits and vegetables are a part of the regular diet and although they are present in small quantities it would be of importance to know whether they are utilized as certain other polysaccharides are. Pectin is also an important component of the apple diet often successfully employed to combat diarrhea. The usefulness of this treatment is generally recognized (Council on Foods, American Medical Association, '37) but its working mechanism has not yet been satisfactorily explained. No final answer to this question can be expected until the metabolic fate of the various components of apples is clear.

All investigators who have studied this question agree that pectin added to the diet of animals and human subjects cannot be recovered from the feces. Fürth and Engel ('31), Manville, Bradway and McMinis ('36) and others believe that pectin is a digestible carbohydrate, hydrolysed and utilized in the animal body, but there is no evidence supporting this assumption. Murer and Crandall ('40) suggest that pectin is not hydrolysed in the digestive tract of the dog.

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An investigation of the working mechanism of the apple diet was begun at this Experiment Station some time ago and in connection with it various tests were conducted to obtain information on the fate of pectins in the animal body. The results obtained are presented herewith. A method for the determination of possible slight decomposition of pectins is also described with the hope that it will facilitate further investigations in this field.

METHODS AND MATERIALS

The powdered citrus pectin used was exhaustively extracted with cold 50% alcohol and contained 88.4% uronic acid (Lefèvre and Tollens, '07, expressed as polygalacturonic acid) and 10.28% CH_3O determined by saponification. It also contained 0.49% ash, and 1 gm. of the pectin gave 1.09 gm. of calcium pectate (Carré and Haynes, '22). The pectin was thus composed almost exclusively of polygalacturonic acid in which most of the carboxyl groups were esterified.

It may be noted that a preliminary precipitation of the pectins with acidified alcohol is usually desirable. The alcohol precipitate is dissolved in a small volume of hot water and this solution used for the determination of calcium pectate and also of the relative viscosity by an Ostwald viscosimeter at 30°C.

In these experiments it was necessary to use a method by which any possible small digestion could be determined on a portion of the pectin applied. It has been reported previously (Kertesz, '39) that upon the slightest decomposition of pectin, there is a great decrease in its viscosity. This causes the ratio $\frac{\text{calcium pectate}}{\text{relative viscosity}}$ to increase materially. The method is applicable only in cases of slight decomposition because upon extensive hydrolysis the calcium pectate precipitate disappears completely.

In all in vitro experiments 30°C. was used rather than body temperature because the degeneration of the viscous properties of pectin is slower at this temperature. There was also less likelihood of slow heat inactivation of the various en-

zymes at this temperature during prolonged digestion. Finally it was also desirable to have the reactions proceed at the same temperature at which the viscosity determinations were performed. In order to avoid hydrolysis or saponification of the pectin by the medium, all in vitro experiments were made at pH 3.4 and 6.4.

EXPERIMENTAL RESULTS

The observation that pectin added to the diet is digested in the body was substantiated by several experiments on dogs as well as on human subjects. In not a single case could any pectin be found in the feces, indicating that it was completely hydrolysed. To discover the agencies responsible for this hydrolysis various digestive secretions were examined for their effect on pectin.

Saliva collected from human subjects, dogs and cows had no effect on pectin at pH 3.4 and 6.4 during several weeks of incubation in the presence of toluol.

Jejunal juice was collected from an isolated auto-transplanted segment in a dog; about 12 ml. were obtained in 4 hours. It contained very active saccharase, amylase and peptidase. To test its possible pectolytic activity 0.5 ml. portions of it were digested with 5.0 ml. of a 1.5% pectin solution in the presence of toluol for several days. Tests performed at various times up to 3 days gave no indication of the slightest decomposition.

Attempts to digest pectin in the stomach and small intestine of the dog failed. The dog used in this experiment weighed 14 kg. and had an enterostomy in the lower end of the jejunum. Previous experiments on the same dog indicated that, of 48 gm. available carbohydrate in test meal ingested, only 15 to 20% could be recovered from the fistula. With the aid of a stomach tube 45 ml. of a 2.0% solution of pectin, followed by about 25 ml. of water, was introduced. The dog showed no signs of discomfort.

At intervals of 60, 90 and 160 minutes 36 ml., 41 ml. and 10 ml. liquid respectively were obtained. In the combined

samples the pectin was precipitated and washed with alcohol, dried at room temperature and dissolved in water; 98.8% of the pectin introduced was recovered. The relative viscosity of this solution when compared with that of the original solution of the same pectin content gave no indication of hydrolysis.

In order to determine whether pectin is digested in the human stomach 200 ml. of a 1.23% solution of pectin was ingested by an adult male. Several samples of 13 to 25 ml. were

TABLE 1

Digestion of pectin in the human stomach and by pectinase

NO.	SAMPLE	DURATION OF EXPERIMENT	PECTIN PURIFIED FROM THE STOMACH CONTENTS OR DIGEST			CALCIUM PECTATE / RELATIVE VISCOSITY OF REFERENCE SOLUTION OF SAME PECTIN CONTENT (2)	DIFFERENCE BETWEEN (1) AND (2)
			Calcium pectate	Relative viscosity (water = 1)	Calcium pectate / Relative viscosity (1)		
		minutes	%				
1	Recovered by stomach tube	45	0.230	2.11	0.109	0.102	+0.007
2	Same as no. 1	90	0.137	1.79	0.077	0.081	−0.004
3	Incubated with juice from stomach	1440	0.393	3.25	0.121	0.117	+0.004
4	Vomited	30	0.117	1.46	0.076	0.071	+0.005
5	Digested with 0.1% commercial pectinase	5	0.452	2.52	0.179	0.129	+0.050
6	Same as no. 5	15	0.428	2.01	0.213	0.129	+0.084

recovered by stomach tube at intervals up to 90 minutes. The person on whom the experiments were performed was accustomed to swallowing the stomach tube and not the slightest discomfort was indicated by him from this operation or from any effect of the pectin. The samples obtained were examined separately and the results are shown in table 1. This table also shows the results obtained in an experiment in which a 5 ml. sample of juice taken from the stomach of the same person was incubated with 10 ml. pectin solution for 24 hours.

All results indicate the absence of digestion. Experiments with pectin ingested by an adult and vomited after 30 minutes also gave negative results.

Tests were conducted to determine the possible digestion of pectin by trypsin, pepsin, rennet and pancreatic amylase. Analyses of the reaction mixtures after 12 days' incubation showed that the pectin was intact.

To study the effect of feces on pectin a suspension was made of human feces and the mixture was filtered through cloth to remove the larger particles. From this suspension (containing 6.31% solids) 50 ml. aliquots were mixed with 25 ml. of a 1.25% pectin solution and the mixture incubated at 30°C. At

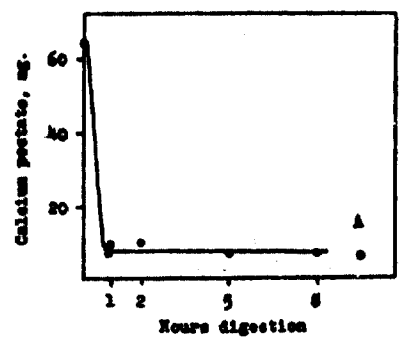

Fig. 1 Digestion of pectin with **feces suspension.** Sample "A" was **digested with feces suspension** plus 1% very active pectinase for 48 hours

intervals up to 8 hours the digests were mixed with 300 ml. of 95% alcohol, the precipitate was dissolved in hot water, made up to volume and an aliquot representing one-fifth of the original mixture was used for the precipitation of calcium pectate. The results obtained are presented in figure 1. The pectin was rapidly digested by the feces suspension. Under these conditions a small quantity of material present in the feces suspension gave a precipitate which did not disappear after prolonged digestion with pectinase. The pectin was also decomposed by feces in the presence of toluol although the progress of the digestion was irregular and much slower in

this case. Canine feces gave results similar to those shown in figure 1.

DISCUSSION

Saliva and the secretions of human and canine stomachs as well as the small intestine of dogs, are devoid of enzymes acting upon pectin. On the other hand pectin incubated with a suspension of feces is rapidly decomposed. This suggests that the pectin passes through the esophagus, stomach and small intestine without decomposition but is subsequently decomposed by the enzymes produced by the abounding flora of microorganisms rather than by enzymes excreted by the large intestine.

Obviously the validity of these conclusions depends on the acceptance of certain assumptions. There is some doubt as to whether the observations made on dogs may be used in predicting the fate of pectin in human beings. Nutritional and other conditions may also be of importance. Murer and Crandall ('40) mention that pectin fed to fasting animals is mostly excreted. Excretion of pectic materials is also apparent when the apple diet is used on patients (or animals) suffering from diarrhea. Perhaps in such cases the normal bacterial flora in the large intestine is altered; this results in a failure to decompose the pectin. There is little excretion of enzymes in the large intestine and it appears safe to assume that the pectin reaching the colon is decomposed there by bacterial enzymes.

The matter of carbohydrate absorption from the large intestine is still a controversial subject. The observations of Imhäuser ('32) that pectin orally administered to dogs does not increase the blood sugar make it probable that the galacturonic acid derived from the pectin is fermented rather than absorbed. Imhäuser also states that the decomposition products of pectin were not excreted. Tests conducted on the fermentation of pectin and galacturonic acid by various intestinal microorganisms (Saunders and Kertesz, '40) indicate that most microorganisms capable of hydrolysing pectin also ferment galacturonic acid.

It is not within the scope of this article to discuss the significance of the present findings in regard to the working mechanism of the apple diet. But it may be suggested that if pectin is not decomposed until it reaches the large intestine its therapeutic action may depend on its colloidal properties. The galacturonic acid derived from the pectin upon hydrolysis may be available for detoxication (Manville, Bradway and McMinis, '36) after absorption from the colon. It is also possible, although not very probable, that some galacturonic acid would reach the ileum by regurgitation (Manville, '40), be absorbed and participate in detoxication reactions.

The author wishes to express his appreciation to Dr. E. S. Nassett of the University of Rochester and to Dr. J. A. Dye of Cornell University for their kind cooperation in performing some of the experiments reported in this paper.

SUMMARY

Tests made with human subjects and dogs indicated that saliva and gastric juice do not contain enzymes acting upon pectin. Pectin passed through the stomach and part of the small intestine of a dog could be recovered without loss. Trypsin, pepsin and rennet had no effect on pectin in vitro, but pectin incubated with feces was rapidly decomposed.

It appears probable that pectin taken orally is not attacked until it reaches the large intestine where it is completely hydrolysed by bacterial enzymes.

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Fiber and Pectin in the Diet and Serum Cholesterol Concentration in Man.* (26401)

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Some populations characterized by low serum cholesterol values subsist on diets high in fiber or cellulose and low in saturated fats, and it has been suggested that fiber may play a role(1,2). Our work in Italy(3,4) yielded somewhat lower serum cholesterol values than seemed to correspond with our findings in controlled experiments in man when comparisons were based solely on dietary fats (5). This discrepancy was confirmed in detailed analyses of data from Italy by means of the prediction formula, based on dietary fatty acids, developed from many controlled diet experiments on man(6,7).

Accordingly, direct controlled experiments were made in which groups of men subsisted alternately on "American" and "Italian" types of diet, comparable in calories, proteins and in kind and amounts of fats but differing in the sources of carbohydrates, an abundance of fruits and vegetables in the Italian types replacing equivalent calories in simpler carbohydrates in the American types. Lower serum cholesterol levels were consistently found with the Italian diets and the differences, though not large, were statistically significant(8).

We noted that the Italian type diets "tend to be high in complex carbohydrates such as pectin, hemicelluloses and fiber"(8), so new experiments were devised to examine the possible effects of these variables. This paper presents the results. The general nature of the findings was reported in abstract form to the Council on Arteriosclerosis of American Heart Assn.(9).

Subjects, methods and procedure. Methods and procedures were the same as in experiments at the Hastings State Hospital previously reported(5-9), and the subjects were similar, *i.e.*, physically healthy, middle-aged, male mental patients maintained under completely controlled conditions in a metabolic unit. In each experiment men were maintained on a standard diet for a stabilization period of 3 weeks, then were assigned to dietary sub-groups matched as to age, relative body weight (obesity), activity habits and general level of serum cholesterol. Thereafter, for successive 3-week periods, the sub-groups subsisted on the experimental diets in a switch-back or reversal pattern so devised as to compensate for possible time trends. The design of the present experiments is summarized in Table I.

One series of experiments tested the effect of fiber or cellulose with each of 2 different diets and another tested the effect of pectin with each of 2 somewhat different diets. Blood samples were drawn from an arm vein from each man on 2 occasions at the end of each dietary period and the serum was analyzed for total cholesterol, in duplicate, by our modification of the method of Abell *et al.*(10).

Diets. Each diet was composed of natural foods in a rotating series of 7 daily menus of 3 meals each corresponding with ordinary American customs. All of the diets were closely similar in calories, fats and cholesterol but they formed 2 pairs. The diets in the S pair of experiments differed in the source of part of the carbohydrate, bread and potatoes in Diet SB being replaced in part in Diet SS by equal calories in sucrose. In the U pair of experiments, Diet UB provided a considerable amount of carbohydrate in legumes which was isocalorically matched in sugar in Diet US. The small differences in proteins resulting from these food source dif-

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TABLE I. Design of Experiments S and U. Diets fed during days indicated. In each case the same standard stabilization diet was fed during days 1-21. "C" = Alphacel, "P" = Pectin, N.F.

Group	No. men	Days 22-42	Days 43-63	Days 64-84	Days 85-105
Q	6	SB	SB + C	—	—
R	6	SB + C	SB	—	—
S	7	SS	SS + C	—	—
T	6	SS + C	SS	—	—
W	6	UB	UB + P	UB + P	UB
X	6	UB + P	UB	UB	UB + P
Y	6	US	US + P	UB + P	UB
Z	6	US + P	US	UB	UB + P

ferences were equalized by the use of wheat gluten and soy bean protein concentrate. The food items selected assured an abundance and relative constancy of vitamins and minerals in all diets. Variability in the foods themselves was avoided by laying in sufficient stocks of staples before the start of each experiment.

The nutrient contents of these diets were estimated from standard food tables. Individual differences in calorie requirements were observed during the pre-experiment stabilization periods and individual allowances were made on this basis, adjustments being made in amounts of simple carbohydrate foods provided (bread, sweets). All servings were individually measured and rejections and plate waste were recorded. Individual allowances were further adjusted when indicated from the body weights (nude) measured at frequent intervals.

A constant, closely supervised regimen of rest, exercise and recreation helped to maintain a high degree of calorie constancy. This is indicated by the actual calorie intakes and body weight data in Table II, which also shows the close correspondence between intakes of all nutrients in the basal diets and the cellulose or pectin supplemented periods with those diets.

The effects of cellulose (fiber) and of pectin were judged by comparing the same men on the same diet with and without additions of these substances at the level of 15 g daily. Cellulose ("Alphacel") was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio. Pectin N. F. (U. S. National Formulary) was donated by Sunkist Growers Co., Ontario, Calif. (Pure Citrus Pectin, lot 444-H). The "Alphacel" and pectin were incorporated in special biscuits. During periods when these supplements were not given the men ate biscuits similar in appearance and equivalent in calories.

Results. Each sub-group consisted initially of 7 men but during the experiments a few men were dropped for medical reasons, failure in cooperation or excessive variability in

TABLE II. Mean Nutrients Actually Consumed Daily during Dietary Periods Indicated in Table I. Sat., mono., and poly. refer to glycerides of saturated, monoene and polyene fatty acids. "CHO" = carbohydrate. Mean body wt pertain to the end of the periods on diets indicated by column headings. "Veg." = leafy vegetables only.

Item	SB	SB + C	SS	SS + C	UB	UB + P	US	US + P
Mean body wt, kg	69.4	69.5	67.5	67.6	70.4	70.4	70.2	70.2
Calories	3060	3025	3043	3104	3025	3033	3055	3038
Proteins, g	97	97	96	97	124	123	125	124
Total fat, g	138	137	138	139	140	140	137	137
CHO in sucrose, g	28	28	155	164	19	19	142	142
" " legumes, g	4	4	4	4	118	118	3	3
" " veg., g	2	2	2	2	5	5	5	5
" " fruits, g	35	35	35	35	21	21	21	21
Fiber, g	5	20	5	19	10	10	10	10
Cholesterol, mg	569	564	570	571	577	576	577	575
% cal. from sat.	19.7	19.8	19.9	19.7	19.9	19.8	19.7	19.8
" " " mono.	17.6	17.5	17.6	17.5	17.8	17.8	17.7	17.7
" " " poly.	1.9	1.9	1.9	1.9	2.0	2.0	2.0	2.0

TABLE III. Dietary Fiber (Cellulose). Mean serum cholesterol, mg/100 ml, at the ends of dietary periods. Δ and stand. error (S.E.) values calculated from the individual differences on the diets compared. "C" = added cellulose.

Groups	No. men	Diet type	Serum cholesterol, mg % No C	+ C	Δ	S.E.
Q, E	12	SB	204.8	212.9	8.1	± 5.0
S, T	13	SS	234.4	236.7	2.3	± 4.1

Exp. SB, t = 1.62, p = >.1
" SS, t = .56, p = >.5

eating, etc. The others remained well and showed no variations in behavior. Serum cholesterol findings are summarized in Tables III and IV.

Table III shows no effect of the cellulose supplement in either of the diets in which it was tested. Contrary to some speculations (1,2) there is no tendency for serum cholesterol concentration to fall when the diet is high in fiber or cellulose. Actually, in both experiments mean cholesterol value rose slightly but the change is not statistically significant.

Table IV shows a small but statistically significant decline in serum cholesterol concentration in both experiments when pectin was added to the diet. The average effect, a fall of about 5% from the level without the pectin supplement, is less than previously observed in comparisons of Italian and American type diets. From this it might be inferred that only a part of the differences observed on these diets could be ascribed to differences in their pectin content.

Discussion. A recent study, reported in abstract, indicates that pectin amounting to 5% of the diet fed to very young rats had no effect on cholesterol level in either plasma or liver unless the diet also included one per cent cholesterol, in which case the pectin seemed to counteract some of the cholesterol rise which occurred otherwise(11). Five per cent of the (dry) diet as pectin is of the order of twice the dosage used in the present experiments. Because of major differences among species in cholesterol metabolism, no extrapolation of these findings to man is justifiable.

TABLE IV. Dietary Pectin. Mean serum cholesterol, mg/100 ml, at the ends of dietary periods. Δ and stand. error (S.E.) values calculated from individual differences on the diets compared. "P" = added pectin.

Groups	No. men	Diet type	Serum cholesterol, mg % No P	+ P	Δ	S.E.
W,X,Y,Z	24	UB	202.4	192.7	− 9.7	± 2.6
W,X,Y,Z	24	US	221.5	211.3	−10.2	± 4.3

Exp. UB, t = 3.72, p = .001
" US, t = 2.40, p = .02

The paucity of data on fiber and particularly on pectin contents of human diets makes it difficult to relate the present experiments to diets as eaten naturally. The amount of cellulose used here is certainly higher than commonly ingested in so-called civilized diets. In the men at Hastings the cellulose supplement was well tolerated and no serious diarrhea resulted but we concluded that larger dosage would not be desirable. Alterations in gastro-intestinal function were clearly shown by changes in motility revealed by serial roentgenograms.

Calculations from such rough data as are available(12,13) suggest that 20 g of cellulose or fiber daily would seldom be attained in diets in Italy or even in the Bantu diets we studied in South Africa(14). It seems, therefore, unlikely that dietary fiber or cellulose plays any significant role in producing low serum cholesterol levels observed in Italy (13) or among the Bantu(14).

There are no reliable estimates of the amount of pectin in human diets. Pectin is present in most fruits and berries, particularly in apples, citrus fruits, and all fruits and berries notable for their value in making jellies. In citrus fruits most of the pectin is in the white inner rind and the connective tissue so it is largely lost as these fruits are ordinarily eaten, particularly in the form of juice. The largest usual source of pectin in human diets is probably in apples but 15 g daily, as used here, would be obtained from this source only by a most enthusiastic apple eater.

It is possible, of course, that more prolonged subsistence on diets containing large amounts of fiber or of pectin would have results different from those reported here.

However, the effects of dietary fats are largely or wholly achieved in a few weeks, with little or no subsequent change.

Summary. Rigidly controlled experiments on middle-aged men subsisting on diets of natural foods with and without supplements of 15 g daily of either cellulose (fiber) or pectin failed to show any significant effect on serum cholesterol concentration from the cellulose but they did consistently show an effect from the pectin. The pectin effect was apparent in 3 weeks and amounted to an average fall of about 5% below the level on the same diet without pectin supplement. It is suggested that the amounts of cellulose and pectin used correspond to the upper levels of these substances provided in natural human diets.

We are grateful to Mrs. Helen Williams for supervising the dietary work and to Mrs. Nedra Foster for aid in devising diets as well as supervision of the technicians responsible for cholesterol and food analyses. This work would have been impossible without the cooperation of Dr. William F. Sheeley, Supt. of Hastings State Hospital, and the devoted assistance of volunteer aides from the Church of the Brethren: Gary McCann, Richard W. Roller, John Hall and John Buehrer.

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Studies of Pectin Administration to Patients Not in Shock.*

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Pectin solution as a plasma substitute has been recommended by Hartman *et al.*[1,2] They demonstrated its effectiveness in experimental shock and in the prevention and treatment of shock in man. It was furthermore shown that pectin raises the plasma volume.[3]

To evaluate the usefulness of pectin solution as a plasma substitute, we studied its ability to cause hemodilution, which is a basic requirement of a plasma substitute; in addition, any untoward results following its administration were looked for, especially pseudo-agglutination of the erythrocytes. The latter has been considered an important contra-indication to the use of plasma substitutes.[4,5]

For better comparison, this study was made on 20 convalescent patients not in shock. Eight patients received a 0.75% and 12 a 1.5% solution of pectin‡ in approximately 90 minutes. The following were determined before and after the administration of pectin: (1) Arterial and venous pressure, and (2)—on venous blood drawn without tourniquet and placed into test tubes containing heparin§—hemoglobin, hematocrit, total protein (by the falling drop method), albumin and globulin (by Nesslerization), non-protein nitrogen, and sedimentation rate. Furthermore, complete hematologic data were obtained from 6 patients before and after pectin administration.

For comparison with pectin, 12 patients received a few days before or after the pectin administration, 1,000 cc of 2.5% dextrose in 0.4% saline intravenously in a period of 90 minutes.

As seen in Table I, after pectin administration the hematocrit, hemoglobin, and the total protein concentration and N.P.N. of the plasma decreased consistently, hemoglobin to a lesser degree. An increase of the mean corpuscular hemoglobin concentration was found in many instances. The albumin-globulin ratio did not change significantly. Systolic and diastolic arterial pressure and venous pressure rose in most instances. The sedimentation rate which was not at a normal level in a number of cases at the beginning of the experiment, because of the underlying disease, rose invariably. All reported constants usually returned to normal within 24 hours, in a few instances in 48 hours. The sedimentation rate, however, was sometimes found markedly elevated, even after 48 hours. Hematologic examinations revealed rouleau formation in 5 out of 6 patients and was present up to 72 hours. No other significant hematological changes were found.

Fifteen of the patients had received, prior to and following the pectin administration,

* Supported by a grant from Frederick Stearns and Company, Detroit, Mich.

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2 Hartman, F. W., Schelling, V., Harkins, H. N., Brush, B., and Warren, K. W., Scientific Exhibit, A.M.A., Atlantic City, 1942.

3 Jacobson, S. D., and Smyth, C. J., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 218.

4 Hanzlik, P. G., and Karsner, H. T., *J. Pharm. and Exp. Therap.*, 1920, **14**, 379.

5 Ivy, A. C., Greengard, H., Stein, I. F., Jr., Grodins, F. S., and Dutton, D. F., *Surg., Gynec. and Obst.*, 1943, **76**, 1.

‡ The pectin solution was obtained from Frederick Stearns and Company and is described as having a molecular weight of between 40-60,000, the 1.5% solution in Ringers' having an oncotic pressure slightly greater than that of human plasma and a viscosity less than that of whole blood. Before administration, the acid solution was buffered with sodium phosphate or sodium lactate to a pH of 7.2.

§ The heparin used was provided by Hoffman-LaRoche Company (Liquaemin).

TABLE I.
Results of Intravenous Administration of Pectin (20 Patients) and of Dextrose and Saline (12 Patients).

Determination	Preliminary (avg)	% of change following administration of 1000 cc of pectin	% of change following administration of 1000 cc dextrose (2.5%) and saline (0.4%)
Total protein (g %)*	7.18	—12.9	— 5.1
A/G ratio	1.41	0	0
Hematocrit, %	43.8	— 9.8	— 4.2
Hemoglobin, g	14.0	— 5.0	
Red cell count	4.45		+ 1.7
Sedimentation rate, mm/hr	53.2	+46.6	+ 6.1
Venous pressure, cm	12.2	+22.1	+15.5
Arterial blood pressure:			
Systolic	123	+14.8	
Diastolic	78.8	+19.6	

* Determined by Barbour-Hamilton Falling Drop Apparatus.

Evans Blue (T-1824) in total doses of as much as 90 mg intravenously without demonstrating the hemorrhagic diathesis reported by others.[3] Hematologic studies failed to reveal such complications.

In none of the cases were reactions observed following the intravenous pectin injections. There was no rise of temperature, no significant change in pulse rate, fever or chills.

The urine output during the pectin (1.5%) administration increased in 8 cases by 500 to 600 cc without a consistent change in protein or nitrogen excretion.

In the patients receiving dextrose and saline solution the total protein and hematocrit were only slightly reduced at the end of 90 minutes; the sedimentation rate rose slightly and the venous pressure moderately.

Discussion. From these studies it appears that pectin solution in the form given is an effective hemodiluting agent in patients not in shock, producing slight rise of arterial and venous pressures. Its hemodiluting effect is much more marked and prolonged than that of dextrose and saline solution and is more marked in the hematocrit than in the hemoglobin determination, which suggests the possibility that part of the reduction of the hematocrit is not true hemodilution but shrinkage of the red cells. The increase in the sedimentation rate due to rouleau formation caused by pectin solution is marked but the resulting sedimentation velocity is not in excess of that commonly found in infections. Whether that represents a contraindication to the use of pectin in the form given must be decided by further studies. Other untoward results were not encountered.

Similar studies using pectin solution in patients in shock are in progress and will be reported separately.

Conclusion. Pectin solution is an effective hemodiluting agent in patients not in shock. It lowers total plasma protein, hematocrit, hemoglobin, and plasma non-protein-nitrogen. It raises the venous and arterial pressure slightly and the sedimentation rate of the erythrocytes markedly.

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The Effect of Polysaccharides on Energy Utilization, Nitrogen Retention and Fat Absorption in Chickens

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A DEPRESSION in the growth of rats was observed when 5% of agar agar or Irish moss was added to the diet of rats (Nilson and Schaller, 1941), while Vaughan *et al.* (1962) reported that carrageenin inhibited the *in vitro* digestion of proteins by pepsin. Booth *et al.* (1963) found that the digestion in rats of agar agar was 21%, pectin 19% and guar gum 76% when fed at levels of 15%, 10% and 6%, respectively.

Ershoff and Wells (1962) reported no significant growth depression in rats with 10% pectin, guar gum, locust bean gum or carrageenan. These materials, however, reduced plasma and liver cholesterol when 1% of cholesterol was fed in the diet. Fisher *et al.* (1964) reported that pectin reduced blood cholesterol in chicks fed cholesterol and Fisher *et al.* (1965) reported further that pectin reduced blood cholesterol in chicks only when cholesterol was fed.

Vohra and Kratzer (1964), and Anderson and Warnick (1964) reported that several natural polysaccharides depressed the growth of chicks when fed in the diet. The growth depressing effects of some of these polysaccharides were destroyed when these materials were treated with suitable enzymes. Riccardi and Fahrenbach (1965) conducted experiments with chicks fed diets containing 3% cholesterol. Under their conditions 3% of guar gum or carrageenan caused a 50% reduction in plasma cholesterol, but there was no gross toxicity as measured by survival, body weight depression or reduced food consumption.

The purpose of the present study is to investigate the mechanism of action of certain deleterious polysaccharides when fed to chickens by specifically studying their effect on energy utilization, nitrogen retention and fat absorption.

EXPERIMENTAL

The basal diets (Table 1) were based on commonly used natural feedstuffs but also contained 0.3% chromic oxide as an indicator to relate feed to feces quantitatively. Additions of polysaccharides were made at a level of 2% at the expense of milo in the conventional basal diet. The protein content of the high fat diet was increased in proportion to the increased level of fat on a calculated metabolizable energy basis. The total weight of the diet was not adjusted to 1 kg. The high protein (approximately 30%) diet was not adjusted in this way

TABLE 1.—*Composition of the experimental diets*

Ingredient	Low fat basal	High fat basal[1]	High protein basal
	gm./kg.	gm.	gm./kg.
Ground milo	270	—	—
Ground yellow corn	300	—	335
Corn starch	—	173	—
Soybean meal (50% protein)	200	288	415
Fish meal (65% protein)	75	105	75
Dried whey	50	50	50
Dehydrated, alfalfa meal (20% protein)	40	40	40
Soybean oil	—	100	20
$CaHPO_4 \cdot 2H_2O$	25	25	25
Cr_2O_3 mix[2]	10	10	10
Sodium chloride[3]	5	5	5
Vitamin mixture[4]	5	5	5
Test material	20	20	20

[1] The total mix was not adjusted to 1 kg.
[2] Mixed in wheat flour dough, baked and ground powder supplied 3 gm. Cr_2O_3.
[3] Iodized sodium chloride contained 0.025 gm. $MnSO_4 \cdot H_2O$/5 gm.
[4] Supplies: riboflavin, 1.1 mg.; niacin, 1.1 mg.; Ca-pantothenate, 1.1 mg.; choline chloride, 5.5 mg.; folic acid, 33.3 μg.; vitamin A, 7,500 I.U.; vitamin D_3, 1,270 I.C.U. (exp. 1 and 3) or 127 I.C.U. (exp. 2); vitamin E, 88 I.U. and bran to make 5 grams.

TABLE 2.—*Effect of 2% levels of various polysaccharides on weight gain, feed consumption, nitrogen retention, and fat absorption in chicks, and the metabolizable energy of the feed containing 1,270 I.C.U./kg. vitamin D_3 (Experiment 1)*

Supplement	Weight gain gm.	Feed intake gm.	Feed / gain	% N retention	% Fat absorbed	Metabolizable energy kcal./gm.
Cellulose	299^{ab}	513	1.72^{ab}	51^{bcde}	76^{ab}	2.93^{bc}
Guar gum	149^{a}	358	2.42^{d}	33^{a}	64^{a}	2.44^{a}
Gum carob	180^{ab}	355	1.98^{b}	53^{def}	82^{d}	2.90^{bc}
Gum karaya	229^{bc}	447	1.95^{b}	45^{b}	74^{bcd}	3.08^{c}
Pectin	231^{bc}	410	1.78^{a}	47^{bcd}	79^{cd}	2.89^{bc}
Me-Et-Cel[1]	304^{d}	543	1.79^{a}	58^{f}	79^{cd}	3.13^{c}
Me-Cel[2]	306^{d}	543	1.76^{a}	56^{ef}	76^{cd}	2.92^{bc}
HO-Pr-Cel[3]	275^{cd}	514	1.78^{a}	53^{cdef}	76^{abc}	2.89^{bc}
CM Cel (4 HP)[4]	236^{bc}	466	1.98^{b}	46^{bcd}	65^{a}	2.89^{bc}

[1] Methylethycellulose.
[2] Methylcellulose.
[3] Hydroxypropylcellulose.
[4] Carboxymethycellulose.
[5] Statistical significance at 1% level is denoted by different letters in a column.

and had a higher protein to energy ratio than the other two diets.

The vitamin D_3 supplements were added to the diets to provide 1270 I.C.U./kg. which is six fold more than the requirement of chicks for this vitamin (200 I.C.U./kg. diet; N.R.C., 1966). However, due to an error in mixing, the level of vitamin D_3 added to the diet in experiment 2 was 127 I.C.U./kg. diet, thus giving a diet marginally deficient in this vitamin.

Day-old Arbor Acres chicks were divided into duplicate groups of 10 chicks each of equivalent weight distribution. The birds were housed in wire cage batteries. Feed and water was supplied *ad libitum* and the experiments were continued for 3 weeks. The method of Dansky and Hill (1952) was used for determining apparent nitrogen retention and fat absorption while metabolizable energy was measured by the method of Hill and Anderson (1958). Data were analyzed statistically by the multiple range method of Duncan (1955).

The polysaccharides tested in this report were either naturally occurring gums or derivatives of cellulose commonly used in food industry. These included guar gum, carob gum, gum karaya, pectin, methylethylcellulose, methylcellulose, hydroxypropylcellulose and carboxymethylcellulose—4 HP or 7 HP.

RESULTS AND DISCUSSION

All natural gums depressed growth in the first experiment (Table 2). Feed efficiency was also reduced by all the natural gums with the exception of pectin. Carboxymethylcellulose in this experiment produced a significant growth depression and reduction in feed efficiency but methylethylcellulose, methylcellulose or hydroxypropylcellulose had no growth depressing properties for chicks.

Nitrogen retention (Table 2) was significantly reduced by guar gum while gum carob, gum karaya and pectin caused no significant change from the control containing cellulose. Fat absorption was also reduced by guar gum but not the other naturally occurring polysaccharides. Metabolizable eneregy, likewise, showed a significant reduction with guar gum. Carboxymethylcellulose (4 HP) produced a slightly lower nitrogen retention but the differences was not statistically significant. Carboxymethylcellulose caused a significant reduction in fat absorption but

TABLE 3.—*Effect of fat and protein level on response of chicks to polysaccharides in diets containing a marginal level of vitamin D_3 (127 I.C.U./kg. diet). (Experiment 2)*

Type of diet	Supplement	Body weight gain, gm.	% Apparent N retention	% Fat absorption	% Bone ash	Pancreas weight, mg./100 gm. body weight
Low fat	Cellulose	311^{ef}	46.9^{def}	63.4^{bcd}	44.4^{ef}	340^{ab}
	Gum karaya	289^{cde}	44.4^{de}	60.3^{bc}	41.0^{e}	355^{a}
	CM Cel (7 HP)	247^{bcd}	49.2^{e}	53.0^{a}	32.6^{ab}	370^{ab}
High fat	Corn	297^{cde}	51.2^{e}	87.9^{ef}	41.1^{e}	345^{a}
	Cellulose	297^{cde}	44.9^{de}	92.6^{f}	41.3^{e}	375^{ab}
	Pectin	246^{bc}	44.1^{cd}	82.8^{e}	35.2^{ab}	375^{ab}
	Guar gum	178^{a}	36.1^{bc}	63.5^{bc}	31.2^{a}	390^{ab}
	CM Cel (7 HP)	219^{cd}	41.0^{cd}	86.1^{e}	—[3]	445^{bc}
High protein	Cellulose	300^{de}	49.3^{e}	85.1^{e}	41.6^{e}	375^{ab}
	Guar gum	194^{ab}	23.7^{a}	65.0^{c}	32.6^{ab}	490^{c}
	CM Cel (7 HP)	236^{b}	30.4^{ab}	72.7^{d}	—[3]	425^{abc}

[1] Statistical significance at 1% level by Duncan's method.
[2] Statistical significance at 5% level by Duncan's method.
[3] Not determined.

there was no effect on metabolizable energy of the diet by the addition of any of the cellulose derivatives.

The deleterious effect of guar gum upon growth, nitrogen retention, fat absorption and metabolizable energy raised the question whether these responses would be mitigated by either raising the energy content of the diet with additional soybean oil while maintaining the energy/protein ratio the same as in low fat diet, or increasing the level of protein alone.

The results of the experiment in which guar gum was fed in high fat or high protein diets are given in Table 3. The diets were marginally deficient in vitamin D_3. The chickens fed 2% carboxymethylcellulose (7 HP), pectin, and guar gum in all diets containing only 127 I.C.U./kg. vitamin D_3 developed rickets which was not noticed with other polysaccharides. The dietary requirements for vitamin D_3 are believed to be 200 I.C.U./kg. The rickets were confirmed by reduction in bone ash for some treatments (Table 3), and appeared to be associated with the growth depressing polysaccharides only. A marked reduction in growth of chickens was observed for carboxymethylcellulose, guar gum, and pectin on low fat, high fat or high protein diets. The depression in growth caused by gum karaya was statistically non-significant. Nitrogen retention and fat absorption data were approximately similar to those of the previous experiment with the exception that guar gum interfered with these parameters more on the high fat diet than was noted previously. Pancreatic weight was significantly increased when the diets contained guar gum in high protein diets or carboxymethylcellulose (7 HP) in high fat diets. In other treatments, there were no significant differences in the pancreatic weights as expressed in terms of the body weight of chickens.

The vitamin D_3 level of the diets used in experiment 3 was again increased to 1270 I.C.U./kg. to determine whether the growth depressing polysaccharides caused rickets only in diets marginally deficient in vitamin D_3. No rickets were observed with any of the treatments in this experiment which is again confirmed by no statistically significant differences in any of the bone (tibia) ash samples (Table 4). As in the

TABLE 4.—*Effect of fat and protein level on response of chicks to polysaccharides containing high levels of vitamin D_3 (1,270 I.C.U./kg. diet) (Experiment 3)*

Type of diet	Supplement	Body wt. gain gm.	% N retained	% Fat absorbed	Pancreas wt. mg./100 g. body wt.	% Bone ash
Low fat	Cellulose	336[efl]	48[cdl]	70[abl]	400[abl]	43.6[al]
	Gum karaya	248[bc]	49[cd]	74[b]	495[abc]	42.6[a]
	CM Cel (7 HP)	243[abc]	39[b]	64[a]	550[cd]	42.8[a]
High fat	Corn	355[f]	54[d]	95[d]	385[a]	41.9[a]
	Cellulose	369[f]	54[d]	94[d]	367[a]	43.7[a]
	Pectin	299[cde]	53[cd]	89[cd]	440[abc]	42.8[a]
	Guar gum	295[cde]	52[cd]	91[cd]	456[abc]	41.8[a]
	CM Cel (7 HP)	211[ab]	47[c]	69[ab]	567[cd]	43.8[a]
High protein	Cellulose	322[ef]	51[cd]	87[c]	477[abc]	43.7[a]
	Guar gum	188[a]	30[a]	75[b]	639[d]	41.0[a]
	CM Cel (7 HP)	273[cd]	32[ab]	87[c]	513[bcd]	42.9[a]

[1] Statistical significance at 1% level in a column by Duncan's method is indicated by different letters.

first experiment, gum karaya and carboxymethylcellulose depressed the growth of chickens significantly on low fat diets. The nitrogen retention was depressed significantly only by carboxymethylcellulose but the fat absorption was unchanged. The depression in growth of chickens also took place on high fat diets with pectin, guar gum or carboxymethylcellulose; and on high protein diets with guar gum or carboxymethylcellulose. Nitrogen retention and fat absorption were depressed by carboxymethylcellulose only on high fat diets. Nitrogen retention and fat absorption were reduced by guar gum on high protein diets but only nitrogen retention was affected by carboxymethylcellulose under these conditions. Carboxymethylcellulose caused enlargement of the pancreas on low fat, and high fat diets but not on high protein diets. Guar gum affected pancreas only in high protein diets.

The growth depressing effect of complex polysaccharides which had been reported previously (Vohra and Kratzer, 1964) was confirmed in these studies.

Guar gum, which depressed growth more severely than other natural gums, was shown to depress nitrogen retention, fat absorption and reduce the metabolizable energy of the whole diet. The greatest aggravation of growth was noted when it was fed in a high protein diet, indicating that its greatest effect was upon nitrogen utilization. On the other hand, carboxymethylcellulose was more growth depressing in a high fat diet. It is not possible to make any further generalizations concerning the effect of the polysaccharides on nitrogen retention and fat absorption.

The significant reduction in the metabolizable energy of the diet by guar gum probably represents a combination of the effect on nitrogen retention and fat absorption. It is analogous to the effect observed by feeding gossypol (Hill and Totsuka, 1964). The enlargement of the pancreas observed in groups fed the growth depressing gums is also similar to that noted in birds fed raw soybean meal.

The lowered bone ash observed in groups fed growth depressing polysaccharides in the diet with a marginal level of vitamin D_3 might be explained by a poor absorption of vitamin D_3, creating a deficiency state which could result in poor bone mineralization. Both Fisher *et al.* (1965) and Riccardi and Fahrenbach (1965) have shown interference with cholesterol utilization caused by pectin, carrageenan or guar gum

feeding. Fisher *et al.* (1965) showed that blood cholesterol was lowered by pectin only when cholesterol was fed. Leveille and Sauberlich (1966) showed that pectin decreased the absorption of bile acids and cholesterol in the rat. It is likely that effect of polysaccharides on calcification is also due to interference in the absorption of a steroid, vitamin D_3.

SUMMARY

The following polysaccharides were fed to chicks at a level of 2 percent of the diet: cellulose, methylethylcellulose, methylcellulose, hydroxpropyl cellulose, carboxymethylcellulose, guar gum, gum carob, gum karaya and pectin.

The natural gums and carboxymethylcellulose depressed growth. Guar gum caused reduced nitrogen retention, fat absorption and metabolizable energy while carboxymethylcellulose caused reduced fat absorption. Growth depression was not overcome by high-fat or high-protein diets but guar gum aggravated growth more severely on a high-protein diet.

At a marginally low level of vitamin D_3, the tibia ash was reduced by the growth depressing polysaccharides.

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Mechanism of the Cholesterol-depressing Effect of Pectin in the Cholesterol-fed Rat [1]

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ABSTRACT The mechanisms by which dietary pectin lowers plasma and liver cholesterol levels in cholesterol-fed rats were studied. Pectin feeding increased fecal bile acid excretion in cholesterol-fed rats. In vitro studies with inverted intestinal sacs demonstrated that pectin decreased taurocholic acid transport by approximately 80%. Rats responded to dietary pectin and cholestyramine, a known inhibitor of bile acid absorption, similarly. Cholesterol-4-^{14}C absorption was somewhat depressed by dietary pectin as evidenced by fecal radioactive cholesterol excretion and deposition of cholesterol-4-^{14}C in liver. The effect of pectin on plasma and liver cholesterol was not altered by dietary succinylsulfathiazole. These data are interpreted to suggest that the hypocholesterolemic effects of pectin are not mediated by an alteration of the intestinal microflora. The results of this study indicate that pectin lowers plasma and liver cholesterol levels in cholesterol-fed rats primarily by inhibiting bile acid absorption and also by reducing cholesterol absorption.

A number of publications have demonstrated a relationship between the incidence of atherosclerosis and serum cholesterol (1). These observations have naturally led to the study of factors influencing cholesterol metabolism and serum cholesterol levels. The control of serum cholesterol levels by dietary means has received particular attention (2). Dietary pectin has been reported to depress serum and liver cholesterol levels of cholesterol-fed rats (3) and chickens [2] and to reduce serum cholesterol levels in man (4).

The mechanism by which dietary pectin exerts its hypocholesterolemic effect has not been elucidated. Apparently, pectin exerts its effect in part by depressing cholesterol absorption (5). Other possibilities are 1) that the effects of dietary pectin might be mediated by an alteration of the intestinal microflora, or 2) that pectin may decrease the enterohepatic circulation of bile acids by interfering with bile acid absorption. These possible mechanisms have been studied in the rat and data are presented which suggest that the hypocholesterolemic effect of pectin is mediated primarily by an inhibition of bile acid absorption and also by a depression of cholesterol absorption.

MATERIALS AND METHODS

Male rats of the Holtzman strain were used for all studies. The animals were housed in stainless steel cages having raised wire floors; the room temperature and humidity were controlled (21° and 50% relative humidity). Diets were supplied ad libitum except in one experiment in which 2 diets were fed alternately; in this experiment animals were allowed access to food from 4:00 PM until 8:00 AM only. Water was supplied ad libitum. Body weight and food consumption were determined weekly. The experimental diets were fed for 3 or 4 weeks as indicated in the tables of results.

The basal diet had the following composition: (in g/100 g) casein (vitamin free), 18; L-cystine, 0.3; non-nutritive fiber, 3; salt mix (USP XIV), 4; corn oil, 5; vitamin mixture,[3] 0.4; choline Cl, 0.3; cholesterol, 1; glucose to 100. All additions to the basal diet, as indicated in the table of results, were made at the expense of glucose.

At the termination of the experiments, blood was obtained by cardiac puncture, while the animals were under light ether anesthesia, using heparin as an anticoagulant. The rats were then killed by exposure to chloroform and the livers were excised, blotted to remove excess blood, weighed and stored at −20° until analysis

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[1] The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed.

[2] Fisher, H., P. Griminger and W. Siller 1965 Retardation of cholesterol-induced atherosclerosis by pectin. Federation Proc., 24: 283 (abstract).

[3] For composition see Leveille et al. (6).

was performed. Plasma and liver lipids were determined as previously described (7, 8).

Fecal lipids were determined on pooled 3-day samples per rat at the termination of the study (table 1). The feces were dried at 60° under reduced pressure, weighed and ground to a fine powder. An aliquot of the dried, ground feces was extracted with chloroform:methanol (2:1, v/v) and 3-β-hydroxy sterols were determined by the same procedure employed for plasma and liver cholesterol. A second aliquot of the dried feces was analyzed for di- and trihydroxy bile acids by the method of Mosbach et al. (9).

Cholesterol absorption was studied with cholesterol-4-^{14}C. Rats fed the experimental diets for 3 weeks were given by stomach tube 2.8 μc of cholesterol-4-^{14}C dissolved in 1 g of corn oil. The feces were collected for 24 hours, saponified and cholesterol was isolated as the digitonide (10) and the radioactivity determined in a Nuclear-Chicago Corporation Model 722 ambient temperature liquid scintillation spectrometer.

In vitro bile acid absorption was studied by the inverted sac technique of Wilson and Wiseman (11). Rats weighing 400 g and fed commercial laboratory chow [4] from weaning were used. The animals were killed by exposure to ether; the lower one-fourth of the small intestine was removed and placed in cold saline containing 0.1% glucose; the intestine was washed with the same saline solution and inverted. Sacs of approximately 3 cm were prepared and filled with Krebs-Ringer bicarbonate buffer containing 100 μg/ml of taurocholic acid. The sacs were incubated for 90 minutes in beakers containing 10 ml of the same buffer containing taurocholic acid, with or without the addition of 0.18% pectin, at 38° in a Dubnoff metabolic shaker at a shaking rate of 90 oscillations/minute. The gas phase was 95% O_2 and 5% CO_2. At the end of the incubation period, the taurocholic acid content of the incubation medium (mucosal) and the medium within the sacs (serosal) was determined by the modified Pettenkofer method of Irwin et al. (12).

The data were statistically evaluated by means of the *t* test.

RESULTS

The effects of dietary pectin on body weight gain, plasma cholesterol, liver lipids, and fecal sterol and bile acid excretion in cholesterol-fed rats are shown in table 1. Pectin did not alter body weight gain. Plasma cholesterol and liver cholesterol levels were significantly reduced by dietary pectin. Fecal sterol excretion was not altered by pectin feeding; bile acid excretion was increased by 31.8% in rats fed pectin; however, this difference did not attain statistical significance at the 5% level.

To further investigate the possibility that pectin lowered cholesterol levels in cholesterol-fed rats by impairing bile acid absorption, the effects of pectin were compared with those of cholestyramine, a resin known to bind bile acids in the intestine

[4] Purina Laboratory Chow, Ralston Purina Company, St. Louis.

TABLE 1

Body weight gain, plasma cholesterol, liver fat and cholesterol and the fecal excretion of sterols and bile acids in rats fed a cholesterol-supplemented diet with or without added pectin

	Additions to diet		P values [1]
	Cholesterol (1%)	Cholesterol (1%) + pectin (5%)	
28-day body wt gain, g	181 ± 5 [2]	173 ± 11	ns
Plasma cholesterol, mg/100 ml	128 ± 3	116 ± 5	< 0.05
Liver fat, % [3]	7.4 ± 0.3	6.6 ± 0.3	ns
Liver cholesterol, mg/g [3]	10.3 ± 0.5	7.5 ± 0.3	< 0.01
Fecal sterols, mg/day [4]	142 ± 5	141 ± 8	ns
Fecal bile acids, mg/day [4]	17.9 ± 2.4	23.6 ± 3.3	ns

[1] Probability of differences being significant; ns = not significant ($P > 0.05$).
[2] Mean ± SE of mean for 10 rats.
[3] Liver lipid values expressed on a wet-weight basis.
[4] Values are means for 5 animals.

TABLE 2

Body weight, liver fat and cholesterol, plasma total lipids and cholesterol of rats fed pectin or cholestyramine with supplemental cholesterol [1]

Dietary treatment	Body wt	Liver [2]		Plasma	
		Fat	Cholesterol	Total lipids	Cholesterol
	g	%	mg/g	mg/100 ml	mg/100 ml
1% Cholesterol	272 ± 14 [3]	7.9 ± 0.3	10.3 ± 0.5	910 ± 114	128 ± 14
1% Cholesterol + 5% pectin	269 ± 9	7.1 ± 0.4	7.2 ± 1.1 [4]	719 ± 81	91 ± 4 [4]
1% Cholesterol + 1% cholestyramine	258 ± 19	5.8 ± 0.2 [4]	4.0 ± 0.2 [4]	736 ± 57	86 ± 4 [4]
Diets alternated daily:					
3% Cholesterol and cholesterol-free	251 ± 12	6.5 ± 0.3	7.8 ± 0.8	723 ± 34	89 ± 3
3% Cholesterol and 5% pectin	235 ± 22	5.4 ± 0.3 [4]	4.2 ± 0.6 [4]	743 ± 38	101 ± 12
3% Cholesterol and 1% cholestyramine	260 ± 6	6.0 ± 0.2	5.0 ± 0.2 [4]	773 ± 32	119 ± 4 [4]

[1] Rats weighing approximately 150 g were fed the diets indicated for 3 weeks.
[2] Liver values are expressed on a wet-weight basis.
[3] Mean for 5 rats ± SE of mean.
[4] Values significantly different ($P < 0.05$) from group fed 1% cholesterol or fed alternately 3% cholesterol and the cholesterol-free diet.

TABLE 3

Effect of pectin on the in vitro absorption of taurochloric acid by sacs of inverted rat intestine [1]

Addition to mucosal fluid	Final serosal conc	Final serosal conc / Final mucosal conc
	μg/ml	
None	297 ± 46 [2]	3.28 ± 0.56
Pectin, 0.18%	158 ± 11	1.52 ± 0.08
P [3]	< 0.05	< 0.05

[1] Intestinal sacs (~ 3 cm) were prepared from the distal one-fourth of the intestine of 400-g rats. Serosal fluid: Krebs-Ringer bicarbonate containing 100 μg taurochloric acid; mucosal fluid: 10 ml bicarbonate buffer ± 0.18% pectin N.F. Preparation incubated 90 minutes at 38° under 95% O_2 and 5% CO_2.
[2] Mean for 4 experiments ± SE of mean.
[3] Probability of the difference being significant.

and thereby prevent their absorption (14). Rats were fed cholesterol-supplemented diets containing either pectin (5%) or cholestyramine (1%) for 3 weeks or were fed on alternate days a diet containing 3% cholesterol and one containing either 5% pectin or 1% cholestyramine.[5] The results of this experiment are shown in table 2. The effects of pectin and cholestyramine were similar whether fed together with cholesterol or separately. These data further suggest that pectin may act by impairing bile acid absorption.

The influence of pectin on bile acid absorption was studied in vitro with inverted intestinal sacs. Sacs from the distal one-fourth of the small intestine were used since it had been demonstrated that bile acid absorption is limited to this area (13). Sacs incubated with or without pectin added to the incubation medium concentrated taurocholate; however, pectin reduced the amount transported by approximately 50% as judged by the serosal-to-mucosal ratio or by the final serosal concentration (table 3). These data support the concept that the hypocholesterolemic effect of dietary pectin in the rat is mediated by an impairment of the enterohepatic circulation of bile acids.

The possible influence of an altered intestinal microflora in pectin-fed rats was studied by feeding pectin to animals receiving 1% dietary cholesterol with or without 1% succinylsulfathiazole. These data are presented in table 4. Succinylsulfathiazole significantly increased plasma cholesterol and liver fat and cholesterol levels ($P < 0.05$). Pectin was as effective in lowering plasma cholesterol and liver lipids in the presence or absence of dietary succinylsulfathiazole, suggesting that its hypocholesterolemic effect is not mediated by an alteration of the intestinal flora.

[5] The cholestyramine used in these studies was generously supplied by Dr. H. D. Brown, Research Laboratories, Merck, Sharp and Dohme, Rahway, New Jersey.

TABLE 4

Plasma cholesterol, liver fat and cholesterol levels of rats fed pectin with or without supplemental succinylsulfathiazole [1]

Dietary variables [2]	Plasma cholesterol	Liver [3]	
		Fat	Cholesterol
	mg/100 mg	%	*mg/g*
Cholesterol	86 ± 5 [4]	5.2 ± 0.4	10.2 ± 1.1
Cholesterol + pectin	77 ± 3	4.2 ± 0.1	4.8 ± 0.1
P [5]	ns [6]	< 0.05	< 0.01
Cholesterol + sulfa	136 ± 8	7.8 ± 0.4	20.7 ± 1.5
Cholesterol + sulfa + pectin	98 ± 3	5.5 ± 0.3	8.5 ± 0.9
P [5]	< 0.01	< 0.01	< 0.01

[1] Rats weighing approximately 50 g were fed the experimental diets for 4 weeks.
[2] Cholesterol 1%; pectin N.F. 5%; Sulfa = succinylsulfathiazole 1%.
[3] Liver values are expressed on a wet weight basis.
[4] Mean for 10 rats ± SE of mean.
[5] Probability of the difference being significant; ns = not significant.
[6] Indicates not significant.

TABLE 5

Liver fat and cholesterol, cholesterol-4-^{14}C deposition in liver and fecal excretion in rats fed cholesterol-supplemented diets with or without added pectin [1]

	Additions to diet		P value [2]
	Cholesterol (1%)	Cholesterol (1%) + pectin (5%)	
Liver fat, % [3]	6.8 ± 0.4 [4]	4.4 ± 0.2 [4]	< 0.001
Liver cholesterol, mg/g [3]	9.4 ± 0.4	6.4 ± 0.2	< 0.001
Cholesterol-^{14}C [5] DPM × 10^3/g liver	51.0 ± 5.8	37.4 ± 0.2	ns [6]
Fecal excretion, % of administered dose	6.3 ± 2.1	13.1 ± 3.4	ns

[1] Rats weighing approximately 250 g were fed the experimental diets for 3 weeks.
[2] Probability of difference being significant; ns = not significant.
[3] Liver values expressed on a wet-weight basis.
[4] Mean for 4 rats ± SE of mean.
[5] 2.8 μc of cholesterol-4-^{14}C in 1 g corn oil were fed by stomach tube; fecal excretion represents radioactivity excreted in 24-hour period following administration.
[6] Indicates not significant.

In table 5 are shown the results of an experiment designed to study the influence of dietary pectin on cholesterol absorption. Again, pectin lowered liver lipids in cholesterol-fed rats. Rats fed pectin excreted over twice as much of the administered radioactive cholesterol as did control animals; however, probably because of the small numbers this difference did not attain statistical significance. However, the difference in excretion of radioactive cholesterol and the lower amount of cholesterol-4-^{14}C in the liver of pectin-fed animals suggests that pectin does in fact depress cholesterol absorption.

DISCUSSION

The series of experiments reported was designed to evaluate the mechanisms by which dietary pectin depresses liver cholesterol levels in the cholesterol-fed rat. Since pectin is generally considered not to be digested, it follows that its effects would probably be limited to the gastrointestinal tract. The most likely mechanisms appeared to be related to 1) a reduction in cholesterol absorption, 2) a depression of bile acid absorption or recirculation, and 3) an alteration in the intestinal microflora. The possible involvement of these 3 mechanisms were therefore evaluated.

The most important effect of pectin appears to be its influence on bile acid absorption. Pectin clearly impairs bile acid absorption in vitro. Such experiments do not prove that the same effect would be found in vivo; however, when considered together with the greater bile acid excretion observed in pectin and cholesterol-fed rats (table 1), the similarity in response of the cholesterol-fed rat to pectin and a known inhibitor of bile acid absorption, cholestyramine (table 2), and the general agreement between in vitro and in vivo studies of bile acid absorption (13, 15), the data strongly suggest that pectin does in fact impair bile acid absorption.

The decrease in cholesterol absorption observed in this study as a consequence of pectin feeding is in agreement with the reports of Lin et al. (5) and of Hyun et al. (16). However, the impairment in cholesterol absorption is not evidenced when sterol excretion is determined (table 1). This observation is in agreement with the results of Wells and Ershoff (3) who were unable to demonstrate a difference in cholesterol excretion of rats fed a cholesterol-supplemented diet with or without pectin. It appears therefore that the impaired cholesterol absorption induced by dietary pectin is only partially responsible for its hypocholesterolemic effect. This conclusion is also supported by the study in which cholesterol and pectin were fed separately on alternate days (table 2). These results are in agreement with those of Wells and Ershoff (3). The observation that pectin effectively lowers liver cholesterol when fed separately from cholesterol suggests that impairment of cholesterol absorption is relatively unimportant to the overall effect of dietary pectin.

The alterations in the intestinal microflora resulting from pectin feeding do not appear to contribute to the cholesterol-lowering effect of this complex carbohydrate. The lack of effect of succinylsulfthiazole on the pectin response observed in this study is in agreement with the observations of Wells and Ershoff (3) and is in accord with the conclusion that alterations of the intestinal microflora do not influence the cholesterolemic effect of dietary pectin in the rat.

Fisher et al. (17) have recently presented evidence that in the chicken, pectin interferes with absorption of nutrients by increasing passage of feed through the digestive tract. This appears to be unlikely in the rat in view of the consistent lack of effect of dietary pectin on body weight (tables 1 and 2).

The data presented suggest that dietary pectin reduces plasma and liver cholesterol levels in the cholesterol-fed rat primarily by decreasing the absorption of bile acids and secondarily that of cholesterol.

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COMMODITIES

Use of pectin in food processing

by B. Lockwood*

It has been known for centuries that if certain fruits are boiled with water and sugar, a jelly will form when the mixture is allowed to cool. Braconnot was one of the first to realise that this phenomenon resulted from the extraction of colloidal substances from the fruit, and in 1825 he coined the name "pectin", from a Greek word meaning "to congeal". During the present century methods for the commercial production of pectin have been developed, and the availability of pectins of standardised behaviour has helped the manufacture of preserves to be put on a more scientific footing. In recent years manufacturers of confectionery and convenience foods have come to appreciate its unique properties as a stabiliser and gelling agent. Consequently, the amount used in the food industry as a whole has been increasing steadily

Pectic substances occur widely in the primary cell walls and intercellular layers of fruits and vegetables. The most important commercial sources are the residues of apples dried after juice extraction, and citrus peel residues either in the moist or dry form. These raw materials contain a high percentage of extractable pectin and this is of a high gelling capacity.

Pectins are polysaccharides with a structure based on a main chain of anhydro-D-galacturonic acid units (Fig. 1). Although its structural chemistry is more complicated than was once thought, it is convenient to consider pectin as a derivative of polygalacturonic acid, in which a proportion of the carboxyl groups are in the methyl ester form. The number and distribution of ester groups along the molecule play important roles in determining what types of gel the pectin is capable of forming, and also the setting characteristics of the gel (Fig. 2). As extracted from commercial raw materials, pectin has a degree of esterification of 70–80 per cent, and is of the rapid set type. The slow set and low methoxyl types may be produced by hydrolysis of this extract.

As well as galacturonic acid groups, virtually all pectins contain a minor proportion of other sugars, such as galactose, arabinose and rhamnose. These are present in the form of side chains, insertions in the main chain and neutral polysaccharides. Their effect on the gelation properties is not well studied or understood.

COMMERCIAL PECTINS

The first stage in the manufacturing process is the conversion of the native pectic material into water-soluble pectin, accompanied by leaching of the latter out of the raw material. This is achieved by heating apple pomace or citrus peel with dilute acid. The weak extract obtained is separated from insoluble residues by filtration or centrifugation, and concentrated by vacuum evaporation, to give what is known as "liquid pectin". In the UK a large amount of high methoxyl apple pectin is sold in this form with sulphur dioxide as preservative. Powdered pectins are produced by precipitation with alcohol or aluminium chloride or by spray drying. Citrus and low methoxyl apple pectins are only available in this form.

Although the liquid form is cheaper per unit weight of pectin and may be more convenient to use, it contains acid and non-pectic substances extracted from the raw material. Therefore, powdered pectins have certain definite advantages over the liquid, the most important being: (i) superior colour, (ii) the absence of any preservative (when using liquid pectins enough boiling time must be given to remove the SO_2), (iii) greater stability on storage, (iv) less space required for storage.

Since pectin is a natural product, its gelling capacity or grade (see below) varies from one production batch to another due to the variable quality of the raw material. For the user it is important that each consignment should behave identically. Therefore, the pure powdered pectins are blended to a standardised grade with an inert diluent such as dextrose monohydrate.

Buffer salts such as phosphates and citrates are added to certain types of powdered pectins developed for use in bakery and confectionery products. The main function of these salts is to control the solution pH. For example, in the production of confectionery jellies a buffer is necessary to ensure that setting does not start until the pH has been lowered by addition of the final portion of acid. Certain buffers also act as sequestering agents for calcium ions, the presence of which may alter the setting properties of the gel and/or the ease of dissolution of the pectin.

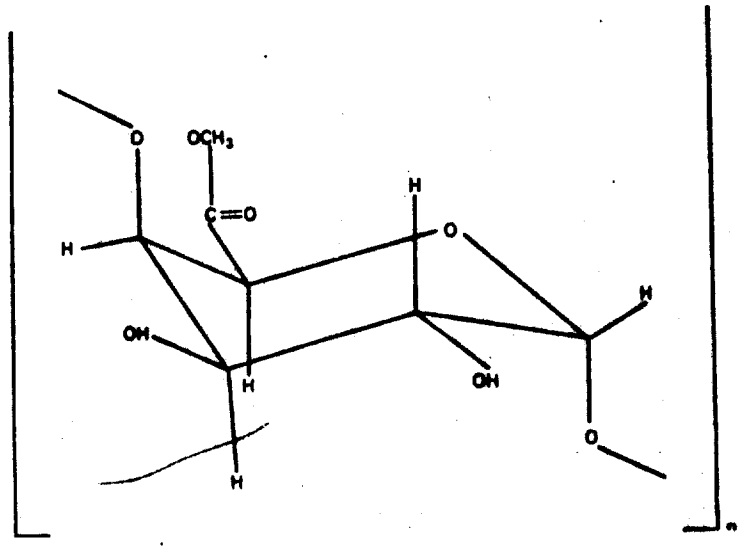

Fig 1: The structure of pectins

* H. P. Bulmer Ltd

Percentage of galacturonic acid groups in form of $-\overset{O}{\overset{\|}{C}}-OH$	$-\overset{O}{\overset{\|}{C}}-OCH_3$	Substances required for gelation	Commercial type
20–30	70–80	Sugar, acid	Rapid Set High Methoxyl
35–45	55–65	Sugar, acid	Slow Set High Methoxyl
60–65	35–40	Calcium ions	Low Methoxyl

GRADE OF PECTIN

This property is defined as the number of grams of sugar which one gram of the pectin is capable of turning into a gel of standard firmness, under standard conditions of acidity and soluble solids content. Many methods of grading exist and for years each producer used a different standardisation procedure. In 1959 a committee of the Institute of Food Technologists reported on this subject and recommended a carefully detailed method for grading high methoxyl pectins. A test jelly having a soluble solids content of 65 per cent is prepared and its strength determined using an Exchange Ridgelimeter. A calibration curve relates the percentage of sag of the jelly to the grade (expressed as "grade—X USA—SAG") of the pectin under test.

Rapid and slow set liquid pectins are usually standardised to 4—6X USA—SAG and unbuffered powders to 100, 150 or 200X USA—SAG. Low methoxyl pectins are also standardised, although no internationally recognised grading method yet exists.

USES OF HIGH METHOXYL PECTIN

(a) *Manufacture of preserves*

The major use of commercial high methoxyl pectin is in the manufacture of jams, jellies and marmalades. For this type of pectin to form a gel network, three conditions have to be fulfilled:

(i) The electrostatic repulsion between molecules has to be decreased by working under acidic conditions in order to depress the dissociation of carboxyl groups. In practice, the range of jelly pH is dependent on the soluble solids content (see Table 1) and on the type of pectin used.

Table 1
Relationship between pH and soluble solids for an acceptable set

Soluble solids %	pH of a 50% solution of the preserve
75–85	3.2–3.5
72–75	3.1–3.4
68–72	3.0–3.3
64–68	2.9–3.1
60–64	2.8–3.0
55–60	2.6–2.8

(ii) Sucrose or similar compounds have to be added in a sufficient amount. The lower limit of sugar content has been shown to be about 50 per cent. In most cases the soluble solids content is considerably higher (65–70 per cent for retail jams in the UK).

(iii) The pectin must be present in a sufficiently high concentration. When deciding on the amount to be used for a particular type of preserve, account must be taken of the type, amount and degree of maturity of the fruit to be used, and of the consistency required in the final product. Natural pectin is extracted from the fruit during the boiling stage. If the amount extracted is large and the grade is high (eg, from damsons or blackcurrants) then less pectin need be added. If the amount of natural pectin is small (eg, from cherries or peaches) or it is of low grade (eg, from raspberries or strawberries) then the gel structure of the finished product must be provided almost entirely by added pectin.

It is important that gelation should occur soon after filling the hot jam into containers in order to prevent settling or floating of the fruit. Therefore, rapid or extra-rapid set pectin is used except (a) when filling large containers, (b) in the production of jelly rather than jam, (c) in preserves having a high soluble solids content (72 per cent or greater). In these situations the use of slow set pectin is preferred. When a vacuum rather than an open boiling pan is being used a slow or medium-rapid set pectin may be found more convenient, and the amount is usually reduced by 5–10 per cent to compensate for the reduced degradation which occurs at the lower temperature.

Pectins are degraded under acidic conditions by cleavage of the glycosidic linkages joining individual units in the chain. This results in a decrease in the average molecular weight and in the jelly grade. The reaction is temperature sensitive, its rate increasing three or four fold for each 10°C rise above 60°C. To minimise degradation, the pectin is added at a late stage in the boiling process.

The majority of pectin used by UK jam manufacturers is in the liquid form. For those wishing to use powder it is advisable to prepare a solution beforehand. This is done using a high-speed mixer and/or by pre-mixing the pectin with four or five times its own weight of sugar in the dry state. These precautions ensure that no undissolved, partially hydrated particles are left in the solution. For home jam making, mixtures of sugar, pectin and tartaric or citric acid are sold on the Continent. These enable the housewife to prepare high-quality jams, even from "difficult" fruits such as strawberries. In the UK and several other countries special liquid pectins are marketed for the same purpose.

Fig 2: Setting characteristics of the gel

(b) *Confectionery jellies*

In the confectionery industry, pectin jellies are made in many forms, including jellied fruits, chocolate centres and fruit slices. For these products the use of slow rather than rapid set pectin is essential. Only in this way can enough time be available for depositing a whole batch of jelly when the required solids content is high. Jellies should normally have a refractometer solids content of 76–80 per cent when deposited. This ensures that only a small amount of drying out will take place under normal storage conditions. The pH of the jellies should be in the range 3.3–3.6.

For some products (eg, fruit slices) the solids content may be as high as 83 per cent. Under these conditions it is advisable for two reasons to work at a pH of up to 3.8:

(i) By raising the solids content the rate at which setting takes place will increase. A higher pH will compensate for this, so maintaining enough time for depositing.

(ii) The longer boiling time required and the higher temperature reached would result in too great an inversion of sucrose into glucose and fructose, if the pH were not raised.

(c) *Bakery products*

(i) Jams, which are filled into tarts or other pastry casings before being baked, must be resistant to caramelisation and must not "boil out" during the baking process. Although a medium-rapid or slow-set pectin is normally used, it can be advantageous to employ a low methoxyl type (see below), enabling the pH to be raised to 3.5–4.0.

(ii) For biscuit fillings, where a very stiff jam or jelly is required, it is usual to work at a high soluble solids content, ie, 75–85 per cent. Under these conditions a slow-set pectin is necessary to prevent pregelation.

(iii) Cold set and flan jellies may be made by adding citric acid solution to a syrup containing sugar and buffered pectin. The pH is thus reduced from 4.0 to 2.8–3.0 enabling gel formation to occur within a short time.

LOW METHOXYL PECTINS

The range of products in which high methoxyl pectins are used as the gelling agent is somewhat limited. On the other hand, low methoxyl (LM) pectins are considerably more flexible since they are capable of forming gels under two distinct sets of conditions.

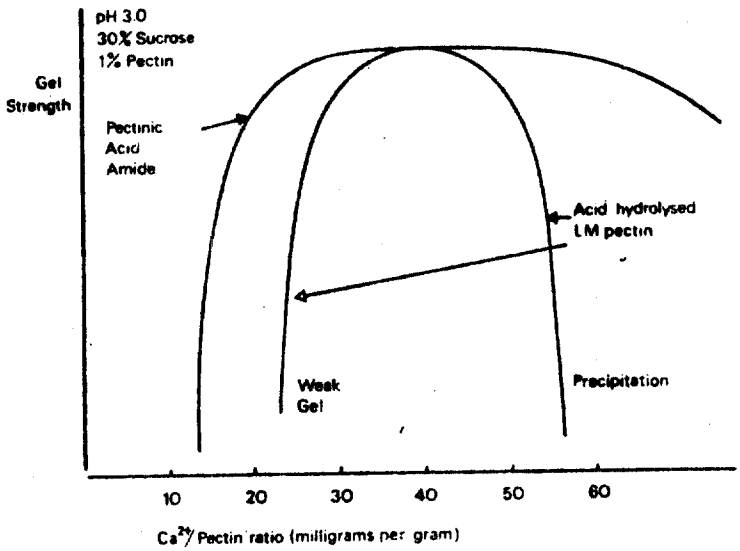

Fig 3: Pectin response to varying concentrations of calcium ions

The "normal" type of high solids jelly can be produced over a relatively wide range of pH (up to six). If the sugar content is reduced to below 50 per cent gels can still be formed provided a certain concentration of calcium ions is present. Unless the molecular weight of the pectin is particularly high, these gels tend to be rather brittle.

Two distinct types of LM pectin are available commercially, both prepared by removal of methyl ester groups. In one case this is achieved by homogeneous acid hydrolysis, in the other by the action of ammonia on an alcoholic suspension of high methoxyl pectin. In the latter case saponification is accompanied by the formation of a proportion of amide groups and the product is known as a pectinic acid amide. The most important difference between the two types lies in their response to varying concentrations of calcium ions, as illustrated in Fig. 3. It can be seen that the amide pectin is more sensitive to low levels of calcium and less susceptible to precipitation at higher levels. This enables it to be used as the gelling agent in products containing little natural calcium, and less constraint is placed on the user to ensure precise concentrations of calcium salts.

USES

(a) In dietetic jams and jellies containing 30–55 per cent soluble solids the use of LM pectin is exemplified by the following recipe which uses a fruit juice sweetened to 25° Brix and of pH 3.5–3.8:

1 lb pectin is mixed with 3 lb sugar in the dry state. 75 lb fruit juice is heated to 200°F and the pectin-sugar mixture is added slowly, stirring continuously until dissolved. ½ oz anhydrous calcium chloride is dissolved in 25 lb juice, heated to 200°F and added carefully, with stirring, to the other solution. The mixture is poured carefully into moulds and allowed to set. The amount of added calcium will vary, depending on the level of natural calcium in the juice.

(b) For high solids confectionery jellies the main use of LM pectin is in the manufacture of Turkish Delight. This is only slightly acidic, the pH of approximately five being well outside the range for gelation of high methoxyl pectin.

(c) Ripple syrups for ice cream are thickened by a combination of LM pectin and calcium salts. Syrups used in the manufacture of fruit yoghurts may be stabilised by slow set or LM pectin.

(d) A large amount of LM amide is used on the Continent in the manufacture of jams of high soluble solids content, which can later be mixed with water, brought to the boil and allowed to re-set. The lower solids content jams so produced are incorporated in bakery products.

(e) A major market exists in the field of milk desserts and gels which has long been dominated by starches. Other hydrocolloids employed as gelling agents are agar, alginates and carrageenans, all produced from seaweeds. The mechanisms of gelation are incompletely understood partly because the structure of milk is so complex. In the cases of alginates and LM pectins gelation is considered to be due to natural calcium in the milk. This exists mainly in the calcium caseinate-calcium phosphate complex, and only a proportion of the total calcium seems to interact with pectin.

The interaction is utilised in a range of products which has recently appeared on the market. Fruit syrup containing pectin and starch is mixed with milk by the housewife and very quickly a gel structure builds up. This is one example of a whole range of possible applications for LM pectins.

Although the market for preserves is fairly static, it is certain to remain the major outlet for pectin. The majority of product development work now being carried out, however, involves the application of low methoxyl pectins as gelling and thickening agents in the expanding convenience food market, and there would seem to be considerable scope for further utilisation of their novel properties.

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LETTER TO THE EDITOR

A RAPID ROUTINE METHOD FOR THE DETERMINATION OF THE TOTAL PECTIN CONTENT IN CITRUS PRODUCTS

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All methods generally used for the determination of total pectin in citrus products are based on the precipitation of Ca pectate and are considerably time-consuming. Several more rapid methods have been proposed (*Fellers and Rice* 1932; *Ahrmann and Hooker*, 1926; *Hinton*, 1940; *Deuel*, 1943; *Yackel*, 1942) and many others which have been surveyed by Kertesz (1951). They are quite general, but all of these require a clarified sample. We tried, with a certain amount of success a method which is a modification of the Fellers and Rice method (1932). We determine the soluble and insoluble pectic compounds, the last being bound to the pulp.

Citrus juices can be analyzed without dilution. The concentrated and comminuted products have to be diluted 1:10 and the squashes not less than 1:5 - 1:6. To 20 ml of the sample 2 ml of 10% NaOH solution are added to solubilize the total pectic compounds as Na-pectate. After 15 min the sample is filtered by suction on hard filter paper. 10 ml of the filtrate are put in a 15 ml conical graduated centrifuge tube and 2 ml of 10% HCl are added. The tube is heated for 15 min in a boiling water bath and then cooled down to room temperature. The tube is then centrifuged for 3 min at 2500 r.p.m. (diam. 13.5 cm). The volume of the pectic acid precipitate is converted into % pectin on a graph prepared by plotting the results obtained by the method for known pectin solutions. This method is sufficiently exact for routine determinations, the results being, generally, up to 20% higher than those obtained by the Ca pectate methods. For instance, the following results have been obtained for the % of total pectic substances:

	Calcium pectate method	*Our method*
Shamouti orange juice	0.020	0.025
Grapefruit juice	0.035	0.042

At any rate it is advisable, when carrying out a series of routine analyies on a particular product, to compare for once results with those obtained by Ca-pectate method.

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A PHARMACOLOGIC STUDY OF PLANTAGLUCIDE

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In the phytochemistry laboratory of the KhNIKhFI, several substances were isolated from the leaves of the large plantain (A. G. Gorin and co-authors). The granulated powder of pectins from the plantain is called plantaglucide.

The present work is devoted to a study of the anti-ulcer activity of plantaglucide and its effect on certain functions of the gastrointestinal tract and on inflammatory edema.

Stomach ulcers were induced in rats by intramuscular injection of phenylbutazone, according to the method described earlier (Ya. I. Khadzhay and G. V. Obolentseva). Plantaglucide was introduced internally together with phenylbutazone in doses of from 0.1 to 1.0 g/kg in the form of an aqueous solution. The experiments were conducted on 61 rats.

The results of the experiment indicate that plantaglucide lowered the inhibitory activity of phenylbutazone. Out of 19 rats which received plantaglucide in 1 g/kg doses, two died, while in the control group, almost half the animals died. It must be noted that, under the influence of plantaglucide, in no single case were loci of necrosis observed nor were there inflammations in the areas of phenylbutazone injection. Upon opening the stomachs of the rats in the control group, ulcerous occurrences were found, while in part of the rats which received plantaglucide, there were no ulcers; in the remainder there were fewer ulcers (see the table).

EFFECT OF PLANTAGLUCIDE ON THE FORMATION OF PHENYLBUTAZONE STOMACH ULCERS IN RATS

Group	Dose in g/kg	# of rats	# of rats surviving	# of rats with ulcers (in %)	# of ulcers	Paul's index	Anti-ulcer activity
Control	—	23	13	100	314	24	—
Plantaglucide	0,1	10	6	87	66	7,2	3,3
	0,5	9	7	71	30	3,5	6,6
	1,0	19	17	30	67	1,2	20,0

In order to explain the effect of plantaglucide on the secretory function of the stomach, 30 experiments were conducted on dogs with stomach fistulae, in the manner of Basova. 20-30 minutes before the start of the experiment, all the dogs received a preparation in a 1-g dose, dissolved in 50 ml of water. Secretion of gastric fluids was elicited by a subcutaneous injection of 0.5 ml of 0.1% histamine solution.

It was established that, under the influence of plantaglucide, the amount of gastric fluid set free by the histamine increased 2½ times in two dogs. In the third dog, significant volume changes in gastric fluids were not noted. In all three animals, plantaglucide elicited an increase of free, as well as total, acidity, on the average of 20-23%. In our experiments, plantaglucide did not affect the protein-splitting activity of the gastric fluids.

In order to determine the effect of the preparation on motor activity of the stomach, the X-ray method of investigation was used. The experiments were conducted on 22 male rats weighing 150-200 g. In all the animals the time of evacuation of the irradiated mass from the stomach was determined in a standard and after administration of the preparation. Plantaglucide was given to the rats internally in doses of 0.1-1 g/kg in the form of an aqueous solution 20-30 min before the introduction of the irradiated mass. The experiments indicated that, in the rats which received plantaglucide in 0.1 g/kg doses, the migration of stomach contents into the small intestine slowed down almost two times, and with an increase in dosage to 1 g/kg, migration slowed down 3½ times. It must be noted that during the first 50-60 min, the irradiated mass did not pass out of the stomach. (The stomach is very large and sometimes takes on a bag-shaped form.)

The effect of plantaglucide on the smooth musculature was studied in an isolated slice of rabbit intestine according to the method of Magnus. The preparation was administered experimentally in concentrations of from 10 to 200 mg %. A quantitative analysis of the results was made using the method proposed by Yu. D. Zil'ber. In the experiments it was established that muscle tone in the intestine decreased immediately after introduction of the preparation and, for 20-30 sec thereafter, it rose almost to the original value. With preparation concentrations of 10-20 mg %, an 80% decrease in tone is observed, on the average. A decrease in the height of intestine preservation corresponded to an increase in the dose, reaching 60-100% for concentrations of 100-200 mg %.

The spasmolitic effect of the preparation was determined in rat intestine with a spasm induced by barium chloride and acetylcholine, and in guinea pig intestine with a histamine-induced spasm. In the experiments in which a spasm was induced with barium chloride and histamine, plantaglucide elicited a distinct spasmolitic effect, which increased with an increase in concentration. ED_{50} in the spasm elicited by barium chloride was 112 + or - 10 mg %, and 127 + or - 21 mg % for histamine. In the experiments with an acetylcholine spasm, plantaglucide proved to be inactive in all the experimental concentrations.

The antospasmodic properties of plantaglucide were studied in mice and rats in which inflammatory edema had been induced by injections of formalin and dextrin (Ye. Yu. Strel'nikov). In experiments with formalin-induced inflammatory edema, the preparation was introduced internally in doses of 0.5 and 1 g/kg every two hours before the injection and for four hours afterwards. It was established that in both dosages, the plantaglucide diminished the edema by 35-40%, i.e. the dosages show an anti-inflammatory action of approximately equal strength. In the experiments on rats with dextrin-induced edema, plantaglucide was introduced internally every hour up to the time of dextran injection, in doses of 0.5 and 1 g/kg. Its anti-edemic effect was observed from the change in volume of edemic rear extremities of the animals, which were recorded simultaneously in the course of 4-5 hours after the introduction of the preparation. The results obtained in these experiments indicated that if, under the influence of dextran in the control group, the volume of the paw increased towards the end of the 4-hr observation by 42%, the edema was less in the group of animals which received plantaglucide.

Toxicity and general effect of plantaglucide were studied in mice, rats, and dogs. The mice received a preparation in the form of 5-10% aqueous solutions, given intraperitoneally. The LD_{50} preparation contains 1.7 (1.12-2.58) g/kg (according to Litchfield and Wilcoxson).

Two groups of young rats weighing 70-80 g (10 animals in each group) were given plantaglucide internally daily in 2- and 3-g/kg doses in the course of 28 days. During the entire observation period, no visible changes

were noted in the state and weight of the animals.

In the experiments conducted on two dogs, with daily introduction of the preparation in 3- and 5-g doses (weight of the dogs was 12 and 14 kg), no changes were observed in the general state and behavior over a 3-wk period. In one dog, which had received a daily 5-g preparation, a weakening effect was noted within four days after the beginning of dosage administration. These studied supported data on the good transportability of plantaglucide.

IN CLOSING

As a result of the studies of the pharmacological properties of the pectin preparation from the leaves of the large plantain, it was established that it is an anti-ulcer, anti-edemic, and a spasmodic agent. In the study of the preparation's effect on motor-secretory function of the stomach, it was shown that plantaglucide slows down the process of evacuation from the stomach in the small intestine. In addition, the preparation stimulates somewhat stomach secretion. According to the data of Manville and the co-authors, Winters and Peters, the introduction of pectin into the animals' gastrointestinal tract protects the mucous membrane from ulcers caused by the action of menthol and Atofan. Sasson used, in the treatment of people with ulcers, a combination of tablets made from other pectinoid substances, and he observed positive results in 31 out of 36 cases. Wompkins and his co-authors established from their observations that pectin contributed to the epithelialization of tissues during the treatment of burns and infected wounds, thereby hastening their healing.

The data in the literature on the effect of pectin-like substances and the indicated properties for plantaglucide permit us to recommend it for treatment of gypsacide gastritis and ulcers of the stomach and duodenum with decreased and normal acidity. Plantaglucide is recommended by the Pharmacological Committee of the USSR Ministry of Health Conservation for medicinal purposes.

CONCLUSIONS

1. A pectin-like preparation made from the leaves of the large plantain (plantaglucide), and used in experiments on rats with phenylbutazone-induced stomach ulcers, exhibits a distinct anti-ulcer effect.
2. The preparation decreases the smooth muscle tone of the stomach and intestine and possesses an anti-spasmodic effect which is clearly expresses for spasms caused by barium chloride and histamine.
3. Exudation processes during experimental inflammation decrease noticeably under the effect of plantaglucide.
4. In experiments on dogs, plantaglucide exhibits a somewhat stimulatory effect on secretion of stomach fluids.
5. Plantaglucide does not exhibit a toxic effect in experiments on mice, rats, and dogs.

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УДК [illegible]

ФАРМАКОЛОГИЧЕСКОЕ ИССЛЕДОВАНИЕ [illegible]ГЛИКОЗИДА

[illegible]

Лаборатория [illegible]

[illegible]

[illegible]

[illegible]

Результаты опытов показали, что плантаглюцид снимал угнетающее действие бутадиона. Из 19 крыс, получавших плантаглюцид в дозе 1 *г/кг*, погибло 2, в то время как в контрольной группе погибла почти половина животных. Нужно отметить, что под влиянием плантаглюцида ни в одном случае не наблюдалось очагов некроза и воспаления в местах инъекций бутадиона. При вскрытии желудков у всех крыс контрольной группы обнаружены язвенные поражения, тогда как у части крыс, получивших плантаглюцид, язв не было совсем, а у остальных их было меньше (см. таблицу).

Влияние плантаглюцида на образование бутадионовых язв желудка у крыс

Группа	Доза (в г/кг)	Всего крыс	Число [illegible]	Число [illegible]	Всего язв	[illegible]	Противоязвенная активность
Контроль	—	23	13	100	311	24	—
Плантаглюцид	0,1	10	8	87	66	7,2	3,3
„	0,5	9	7	71	36	3,6	6,6
„	1,0	19	17	30	67	[illegible],2	20,0

С целью выяснения влияния плантаглюцида на секреторную функцию желудка проведено 30 опытов на собаках с фистулами желудка по Басову. За 20—30 мин. до начала исследования все собаки получали препарат в дозе 1 *г* на животное, растворенный в 50 *мл* воды; секреция желудочного сока вызывалась подкожной инъекцией 0,5 *мл* 0,1% раствора гистамина.

Установлено, что под влиянием плантаглюцида у 2 собак количество выделяющегося на гистамин желудочного сока увеличилось в $2^1/_2$ раза. У третьей собаки существенных изменений объема желудочного сока не отмечено. У всех 3 животных плантаглюцид вызывал повышение как свободной, так и общей кислотности в среднем на 20—23%. Плантаглюцид в наших опытах не оказывал влияния на протеолитическую активность желудочного сока.

Для определения влияния препарата на двигательную активность желудка использовали рентгеновский метод исследования. Опыты проведены на 22 крысах-самцах весом 150—200 *г*. У всех животных определяли время эвакуации рентгеноконтрастной массы из желудка в норме и после введения препарата. Плантаглюцид вводили крысам внутрь в дозах 0,1—1 *г/кг* в виде водного раствора за 20—30 мин. до [illegible] рентгеноконтрастной массы. Опыты показали, что у крыс, получивших плантаглюцид в дозе 0,1 *г/кг*, переход содержимого желудка [illegible] отдел кишечника замедлился почти в 2 раза, а при увеличении [illegible] до 1 *г/кг* — в $3^1/_2$ раза. Следует отметить, что на протяжении [illegible] 60 мин. рентгеноконтрастная масса из желудка [illegible] был больших [illegible]

[illegible]

[illegible] испытывали в конце [illegible] ботку результатов [illegible] ром. В опытах установлено, что [illegible] сразу же после введения препарата [illegible] ливается почти до [illegible] не [illegible] снижен [illegible]

Спазмолитический эффект препарата определяли на кишке крысы со спазмом, вызванным хлористым барием и ацетилхолином, и кишке морской свинки с гистаминовым спазмом. В опытах со спазмом, вызванным хлористым барием и гистамином, плантаглюцид вызывает отчетливое спазмолитическое действие, возрастающее при повышении концентрации. ED_{50} при спазме, вызванном $BaCl_2$, составляет 112±10 *мг*%, при гистаминовом — 127±21 *мг*%. В опытах с ацетилхолиновым спазмом плантаглюцид во всех испытанных концентрациях оказался неактивным.

Противовоспалительные свойства плантаглюцида изучали на белых мышах и крысах, у которых воспалительный отек вызывали инъекциями формалина и декстрина (Е. Ю. Стрельников). В опытах с формалиновым воспалительным отеком препарат вводили внутрь в дозах 0,5 и 1 *г/кг* за 2 часа до инъекции формалина и через 4 часа после нее. Установлено, что в обеих дозах плантаглюцид уменьшает воспалительный отек на 35—40%, т. е. оказывает противовоспалительное действие примерно равной силы. В опытах на крысах с декстрановым отеком плантаглюцид вводили внутрь за час до инъекции декстрана в дозах 0,5 и 1 *г/кг*. О противоотечном действии судили по изменению объема отечных задних конечностей животных, которое регистрировали онкометрически в течение 4—5 часов после введения препарата. Полученные в этих опытах результаты показали, что если под влиянием декстрана у контрольной группы животных объем лап к концу 4-го часа наблюдения увеличивался на 42%, то в группах животных, получавших плантаглюцид, явления отека были менее выражены.

Токсичность и общее действие плантаглюцида изучали на мышах, крысах и собаках. Мышам препарат вводили в виде 5—10% водных растворов внутрибрюшинно. LD_{50} препарата составляет 1,7 (1,12÷÷2,58) *г/кг* (по Литчфилду и Уилкоксону).

Двум группам молодых крыс весом 70—80 *г* (по 10 животных в каждой группе) плантаглюцид вводили внутрь ежедневно в дозах 2 и 3 *г/кг* в течение 28 дней. В течение всего периода наблюдений видимых изменений в состоянии и весе животных не отмечено.

В опытах, проведенных на 2 собаках, при ежедневном введении препарата в дозах 3 и 5 *г* на животное (вес 12 и 14 *кг*) в течение 3 недель изменений общего состояния и поведения не наблюдалось. У одной собаки, получавшей по 5 *г* препарата ежедневно, через 4 дня после начала введения отмечен послабляющий эффект. Эти исследования подтвердили данные о хорошей переносимости плантаглюцида.

Заключение

В результате исследования фармакологических свойств пектинового препарата из листьев подорожника большого установлено, что он оказывает противоязвенное, противовоспалительное и спазмолитическое действие. При изучении влияния препарата на моторно-секреторную функцию желудка оказалось, что плантаглюцид замедляет процессы эвакуации из желудка в тонкий отдел кишечника, кроме того, препарат несколько стимулирует секреторную функцию желудка. Полученные нами данные согласуются с литературными сведениями о действии пектиновых веществ. По данным Manville с соавторами, Winters и Peters, введение пектина в желудочно-кишечный тракт животных предохраняет слизистые оболочки от язвенных повреждений, вызываемых действием ментола и атофана. Sasson применил для лечения язвенных больных комбинированные таблетки, содержащие наряду с другими веществами пектиноид, и наблюдал благоприятные результаты у 31 из 36 больных. Наблюдениями Tompkins с соавторами установлено, что пектин способствует эпителизации тканей при лечении ожогов и зараженных ран, ускоряя тем самым их заживление.

Литературные данные о действии пектиновых веществ и указанные свойства плантаглюцида позволили рекомендовать его для лечения гиперацидных гастритов и язвенной болезни желудка и двенадцатиперстной кишки с пониженной и нормальной кислотностью. Плантаглюцид рекомендован Фармакологическим комитетом Министерства здравоохранения СССР для медицинского применения.

Выводы

1. Пектиновый препарат из листьев подорожника большого (плантаглюцид) в опытах на белых крысах с бутадионовой язвой желудка оказывает выраженное противоязвенное действие.

2. Препарат понижает тонус гладкой мускулатуры желудка и кишечника и обладает спазмолитическим действием, которое отчетливо выражено при спазмах, вызванных хлористым барьем и гистамином.

3. Экссудативные процессы при экспериментальном воспалении под влиянием плантаглюцида заметно уменьшаются.

4. В опытах на собаках плантаглюцид оказывает некоторое стимулирующее влияние на секрецию желудочного сока.

5. Плантаглюцид в опытах на мышах, крысах и собаках не оказывает токсического действия.

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PHARMACOLOGICAL ANALYSIS OF PLANTAGLUCIDE

G. V. Obolentseva, Ya. I. Khadzhai

In experiments conducted on animals it was ascertained that plantaglucide (a pectin preparation made from plantaginis majoris folia) produces a well accentuated effect, and being given in a dose of 1 gm/kg it cuts down the ulceration index in the rat's stomach as much as 20 times. The drug intensifies somewhat the secretion of gastric juice in dogs. It lowers the tone and reduces the range of contractions in an isolated rabbit's intestine and also has a spasmolytic effect which becomes distinct in spasms caused by barium chloride and histamine. In experiments on rats plantaglucide lengthens the X-ray contrast medium stomach evacuation time. The preparation helps to reduce an inflammatory edema provoked by formalin and dextran. Its prolonged enteral administration to rats and dogs does not produce any toxic effects.

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Effect of Pectin Dose on Serum Cholesterol Levels

GRANT H. PALMER, A.B.* AND DAVID G. DIXON, M.D.†

ELEVATED serum cholesterol levels have assumed added importance to the clinician because of the possible relationship of cholesterol to the etiology of atherosclerosis. The lipid content of human aortas has been studied by Böttcher et al.[1] and by Mead and Gouze[2] who noted that the cholesterol or sterol fraction of aortic lipids increases as the severity of atherosclerosis becomes more pronounced.

Aside from limiting the dietary intake of cholesterol and cholesterol precursors, the control or reduction of cholesterol concentration in the blood has been approached through a variety of physiologic pathways, such as limiting cholesterol production, prevention of cholesterol transfer across the intestinal wall, increased cholesterol utilization and increased fecal output of cholesterol and its conjugates.

Oral ingestion of pure citrus pectin has been observed to increase fecal cholesterol output.[3] Other investigators using Pectin N.F. XI, a pure citrus pectin, reported the lowering of serum cholesterol levels.[4-7] A recent study on poultry suggests "that pectin may be efficacious in retarding spontaneous avian atherogenesis."[8] These observations, particularly the clinical report of Keys and associates,[4] raise the question of what is an effective dose of pectin for reducing blood cholesterol concentrations. With this thought in mind the present study was undertaken.

METHOD

Eighteen volunteer adult male subjects entered into this double-blind study. Sixteen completed four of the six four week test periods, fourteen completed five and ten subjects completed all six periods.

This investigation purposely was designed not to include dietary control in order to evaluate pectin intake under conditions generally present in outpatient clinics. However, all subjects were urged to continue on their usual diet, smoking habits and exercise patterns throughout the study.

One of the eighteen subjects was lost to the study after the first test period through a change of employment. After the first two treatment periods another subject withdrew from the study because he found it difficult to take the required 20 capsules each day.

Serum cholesterol levels were determined by the method of Pearson and co-workers[9] using a pooled serum reference standard with each series of determinations. Blood samples were obtained from each subject just before the noon meal on Monday, Wednesday and Friday prior to the start of the study for use as each subject's reference cholesterol level. Subsequent blood samples were taken at the same hour during the final week of each treatment period, also on Monday, Wednesday and Friday.

All participants received several packets containing 10 capsules each at the start of every test period and were instructed to take the capsules of one packet in the morning after breakfast and the capsules of a second packet in the evening after dinner every day. The capsules contained either Pectin N.F. XI or Alphacel®. The selection of Alphacel for use as the placebo material was based on the finding that it did not significantly effect serum cholesterol concentrations.[4,10]

The daily pectin intake was varied by substituting Alphacel capsules for pectin-containing capsules. Each number zero capsule contained 500 mg. of pectin or 170 mg. of Alphacel. The maximum daily dose of 20 pectin capsules, therefore, was 10 gm. The daily pectin intake was changed by 2 gm. increments by replacing 4 pectin capsules with capsules filled with Alphacel.

In Table I the order of pectin intake during the several test periods assigned to each participant is

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Table I
Pectin Dosage Schedule

Subject No.	Treatment Periods* 1	2	3	4	5	6
1	4–16	8–12	12–8	16–4	20–0	0–20
2	8–12	12–8	16–4	20–0	0–20	4–16
3	16–4	20–0	0–20	4–16	8–12	12–8
4	20–0	0–20	4–16	8–12	12–8	16–4
5	4–16	8–12	12–8	16–4	20–0	...
6	8–12	12–8	16–4	20–0	...	...
7	12–8	16–4	20–0	...	4–16	8–12
8	16–4	20–0	0–20	4–16	8–12	12–8
9	20–0	0–20	4–16	8–12	12–8	16–4
10	0–20	4–16	8–12	12–8	16–4	20–0
11	4–16	8–12	12–8	16–4	20–0	0–20
12	8–12	12–8	16–4	20–0	0–20	4–16
13	12–8	16–4	20–0	0–20	4–16	8–12
14	16–4	20–0	0–20	4–16	8–12	...
15	.	4–16	8–12	12–8	16–4	20–0
16	...	...	20–0	4–16	8–12	12–8

*Four weeks each. The figures in these columns refer to the number of pectin or Alphacel capsules assigned to be taken in divided daily doses.

Table II
Serum Cholesterol Values

Treatment	Subject No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Initial	278	212	212	230	249	236	212	286	184	206	206	249	...	218	236	270
	257	200	212	243	286	230	218	302	195	249	224	271	224	230	218	264
	263	218	189	263	249	218	206	320	195	195	229	256	200	206	212	249
2 gm. pectin/day	256	...	174	...	263	...	206	278	184	211	243	...	184	243	235	264
	286	212	179	256	263	..	212	285	189	190	256	263	194	228	224	263
	271	211	184	249	271	.	200	270	179	194	224	249	206	184	206	249
4 gm. pectin/day	223	230	200	249	285	218	...	249	195	200	211	243	...	212	212	292
	184	236	194	242	271	243	230	263	179	205	195	236	206	217	205	278
	249	230	184	249	242	236	217	270	174	212	217	256	179	...	218	263
6 gm. pectin/day	257	217	...	236	224	229	243	..	189	195	218	250	206	...	212	...
	263	212	184	242	224	218	243	271	189	184	218	236	205	...	200	278
	286	...	179	236	206	200	243	270	184	206	212	223	218	..	206	235
8 gm. pectin/day	243	230	176	...	271	206	211	278	...	195	224	228	195	206	195	...
	263	224	195	263	223	205	190	310	184	200	206	236	190	249	200	...
	249	...	200	242	249	200	189	278	189	200	195	223	164	206	206	...
10 gm. pectin/day	..	189	179	286	200	212	200	270	184	...	224	264	189	211	...	243
	270		160	270	241	206	195	286	205	184	223	242	178	206	189	286
	278	200	164	263	.	211	207	270	200	194	223	...	179	206	179	278
Placebo	...	200	179	249	...	.	.	278	169	206	...	...	200	212	...	...
	294	200	173	243	..	.	...	271	184	205	236	. .	184	223	...	...
	249	.	179	276	.	...	..	263	174	200	211	256	189	...	...	...
10 wk. after final treatment	278	223	184	256	...	...	223	270	173	194	229	268	194	212	229	256
	271	218	195	271	...	...	212	279	184	224	243	264	206	230	212	264
	249	271	200	249	..	...	...	263	189	206	...	...	179	230	218	...

presented. The daily pectin intake sequence followed the general pattern of increasing the pectin dose by 2 gm. at the beginning of each four week treatment period. The Alphacel placebo was taken after the treatment with 10 gm. of pectin. To nullify any seasonal variation, as the study progressed, the six doses of pectin under study were assigned in rotation to the several subjects at the start of the investigation.

RESULTS

The values determined for cholesterol in each blood sample, as milligrams per 100 milliliters of serum, are presented in Table II. Comparison of the average initial values with those obtained after each of the several pectin dosage periods, using the Student's t test, indicates a significant reduction of cholesterol concentration at daily pectin intakes of 6 gm. ($P = 0.05$), 8 gm. ($P = 0.01$) and 10 gm. ($P = 0.01$).

Applying the analysis of variance procedure* to the data presented in Table II confirms this cholesterol reduction effect at daily pectin intake levels of 6 gm., 8 gm. and 10 gm. Comparison of the F ratios from the analysis of variance calculations with a F distribution table shows the responses at treatment levels of 6 gm., 8 gm. and 10 gm. pectin were significant at the 0.025 confidence level. The individual subject variability as well as the interaction between the subjects on self-selected diets and pectin treatment were highly significant at the 0.001 level indicating that, within the dose range tested, all subjects did not respond equally to the influence of pectin on lowering serum cholesterol levels.

The cholesterol values obtained for each subject throughout the study were compared with the pectin dosage by linear correlation.

* In order for all treatment rows to have the same number of items per cell for each subject, as needed for the analysis of variance calculation procedure, in those subjects from whom only two cholesterol values were obtained the average was included as the third cell item. This lack of complete figures for cholesterol values occurred on occasion due to vacations or the unavailability of the subject when blood samples were collected.

Correlation Coefficients

Subject No.	r	df	P
1	0.0608	4	
2	0.1919	4	
3	−0.6393	4	
4	0.5874	4	
5	0.7376	4	0.1
6	0.8132	3	0.1
7	−0.1969	4	
8	−0.9527	4	
9	0.4222	4	
10	−0.8251	4	0.05
11	−0.3319	4	
12	−0.5462	4	
13	−0.7012	4	
14	−0.5222	3	
15	−0.9668	4	0.005
16	0.2707	3	

The significance of the correlation coefficient (r) for each subject was ascertained from the percentage points table of correlation coefficients. The calculated r values, the degrees of freedom (df) and the indicated probability (P) for all subjects are summarized in Table III.

Negative correlation coefficients indicate that as the daily pectin dose was increased, the corresponding serum cholesterol value decreased. Of the sixteen subjects in this study, twelve showed a negative r value. Two of these values were significant at the 0.05 level.

Least squares lines for subjects 10 and 15 (the two subjects having a statistically significant cholesterol reduction) were calculated and plotted (Fig. 1). Figure 1 graphically illustrates the over-all effect of orally ingested pectin on these subjects. It also indicates that a daily pectin dose of about 5 gm. for subject 10 and about 7 gm. for subject 15 should maintain their serum cholesterol near the 200 mg. per 100 ml. level.

The standard error of the mean times the range values obtained from the Student's t test at $p = 0.05$ (5 per cent) and $p = 0.01$ (1 per cent) give the shortest significant range figures for Duncan's range test. In Duncan's range test these values serve as a guide to indicate significance of the differences between

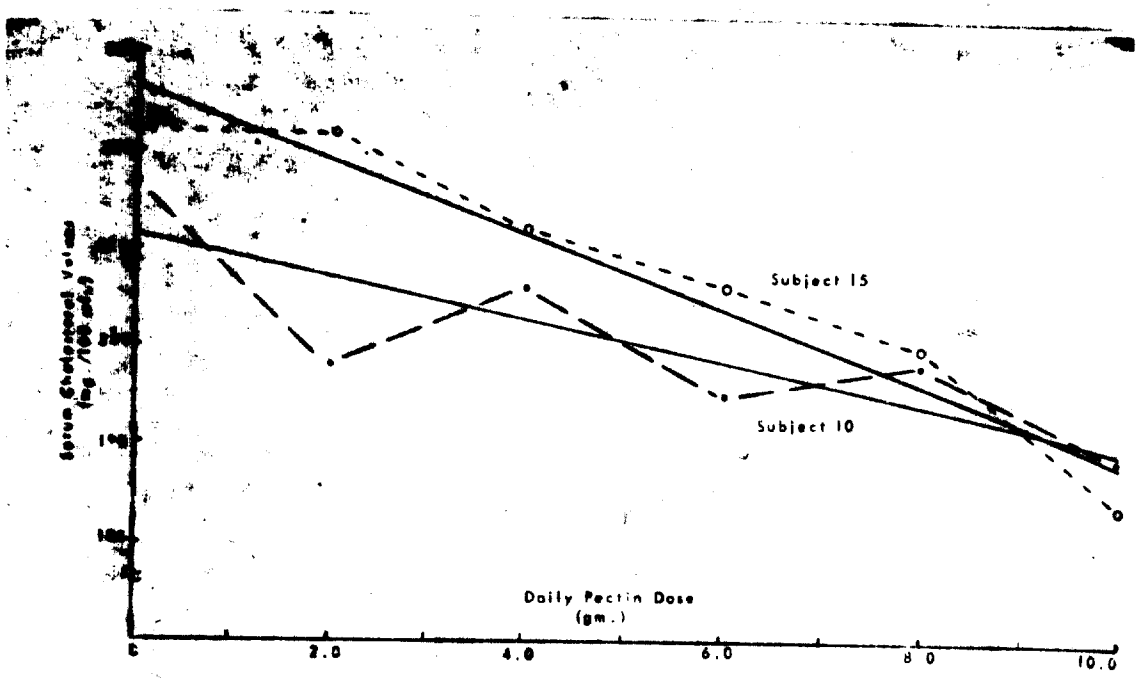

FIG. 1. Linear regression.

TABLE IV
Duncan's Range Tests

Pectin Dose Compared	Subjects at Dose Levels Compared/df	Initial	Average Cholesterol Values 2 gm.	4 gm.	6 gm.	8 gm.	10 gm.	Items in Range	Shortest Significant Range 5%	1%
Initial to 2-10 gm.	13/60	232.5	227.6	222.0	221.3	220.1	218.2			
			4.9	10.5	11.2	12.4*	14.3*	6	12.19	15.92
				5.6	6.3	7.5	9.4	5	11.98	15.67
					0.7	1.9	3.8	4	11.72	15.37
						1.2	3.1	3	11.35	14.95
							1.9	2	11.03	14.34
Initial to 4-10 gm.	14/59	232.1		222.7	220.9	218.9	217.6			
				9.4	11.2*	13.2*	14.5*	5	11.66	15.25
					1.8	3.8	5.1	4	11.40	14.95
						2.0	3.3	3	11.04	14.55
							1.3	2	10.49	13.96
Initial to 6-10 gm.	14/39	232.1			220.9	218.9	217.6			
					11.2*	13.2*	14.5†	4	10.76	14.21
						2.0	3.3	3	10.42	13.83
							1.3	2	9.91	13.26
Initial to 8-10 gm.	15/28	231.2				219.0	216.9			
						12.2†	14.3†	3	9.34	12.48
							2.1	2	8.88	11.96
Initial to 10 gm.	16/15	233.1					220.2			
							12.9*	2	10.44	14.44

Significant at the 5% level.
Sig. at the 1% level

the various cholesterol levels. The data in Table IV demonstrate that daily doses of 8 to 10 gm. of pectin, added to the dietary regimen, caused a significant decrease in serum cholesterol in this group of subjects.

COMMENTS

Treatment of elevated serum cholesterol levels has been based on daily medication or control of the kind and amount of dietary fat intake. Generally, medications designed to limit cholesterol accumulation have been found to produce unwanted or detrimental side reactions when tested adequately in the clinic. As a result many therapeutic formulas never become accepted by the medical profession.

Physicians generally recommend and prescribe dietary regimens. At the same time they recognize that adherence to restricted diets by the patient is usually temporary.

Pectin, a normal constituent of fruits and vegetables, may serve as a therapeutic measure for the reduction of serum cholesterol concentrations. In this study citrus Pectin N.F. XI was added to self-selected diets. Although the initial serum cholesterol levels of these subjects were nearly all within the normal range pectin effected a reduction of total cholesterol.

The means by which pectin reduces serum cholesterol has not been evaluated. Studies on the metabolic fate of pectin indicate that it passes through the intestinal tract essentially unchanged until it reaches the colon where it is broken down by the normal bacterial flora.[11–15] This action indicates that whatever effect pectin may have, it apparently takes place either in the stomach or small intestine.

SUMMARY

Evaluation of the daily pectin intake, in 2 gm. increments, has been carried out in a double-blind clinical study of sixteen volunteer subjects. The treatment time extended over six four week test periods followed by a ten week nontreatment period. Total serum cholesterol determinations were made on blood samples obtained prior to treatment and during the final week of each observation period.

Four statistical procedures were used to [illegible] the results of the Student's t test, analysis of variance and Duncan's range test all indicated that a daily dose of 6 gm. to 10 gm. of Pectin N.F. XI significantly reduced the serum cholesterol level in this group of subjects. The correlation coefficient calculation for each subject was the fourth statistical procedure used to evaluate the data.

The results of the paired Student's t test implied that possibly there was a carry over effect at the 10 gm. intake level which appears to have been dissipated in two to three months.

In addition to evaluating the effect of treatment with various pectin doses, the analysis of variance indicated that all subjects on self-selected diets do not respond equally well to pectin therapy.

From the correlation coefficient calculations it was noted that twelve of the sixteen subjects had negative coefficients signifying a reduction of serum cholesterol with increased pectin doses. Two of these twelve subjects had negative correlation coefficients which were significant at $P = 0.05$.

A graph of the least squares lines for these two subjects (Fig. 1) illustrates the over-all effect pectin treatment had on these subjects and indicates that some variability of pectin dosage is needed by different people in order to maintain lowered cholesterol levels.

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Effect of Pectin, a Hypocholesterolemic Polysaccharide, on Vitamin A Utilization in the Rat

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ABSTRACT To study the effects of dietary pectin on the utilization of vitamin A and carotene, rats were fed either a ground cube diet or a semipurified casein-sucrose diet, supplemented with either cholesterol, pectin or both. Pectin was hypocholesterolemic when the diet contained cholesterol, and was without effect in a cholesterol-free diet. The hypocholesterolemic properties were demonstrated without the adverse effect of limiting the absorption of vitamin A or utilization of the provitamin β-carotene. The data suggest a mechanism of action different from that of cholestyramine.

Dietary regimens and various drugs have been used to lower serum cholesterol as a result of the association of atherosclerotic disease and increased serum cholesterol. These treatment patterns either limit the amount or absorption of dietary cholesterol, increase excretion, or decrease endogenous cholesterol biosynthesis. Therapeutic or prophylactic regimens of these types have nutritional significance since factors which influence the absorption or transport of a specific lipid, cholesterol, may also modify the metabolism of other lipid-soluble substances, namely, the fat-soluble vitamins.

The vitamin A status of rats treated with *p*-chlorophenoxyisobutyrate (CPIB, Atromid S), a compound limiting cholesterol transport (1), and a group of inhibitors of cholesterol synthesis, Benzmalecene, SK&F No. 525A and AY9944 (2) have been studied and appear to have no adverse effects in this regard. The present investigations were undertaken to study a compound limiting exogenous cholesterol absorption.

Pectin ingested orally lowers serum cholesterol in man (3) as do numerous mucilaginous polysaccharides in chickens (4) and rats (5, 6). Although the precise mechanism of action of these compounds is not known, the effects appear to be related to a reduction of cholesterol absorption. Cholestyramine (7) also limits cholesterol absorption by interfering with bile acid metabolism and at the same time decreases the utilization of fat-soluble vitamins (8). Whether pectin influences the metabolism of the fat-soluble vitamins was not known. The present studies were designed to examine the effect of pectin on the utilization of vitamin A and β-carotene in the rat.

EXPERIMENTAL

Materials and methods. Weanling male rats of the Wistar strain were used in all experiments. Diets and water were given ad libitum. The basal diets were either ground stock cubes to which was added 3% corn oil, or a semipurified casein-sugar type diet. The composition of the latter diet was: (in percent) sugar, 70; vitamin-free casein, 20; cottonseed oil, 5; salt mix USP XIV, 4; and a vitamin A-free vitamin mixture,[1] 1. Liver vitamin A was determined as previously described (9) and serum cholesterol by the method of Ferro and Hamm (10). The vitamin A status of our laboratory animals is routinely checked and found to be relatively low, approximately 50 to 55 μg vitamin A/liver.

Experimental design. This paper summarizes the results of three experiments. In experiment 1, 1% cholesterol, 3% pec-

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[1] Vitamin mixture was composed of: (in grams) thiamine hydrochloride, 0.5; riboflavin, 1.0; pyridoxine hydrochloride, 0.4; calcium pantothenate, 4.0; niacin, 4.0; *p*-aminobenzoic acid, 10.0; biotin, 0.02; folic acid, 0.2; vitamin B_{12}, 0.002; inositol, 25; choline bitartrate, 200; calciferol (250,000 IU/g), 0.118; *dl-α*-tocopherol, 10; menadione, 0.5; and alphacel to make 1000 g. This mixture was incorporated into the diet at the 1% level.

tin[2] or both were incorporated into the casein-sucrose type diet containing supplemental vitamin A (Animal Nutrition Research Council reference standard equivalent to 3000 μg vitamin A alcohol/kilogram diet). Weanling rats were fed for 18 days. In experiment 2, 3% pectin was incorporated into the ground cube diet in which vitamin A activity was derived from both carotenoids (4000 μg β-carotene/kilogram) and preformed vitamin A (2000 μg/kilogram). This diet or the control ground cube diet without pectin was fed for 21 days. In experiment 3, male weanling rats were divided into two groups and fed the casein-sucrose vitamin A-free basal diet or this diet containing 2% pectin. After 20 days feeding, 10 animals in each group were dosed by stomach tube with 537 μg vitamin A acetate in 0.5 ml of cottonseed oil while an additional 10 animals in each group were dosed with 5.40 mg β-carotene in 0.5 ml cottonseed oil. Forty-eight hours after dosing, the animals were killed and liver vitamin A was determined to study the effect of pectin on the utilization of vitamin A or β-carotene from a single oral dose.

RESULTS

The effect of pectin on liver storage of vitamin A in the rat was studied in three separate experiments.

The incorporation of cholesterol, pectin or both into the casein-sucrose type diet containing supplemental vitamin A (experiment 1) did not influence the rate of growth when fed for a period of 18 days (table 1). Cholesterol feeding significantly increased ($P \leq 0.01$) serum cholesterol levels. Dietary pectin when fed concomitantly with cholesterol abolished the increment in serum cholesterol resulting from dietary cholesterol. Pectin was without effect on serum cholesterol of animals fed the cholesterol-free diet. Liver storage of vitamin A was significantly reduced by dietary cholesterol. Pectin, however, did not significantly influence liver vitamin A levels regardless of cholesterol intake. Pectin

[2] Pectin generously supplied by General Foods, Toronto, Ontario.

TABLE 1

Effects of pectin in a semipurified diet on serum cholesterol and liver vitamin A of the rat

Pectin, % of diet	0	0	3	3
Cholesterol, % of diet	0	1	0	1
Number of animals per group	15	15	15	15
Body weight, g	269 ± 5.6[1]	282 ± 8.3	265 ± 14.1	265 ± 6.9
Liver weight, g	12.1 ± 0.39	14.9 ± 0.68	12.1 ± 0.80	12.2 ± 0.53
Vitamin A				
μg/g liver	23.5 ± 1.34	14.2 ± 0.80	20.6 ± 0.87	17.0 ± 0.86
μg/liver	284 ± 17.4	209 ± 12.0	250 ± 19.9	204 ± 7.4
Serum cholesterol, mg/100 ml	94.7 ± 5.94	116.9 ± 5.10	91.3 ± 3.13	85.6 ± 3.72

[1] Mean ± SEM.

TABLE 2

Effects of pectin in a practical type diet on serum cholesterol and liver vitamin A

Dietary pectin	No. of animals per group	Body weight	Liver weight	Vitamin A		Serum cholesterol
%		g	g	μg/g liver	μg/liver	mg/100 ml
0	20	221 ± 5[1]	8.6 ± 0.2	114 ± 3	966 ± 37	86.7 ± 3.9
3	20	218 ± 5	8.9 ± 0.2	104 ± 2	921 ± 30	90.8 ± 3.0

[1] Mean ± SEM.

TABLE 2

Effect of dietary pectin on the utilization of a single oral dose of carotene or vitamin A

Dietary pectin, % / Oral supplement	0 / nil	2 / nil	0 / Vitamin A	2 / Vitamin A	0 / Carotene	2 / Carotene
Body weight, g	198 ±6[1]	160 ±3	141 ± 3	162 ± 5	141 ± 3	152 ± 8.0
Liver weight, g	7.4±0.5	8.0±0.2	9.0± 0.2	8.4± 0.4	8.3± 0.3	9.1± 0.6
μg vitamin A/g liver	0.8±0.2	1.3±0.3	16.7± 1.5	15.7± 1.4	23.3± 1.8	28.9± 2.0
μg vitamin A/liver	5 ±1	10 ±2	152 ±17	131 ±10	183 ±19	211 ±16

[1] Mean ± SEM.

prevented the hypocholesterolemia induced by dietary cholesterol, and did so without interfering with vitamin A absorption. It did not, however, prevent the lowering of liver vitamin A induced by cholesterol feeding. These interesting observations cannot be explained on the basis of present knowledge.

Pectin (3%) was incorporated into the ground cube diet in which vitamin A was derived from both carotenoids and preformed vitamin A (experiment 2). Ad libitum feeding of this diet for 21 days did not significantly affect body weight, liver weight or serum cholesterol when compared to animals fed the ground cube diet without pectin (table 2). Total liver vitamin A was not significantly influenced by pectin. The concentration of liver vitamin A was significantly lower ($P < 0.01$) which probably reflected the slightly higher liver weights of pectin-fed animals. In experiment 3 the 20-day predosing feeding period (table 3) reduced liver stores of vitamin A to negligible amounts. The oral administration of either vitamin A or carotene produced significant liver stores of vitamin A. The feeding of 2% pectin did not affect the resulting liver stores, demonstrating no effect on the utilization of either vitamin A or β-carotene.

DISCUSSION

The observations reported in this study, using rats, on the ability of dietary pectin to decrease the elevation of serum cholesterol resulting from ingestion of diets containing 1% cholesterol are in agreement with previous reports (11, 12). Leveille and Sauberlich (12) studied the mechanism of the cholesterol-depressing effect of pectin in the cholesterol-fed rat and suggested that the hypocholesterolemic effects are not mediated by an alteration of the intestinal microflora but rather by inhibiting bile acid absorption and reducing cholesterol absorption. The latter mechanism is suggested mainly by the similarity in response of the cholesterol-fed rat to pectin and a known inhibitor of bile acid absorption, cholestyramine. These authors further state, however, that the impaired cholesterol absorption is only partially responsible for its hypocholesterolemic effect.

The present experiments would support a concept of different mechanisms for the action of pectin and cholestyramine since cholestyramine is known to limit vitamin A absorption through its effect on bile acid metabolism (8) while pectin was without effect on vitamin A utilization.

Recent surveys in Canada (13, 14) on human liver vitamin A stores have demonstrated a surprisingly high incidence of low or nondetectable reserves of the vitamin despite a high per capita disappearance of the vitamin and provitamins from the consumer market. Further emphasis on studies of factors affecting the utilization of vitamin A (e.g., food additives, therapeutic agents and environmental contaminants) appears warranted. In the present series of experiments other mucilaginous polysaccharides, sodium carrageenan [5] and a refined psyllium-seed hydrophilic muciloid [6] were also studied in relation to the utilization of vitamin A and carotene. The results were similar in all respects to those observed following the feeding of pectin and are thus not repeated in tabular form. These data clearly demonstrate that the mucilaginous polysaccharides have hypocholesterolemic properties in the rat without the adverse effect of limiting absorption of vitamin A or the utilization of the provitamin β-carotene, thus suggesting they may have significant application therapeutically or prophylactically for man.

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Effect of Guar Gum and Pectin N. F. on Serum and Liver Lipids Of Cholesterol Fed Rats. (31844)

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Previous studies in our laboratory(1) have demonstrated the hypocholesterolemic effect of 16 mucilaginous polysaccharides in White Leghorn cockerels fed a semi-purified diet supplemented with dietary cholesterol. Because our results on the relative order of activity of the various mucilaginous polysaccharides in the chick did not agree with those of Ershoff and Wells in the rat(2), it appeared of interest to carry out several experiments in rats comparing guar gum and pectin N. F. under similar conditions.

Methods. Two basal diets were employed. The first of these was a casein-sucrose basal diet similar to that employed by Ershoff and Wells(2); the composition of this diet has been previously reported(3). The second diet was powdered Purina Lab Chow. In the casein-sucrose basal diet, test substances (including 1% cholesterol and 10% corn oil) were added at the expense of carbohydrate; in the Purina Lab Chow basal diet, test substances (including 1% cholesterol and 5% corn oil) were added at the expense of the

TABLE I. Effects of Dietary Supplements on Serum and Liver Cholesterol and Liver Total Lipids of Rats Fed a Cholesterol-Supplemented Casein-Sucrose Diet(1) (5 Animals/Group).

Group	Supplements fed with basal ration*	Results at the end of 28 days: Body wt (g)†	Serum total cholesterol (mg/100 ml)†	Liver total cholesterol (mg/g)†	Liver total lipid (%)†
1	None	219 ± 4.2	106 ± 3.3	1.81 ± .07	4.50 ± .22
2	1% cholesterol	227 ± 15.0	146 ± 14.6	15.50 ± 1.97	9.75 ± .87
	Idem + following supplements:				
3	5% pectin N. F.	226 ± 4.2	147 ± 6.9	10.63 ± 1.48	9.95 ± 1.22
4	10% " "	197 ± 16.6	132 ± 6.3	9.20 ± 1.08	7.87 ± .64
5	5% guar gum	217 ± 2.6	134 ± 8.0	10.12 ± 1.02	8.56 ± .34
6	10% " "	194 ± 5.5	105 ± 12.2	4.53 ± .62	5.82 ± .48

* Pectin N. F. (citrus) obtained from Sunkist Growers, Ontario, Calif.; methoxyl content 10.5% on a moisture-ash-free basis. Guar gum (A-20-D) obtained from Stein, Hall and Co., Inc., New York.
† Mean ± standard error of mean.

diet. Carworth Farms rats (CFE strain),* 42-55 g, were divided into groups of 6, and each animal was housed individually with food and water *ad libitum*. Body weight and food consumption were determined weekly. At the end of 28 days on the experimental diets, final body weight and total food consumption were recorded for each animal, then the rats were anesthetized with sodium pentobarbital, and a 5.0 ml blood sample was taken *via* heart puncture. Livers were excised, blotted to remove excess fluid, weighed and stored in a freezer until analyzed. Total liver lipids were determined by the method of Shipley *et al*(4), and total cholesterol was determined on the serum and liver by a modification of the Leffler procedure(5) adapted for use on a Technicon AutoAnalyzer.

Results. Data on body weight, serum and liver cholesterol and liver lipids produced by feeding a casein-sucrose diet supplemented with 1% cholesterol and 10% corn oil to rats over a 28-day period are summarized in Table I. The feeding of 10% guar gum or 10% pectin N. F. (Groups 4 and 6) appeared to cause a slight decrease in body weight gain; however, these decreases were not statistically significant from the non-cholesterol- or cholesterol-supplemented control groups (Groups 1 and 2) at the 5% probability level.

Small decreases in serum cholesterol were observed when 5% guar gum and 10% pectin N. F. were incorporated into the diet; however these changes were not statistically significant at the 5% probability level (Groups 4 and 5 *vs* Group 2); on the other hand, feeding of 10% guar gum lowered serum cholesterol to the level of the control group unsupplemented with cholesterol (Group 6 *vs* Group 1).

Liver cholesterol (mg/g) was reduced from the cholesterol-supplemented control (Group 2) with all levels of agents tested; however, 10% guar gum produced a significantly greater reduction than 10% pectin N. F. (Group 6 *vs* Group 4, $p \leq 0.05$).

Liver total lipids were also decreased with 10% pectin N. F. and 5% and 10% guar gum. However, statistical significance was obtained only with the 10% level of guar gum (Group 6 *vs* Group 2, $p \leq 0.05$).

A similar experiment was carried out in rats in which the casein-sucrose basal diet was replaced with a powdered Purina Lab Chow diet plus 5% corn oil. The data on body weight, serum and liver cholesterol and liver lipids are summarized in Table II.

Body weight gain was decreased in the groups receiving 10% pectin (Group 4) and 5% and 10% guar gum (Groups 5 and 6) compared to the cholesterol-supplemented control (Group 2); a significant reduction was obtained only with 5% guar gum (Group 5, $p \leq 0.05$).

Small decreases in serum cholesterol levels

* Obtained from Carworth Farms Inc., New City, N. Y.

TABLE II. Effects of Dietary Supplements on Serum and Liver Cholesterol and Liver Total Lipids of Rats Fed a Cholesterol-Supplemented Powdered Purina Lab Chow Diet (6 Animals per Group).

Group	Supplements fed with basal ration*	Results at the end of 28 days: Body wt (g)†	Serum total cholesterol (mg/100 ml)†	Liver total cholesterol (mg/g)†	Liver total lipid (%)†
1	None	212 ± 5.5	96 ± 1.8	1.80 ± .07	4.58 ± .14
2	1% cholesterol	218 ± 5.2	90 ± 3.1	5.76 ± .31	6.03 ± .18
	Idem + following supplements:				
3	5% pectin N. F.	217 ± 2.7	94 ± 5.3	4.60 ± .50‡	5.60 ± .21
4	10% " "	209 ± 6.0	85 ± 3.8	4.28 ± .53	5.85 ± .25
5	5% guar gum	188 ± 3.9	87 ± 8.2	3.10 ± .28‡	5.15 ± .28
6	10% " "	196 ± 9.3	76 ± 6.3	3.58 ± .58	5.60 ± .37

* Pectin N. F. (citrus) obtained from Sunkist Growers, Ontario, Calif.; methoxyl content 10.5% on a moisture-ash-free basis. Guar gum (A-20-D) obtained from Stein, Hall and Co., Inc., New York.

† Mean ± standard error of mean.

‡ 5% guar gum *vs* 5% pectin N. F., $p \leq 0.05$.

were observed with the high levels of pectin N. F. and guar gum (Groups 4 and 6), but these were not statistically significant at the 5% probability level.

Liver sterols were reduced with all levels of the agents tested, but a statistically significant reduction was obtained only with 5% guar gum (Group 2 *vs* Group 5, $p \leq 0.05$). Five per cent guar gum gave a greater reduction in liver sterols than an equal amount of pectin N. F. (Group 3 *vs* Group 5, $p \leq 0.05$).

Minor reductions were observed in total liver lipids of all treated groups compared to the cholesterol-supplemented control (Group 2), but once again only 5% guar gum (Group 5) was significantly different ($p \leq 0.05$).

The results obtained with the powdered Purina Lab Chow basal diet were not as dramatic as those obtained with the casein-sucrose basal diet; however, it should be noted that supplementation of this diet with 1% cholesterol (Group 2) did not cause an increase in serum cholesterol, and the increases in liver cholesterol and liver total lipids over the non-supplemented control (Group 1) were not as great as those observed when the casein-sucrose basal diet was employed (Table I).

Discussion. The administration of guar gum and pectin N. F. to rats decreased liver sterols and liver total lipids induced by the feeding of 1% cholesterol. Our results on the relative order of activity of these agents in the rat do not agree with those of Ershoff and Wells(2), but do agree with our results in the chick(1); that is, guar gum has a greater order of hypocholesterolemic activity than pectin N. F. Ershoff and Wells(2) stated that pectin N. F. with maximal activity was obtained only when the methoxyl content, on a moisture-ash free basis, was relatively high in the range of 10.7%, and that inactive preparations resulted when the methoxyl content was 5% or less. All of our experiments were carried out with pectin N. F. which assayed 10.5% methoxyl and was also obtained from a citrus source. Therefore, it would appear that this parameter does not account for the different relative order of activity observed with pectin N. F. in our experiments in rats.

The casein-sucrose basal diet employed in these experiments differed from that of Ershoff and Wells(2) in the following respects: their complete diet contained more sucrose (10%), casein (4%) and salt mixture (3%) than our diet; furthermore, our diet contained 2% bone ash, 5% calcium gluconate and 8% gelatin which were not present in their diets. These alterations in the composition of the basal diet could possibly account for the differences in our results.

Supplementation of a powdered Purina Lab Chow diet with 1% cholesterol did not cause

the marked increases in serum and liver cholesterol levels and liver lipids of the rat obtained with the casein-sucrose basal diet; although these elevations were much less, guar gum still demonstrated greater hypocholesterolemic activity than pectin N. F. Similar results had been previously observed in our experiments with chickens(1) fed either the casein-sucrose basal diet or a commercial-type diet prepared from a variety of natural foodstuffs from vegetable or animal sources.

Summary. The oral administration of guar gum and pectin N. F. to rats in a casein-sucrose basal diet greatly reduced the elevations in liver sterol and total liver lipids produced by the feeding of 1% cholesterol. Similar results were also obtained in rats when a powdered Purina Lab Chow diet was employed. Guar gum was considerably more active than pectin N. F., which confirms the results of similar experiments previously carried out with chickens.

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They were killed by ether inhalation at that time. Sections were fixed in Zenker's fluid-formaldehyde solution.

In a second experiment 6 mice were given two intravenous injections of 1 cc. of autoclaved citrus pectin and 6 others received 1 cc. of autoclaved apple pectin. The second injections were given 1 week after the first. These animals were autopsied 6 weeks after the first injection.

A number of mice were given intraperitoneal injections of pectin, but none of them developed any lesions.

Results

With the exception of splenomegaly (spleen up to approximately twice normal size) in several of the animals, no gross abnormalities were observed.

The most striking lesions were seen microscopically in the liver and kidney. Most of the mice had numerous focal lesions of the liver parenchyma. These consisted of disintegrated or disintegrating liver cells which had previously become filled with a material which took a grayish blue and sometimes a purple color when stained with hematoxylin and eosin. This was shown to be pectin, as it was selectively stained a deep red by ruthenium red[2-4] (Fig. 3). Many of these cells had a vacuolation which gave them the appearance of "foam cells." Often their outline was quite well preserved, as was their normal eosinophilic material. In other areas there were only masses of pectin, containing disintegrated nuclei of liver cells (Figs. 1 and 2). Although pectin could be demonstrated also in some liver cells away from the focal areas, it was far less conspicuous there than in the focal lesions. In addition, there were granulomata in several areas where severe damage had been brought about by the pectin. In some of these areas lymphocytes and macrophages were prominent. In others there was beginning fibrosis (Fig. 4). Liver cells in various stages of mitosis were seen in and about some of these granulomata, demonstrating the regeneration of hepatic parenchyma. Pectin was also seen within liver sinusoids and inside some of the reticulo-endothelial cells.

In the kidneys pectin was demonstrated inside glomerular capillaries as well as within some of the endothelial cells themselves. It was prominent in the lumina of proximal and distal tubules but most notably in the collecting tubules. The tubular epithelium in the affected segments was frequently pale, and pectin could be stained within many but not nearly all of the cells involved. There was also a shedding of this epithelium, especially in areas where pectin had plugged the tubular lumen. Pectin casts were a prominent feature in the collecting tubules. Again,

in several of the severely affected portions there was granuloma formation (Figs. 5 and 6). The lesions in the kidneys were less abundant than those in the liver, but they were also focal, and from their general configuration one might be led to conclude that stasis of pectin was an important factor in their genesis.

The lungs of one of the animals showed foam cell formation of the type described by Hueper.[2] There was plugging of pulmonary capillaries by pectin in 2 of the animals.

In the spleens, which were enlarged, there was marked reticulum cell hyperplasia, and pectin could be demonstrated inside some macrophages. The same animals usually had a similar reaction in the lymph nodes where foam cell formation was prominent.

No remarkable changes were seen in other organs.

It should be pointed out that there was no difference in the appearance or distribution of the lesions which could be related to the fact that some of the animals received autoclaved pectin, while others received non-autoclaved pectin. Nor was there any difference between the lesions produced by apple and citrus pectins.

Discussion

It seems plain that apple and citrus pectins can produce focal necrosis in the hepatic parenchyma and degenerative changes in the renal tubules of mice. Such lesions were not reported by Hueper,[2] who worked with rabbits and dogs, nor by Popper *et al.*,[3] who studied humans and rabbits, although Hueper observed a diffuse "foam cellular transformation" of hepatic cells following the injection of pectin, and he reported similar lesions following the injection of acacia and of methyl cellulose.[5,6] Metcalf and Hawkins[7] noted the same in dogs after the injection of acacia and also reported necrosis of the liver of a diffuse type in some of their animals.

It has been said that pectin, a mixture of large carbohydrate molecules of different constitutions,[8] is probably not metabolized by the mammalian organism, since a large proportion of the injected material is excreted unchanged by the kidneys.[9] However, pointed studies to show whether or not some of it is broken down have never been done. That this does occur has been suggested.[10]

The significance of the liver lesions produced by pectin would seem to lie in their analogy to lesions produced by some natural diseases. Focal necrosis of the liver may develop in typhoid fever, tularemia, diphtheria, scarlet fever, poliomyelitis, measles, and other diseases. In diphtheria and scarlet fever these lesions are due to toxins. In typhoid fever, toxic

effects as well as stasis of inflammatory sludges in the liver sinusoids have been invoked as causative mechanisms. In the case of the two virus diseases mentioned it is possible that the macromolecular nature of the virus proteins may play an important rôle in the inability of the invaded cells to handle the virus, as Hueper[1] has suggested. Yet why the lesions are focal remains a mystery.

Stasis does not appear to be an adequate explanation for the hepatic lesions produced by pectin. It may be that different liver cells or different areas of liver cells show different degrees of functional activity, and therefore perhaps of functional vulnerability, at various times. Only cells appearing heavily laden with pectin were necrotic, suggesting that they had been more active in ingesting pectin than the healthy cells. In any event, the sequence of ingestion of pectin molecules by liver cells, focal necrosis, and finally granuloma formation offers a suggestive analogy to that which may be seen in natural diseases. Whether or not the death of cells is related to the size of the noxious agent as well as to its chemical constitution remains unsettled.

As to the changes in the renal tubules, the anatomical evidence points to stasis of pectin as a cause. That the presence of casts of various kinds within the tubules can lead to degenerative changes in the tubular epithelium is a well known fact. How or why these changes occur is a matter of hypothesis. In the present instance we know that at least some of the epithelial cells take up the pectin. But inasmuch as pectin could be demonstrated only in a minority of abnormal cells, no definite conclusions regarding the mechanism of cell alteration in the renal tubules can be deduced.

There are many "foreign" colloids which various parenchymal cells will ingest. This may represent an attempt to get rid of the material via intracellular breakdown which can result in cell death. The old concept of "storage" of such materials may well bear some revision. In the case of pectin, studies on the excretion of galacturonic acid, a constituent of pectin molecules, would provide important information in this regard. The fact that pectin is excreted unchanged in large amounts[9] does not exclude the possibility of some degree of breakdown inside the body.

Summary

Pectin, administered intravenously to mice, produces focal necrosis of the hepatic parenchyma. This appears to be related to its being taken into the affected cells in excessive amounts.

Focal degenerative changes of various degrees were seen in the renal tubules.

Granuloma formation has been observed in areas of focal necrosis in both liver and kidney.

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[*Illustrations follow*]

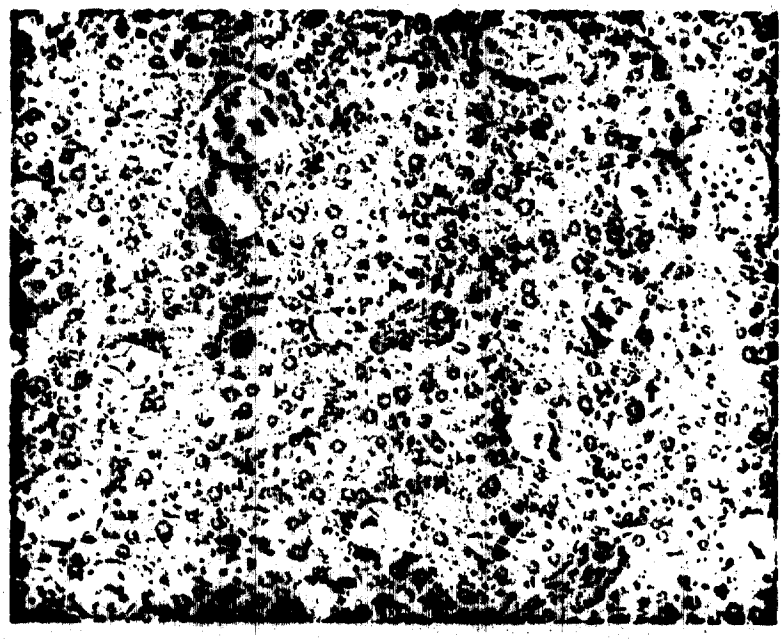
DESCRIPTION OF PLATES

Plate 60

Fig. 1. Distribution of focal lesions in the liver. Hematoxylin and eosin stain. × 180.

Fig. 2. Three areas of focal necrosis in the liver from Figure 1. The cells in these areas are loaded with pectin and are disintegrating. The nuclei are quite pyknotic. The cytoplasm took a grayish blue stain but retained many eosinophilic granules. A number of damaged liver cells which took the stain normally can be seen also. Hematoxylin and eosin stain. × 450.

Fig. 3. Liver. The cells in the areas of focal necrosis stained red, demonstrating the presence of pectin. Ruthenium red stain. × 180.



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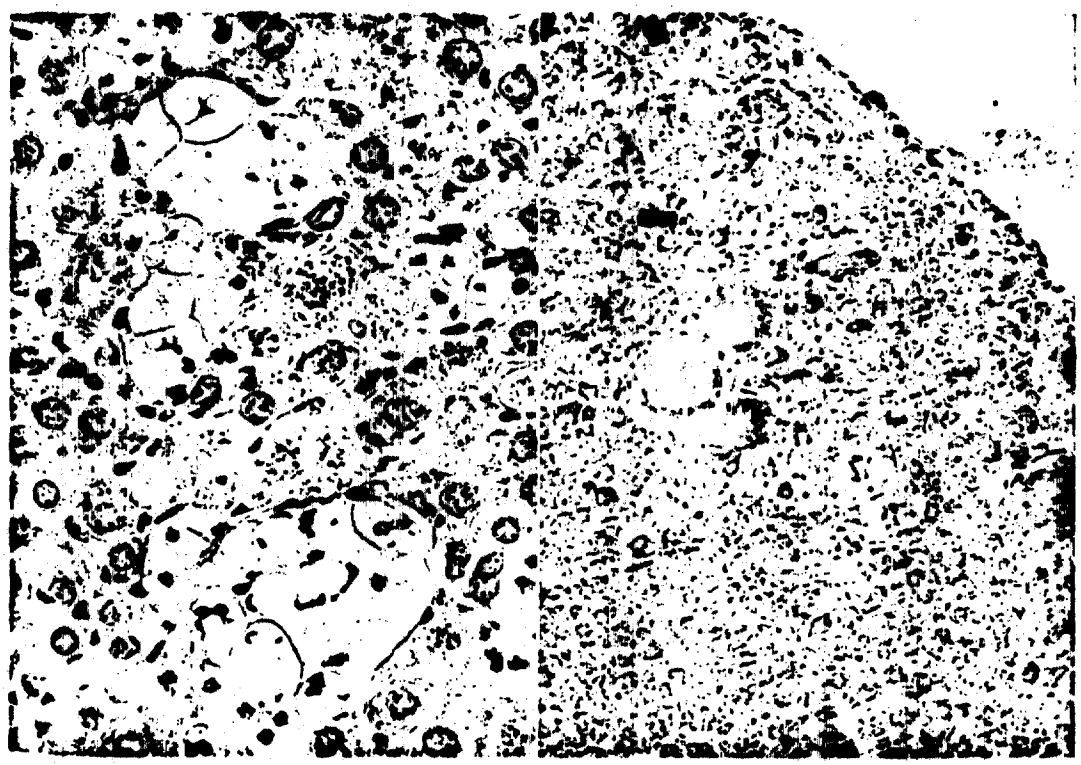

2 3

Richter Parenchymatous Lesions Due to Pectin

PLATE 61

FIG. 4. Granulation tissue and beginning fibrosis in conglomerated areas of focal necrosis in the liver. Nests of disintegrating foam cells like those in Figure 2 are surrounded and invaded by fibrous tissue. Hematoxylin and eosin stain. × 280.

FIG. 5. Granuloma formation in kidney. There are also three pectin casts in tubules, two of which contain polymorphonuclear leukocytes. Hematoxylin and eosin stain. × 450.

FIG. 6. Kidney. In the tubular epithelium are pale cells, many of which stain grayish blue with hematoxylin and eosin and red with ruthenium red. Nuclei are degenerating. Of note are pectin casts in the lumina of the tubules, some of which have been completely blocked. Hematoxylin and eosin stain. × 450.

Richter Parenchymatous Lesions Due to Pectin

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THE EFFECT OF THE CALCIUM DISODIUM SALT OF CYCLOHEXANE-DIAMINOTETRA-ACETIC ACID [$CaNa_2$(CDTA)], THE CALCIUM DISODIUM SALT OF ETHYLENE-DIAMINOTETRA-ACETIC ACID [$CaNa_2$(EDTA)] AND PECTIN ON DISTRIBUTION AND EXCRETION OF ^{59}Fe

A. A. RUBANOVSKAYA

THE TREATMENT of radiation injuries caused by the ingestion into the body of radioactive substances includes the use of preparations to accelerate the excretion of these isotopes.

Many workers have tested preparations, chiefly of the chelinating type, to accelerate the excretion of radioisotopes (Schubert, 1955; Hurch, 1949; Yu. I. Moskalev and L. Budko, 1958; D. I. Semenov and I. P. Tregubenko, 1958; Catsch and Melchinger, 1958; Yu. I. Moskalev, 1959).

The mechanism of action of these compounds is due to the ability to form, with cations, stable water-soluble complexes which are easily and rapidly evacuated in the urine. The compounds proposed have varying efficacy in respect of different isotopes; some of them may have only a limited application. Thus, for example, BAL is effective only with poloaium and has no influence on other radioisotopes in the body.

As is known, one of the most effective chelinating compounds is ethylene-diaminotetra-acetic acid (EDTA), which facilitates excretion from the body of a whole series of radioactive metals: plutonium, yttrium, cerium, thorium, lead, zinc, etc. (D. I. Semenov, 1957; Schubert, 1955; Cohn, Gong and Fischler, 1953; Foreman and Hamilton, 1951; and others). However, even this preparation is not a universal stimulus to excretion of radioisotopes. Moreover, at a long interval after intake, EDTA has only a small effect. Therefore, the trial of new means of increasing excretion of radioisotopes is of considerable interest.

A study has been made of the effect of the calcium-disodium salt of cyclohexane-diaminotetra-acetic acid on excretion and distribution of ^{59}Fe. A ^{59}Fe preparation in the form of iron citrate was used. It should be pointed out that solutions of ^{59}Fe citrate contained a relatively large amount of stable carrier: from 1·5 to 2·5 mg of iron per ml of solution.

The experiments were carried out on rats weighing from 160 to 230 g. In

each variant of the experiment there were 10 rats: 5 experimental and 5 control.

The ^{59}Fe solution was injected intraperitoneally 10 μc per rat. 0·3 ml per 30 mg of the compound was administered to each experimental rat intraperitoneally, intravenously or orally, at the same time 0·3 ml physiological saline was administered to each of the control animals. The number of injections of the compound varied: 1–2 in the course of a day or 2 injections per day for several days (3 and 10 days).

In order to discover the optimal conditions for use of the therapeutic preparation administration of the compound was begun at different periods after injection of ^{59}Fe: after 1–2 minutes, 1 hour, 3½ hours, 2 days and 11 days.

The animals were killed 1, 4 or 11 days after the beginning of treatment (a day after the last injection of the preparation) and the radioactivity of excreta collected during the administration period of the preparation, and of the tissues and organs measured.

The experiments showed that a single intraperitoneal or intravenous injection of the preparation immediately after administration of the isotope (after 1–2 min) produces a sharp increase in excretion of ^{59}Fe in the urine (Table 1).

TABLE 1. *Excretion of ^{59}Fe in Rat Urine Following Injection of $CaNa_2$ (CDTA) Immediately after ^{59}Fe Injection*

No. of experiment	Number of animals in the experiment	Path of injection and dose of $CaNa_2$ (CDTA)	^{59}Fe excreted in one day (% of amount injected)		Experimental as percentage of control	Notes
			control	experimental		
1	10	Intraperitoneal 30 mg	11·8 ± 1·1	27·4 ± 2·2	232·2	
2	10	Intraperitoneal 30 mg	15·4 ± 1·5	32·3 ± 2·0	209·7	
3	10	Intraperitoneal 30 mg	15·6 ± 0·7	29·7 ± 2·0	190·0	
4	10	Intravenous 30 mg	4·3 ± 0·4	74·6 ± 1·1	1734	Carrier—0·07 mg/ml
5	10	Intravenous 30 mg	11·5 ± 0·5	34·1 ± 2·2	296·5	

From 11·5 to 15·6 per cent on average of the injected amount was excreted in the urine per day in the control rats. The experimental animals excreted from 27·4 to 34·1 per cent ^{59}Fe.

Thus, excretion of the isotope in the urine increased by 2–3 times.

The increase in the excretion of ^{59}Fe from the body was accompanied by its reduced deposition in the tissues and organs. The ^{59}Fe content of the liver, spleen and kidneys declined markedly (Table 2). Thus, the liver of control rats a day after ^{59}Fe injection contained from 29·2 to 43·1 per cent of the quantity administered, that of experimental animals from 15·1 to 20·7 per

TABLE 2. *Content of ^{59}Fe of Rat Tissues and Organs Following Injection of $CaNa_2$ (CDTA) 1–2 min after ^{59}Fe Injection* in Whole Organ as a Percentage of Total Received by the Body

No. of experiment	Number of animals in the experiment	Group of rats	Blood	Liver	Spleen	Kidneys	Lungs	Amount of carrier (mg/ml)	Notes
1	10	Experimental Control Experimental (as percentage of control)		15·1 ± 1·2 43·1 ± 0·37 35	1·0 ± 0·09 1·54 ± 0·08 65	0·73 ± 0·02 1·34 ± 0·1 54·4	0·15 ± 0·01 0·36 ± 0·04 41·6	1·5–2·5	Rats killed 1 day after ^{59}Fe injection
2	10	Experimental Control Experimental (as percentage of control)	4·0 ± 0·4 4·5 ± 0·17 88·8	20·7 ± 0·95 34·0 ± 0·95 60·8	1·03 ± 0·2 1·67 ± 0·01 61·6	1·1 ± 0·05 2·22 ± 0·01 50	0·27 ± 0·01 0·28 ± 0·02 100	1·5–2·5	Rats killed 1 day after ^{59}Fe injection
3	10	Experimental Control Experimental (as percentage of control)	4·1 ± 0·36 6·2 ± 1·3 66·1	19·8 ± 1·8 29·2 ± 2·1 67·3	1·07 ± 0·07 1·68 ± 0·26 63·6	0·95 ± 0·12 1·32 ± 0·17 71	0·27 ± 0·03 0·34 ± 0·07 80	1·5–2·5	Rats killed 1 day after ^{59}Fe injection
4	10	Experimental Control Experimental (as percentage of control)	8·5 ± 0·53 40·5 ± 2·9 20·7	3·5 ± 0·18 20·4 ± 1·0 17·1	0·33 ± 0·01 2·9 ± 0·36 11·7	0·62 ± 0·02 0·88 ± 0·07 70·4	0·18 ± 0·02 0·81 ± 0·09 22·1	0·07	Rats killed 1 day after ^{59}Fe injection

cent (from 35 to 67·5 per cent of the control); in the kidneys of control rats there was from 1·32 to 2·4 per cent; in experimental animals, from 0·73 to 1·1 per cent (54–71 per cent of the control).

It should, however, be realized that the amount of ^{59}Fe excreted in the urine and its distribution in the body, as with other radioisotopes, depends on the quantity of carrier in the preparation. The results of experiment No. 4 illustrate this point. In this experiment the rats were injected with a ^{59}Fe citrate solution containing 0·07 mg/ml of stable iron.

Excretion of ^{59}Fe in the urine during one day under these conditions consisted of 4·3 per cent of the injected quantity in the control rats. The increase in excretion of ^{59}Fe caused by the chelinating compound was significantly greater than in the previous experiments—on average by 17 times. Correspondingly, ^{59}Fe content of the organs of the experimental animals was more reduced than when a ^{59}Fe preparation with a greater amount of stable iron was used. The radioactivity of the blood, liver and spleen is reduced particularly sharply. Thus, when the amount of stable iron in the solution is small the chelinating compound is more effective.

The compound proved to have little effect when administered 11 days after the ^{59}Fe injection. By this time the ^{59}Fe was already firmly fixed in the tissues and excretion in the urine and faeces had become established at a low level. Administration of the compound for 3 and 10 days (30 mg each twice a day) at this period produces only a negligible increase of the absolute quantity of ^{59}Fe in the urine: from 0·38 to 1·02 per cent of the amount injected, which cannot be of practical importance. Excretion of ^{59}Fe in the faeces is unchanged.

Shortening the time interval between injection of ^{59}Fe and administration of the preparation increases the efficacy of the latter. Administration (twice a day for 3 days) begun 2 days after ^{59}Fe injection increases the absolute ^{59}Fe content of the urine by a greater amount than injection begun after 11 days (from 1·9 to 3·8 per cent of the amount administered).

Intraperitoneal injection of the preparation, 1 and 3½ hr after ^{59}Fe injection, increases excretion of ^{59}Fe in the urine by 1½–2 times.

It is known that oral administration of chelinating compounds is less effective than intraperitoneal or intravenous injection. Therefore, the compound was administerd orally in considerably greater quantity (60 mg each 1 min before and ½ hr after intraperitoneal injection of ^{59}Fe). As can be seen from Table 3, these quantities of the preparation, when administered orally, had little effect on ^{59}Fe evacuation.

Excretion of ^{59}Fe in the urine under these conditions was 12·4 per cent of the amount administered in the control rats and 15·6 per cent in the experimental animals.

Thus, the therapeutic preparation is absorbed in small quantities from the gastro-intestinal tract.

Measurement of ^{59}Fe in tissues and organs showed that, administration of the compound for 3 days (2 injections per day) commencing 11 days after ^{59}Fe injection, has no effect on the figures obtained. However, more

TABLE 3. *Excretion of ^{59}Fe in Rat Urine and Faeces Following Treatment with $CaNa_2$ (CDTA) at Various Intervals after ^{59}Fe Injection*

No. of experiment	Number of animals in the experiment	Method of administration and dose of $CaNa_2$ (CDTA)	Interval after ^{59}Fe injection	Amount excreted as percentage of dose administered					Notes
				in the urine			in the faeces		
				control	experimental	experimental as percentage of control	control	experimental	
6	10	Intraperitoneally 30 mg twice a day for 3 days	11 days	0·38 ± 0·03	1·02 ± 0·01	269·4	0·62 ± 0·05	0·67 ± 0·02	During 4 days
7	10	Ditto	2 days	1·9 ± 0·14	3·8 ± 0·9	200·0	—	—	Ditto
8	10	Ditto	3½ hr	12·6 ± 2·0	21·4 ± 1·0	170·0	—	—	Ditto
9	10	Ditto	1 hr	12·4 ± 2·0	20·5 ± 2·1	166·3	—	—	During 1 day
10	10	Ditto	1 hr	14·0 ± 1·0	23·7 ± 2·0	169·3	—	—	Ditto
11	10	Orally 60 mg	1 min before and ½ hr after intraperitoneal injection	12·4 ± 0·53	15·6 ± 0·73	126·0	—	—	Ditto

TABLE 4. *Content of ^{59}Fe in Rat Tissues and Organs Following Treatment with* in the Whole Organ as a per cent

No. of experiment	Number of animals in the experiment	Group of rats	Interval after ^{59}Fe injection (days)	Dose and method of injection of the compound
6	10	Experimental Control Experimental (as percentage of control)	11	Intraperitoneally 30 mg twice a day for 3 days
13	10	Experimental Control Experimental (as percentage of control)	11	Intraperitoneally 30 mg twice a day for 10 days
7	10	Experimental Control Experimental (as percentage of control)	2	Intraperitoneally 30 mg twice a day for 10 days
7a	10	Experimental Control Experimental (as percentage of control)	11	Intraperitoneally 30 mg twice a day for 10 days

prolonged injection of the preparation (for 10 days), even at these times has some effect (Table 4).

Treatment for 10 days beginning 2 days after intake of ^{59}Fe results in a significant reduction in the amount of the isotope in tissues and organs.

All the data obtained by us showed that the compound is effective, at the short periods after ^{59}Fe intake. A single injection of the compound immediately, and 1 and 3½ hr after intake of ^{59}Fe sharply increased excretion of the isotope in the urine and reduced its deposition in the tissues and organs.

During the first few days after ^{59}Fe intake prolonged, repeated administration of the preparation also has a considerable effect.

As was pointed out above, one of the most effective chelinating compounds in relation to a series of radioisotopes is EDTA. A comparison of the effectiveness of CDTA and EDTA in respect of ^{59}Fe is of some interest. In the following series of experiments a comparison was made of the effects of two dosages of the calcium-disodium salts of both compounds: 30 mg per rat (0·3 ml 10 per cent solution) and 20 mg per rat (0·2 ml 10 per cent solution).

The rats were injected intraperitoneally with ^{59}Fe solution and after 1–2 min one half of the animals received an intraperitoneal injection of $CaNa_2$ (CDTA), and the other half $CaNa_2$ (EDTA). The animals were killed a day later.

$CaNa_2$ (CDTA) Begun at Different Intervals after Injection of the Isotope
of Total Received by the Body

Blood	Liver	Spleen	Kidneys	Lungs	Bone marrow
9·67 ± 0·70 10·2 ± 0·36 94·8	9·1 ± 0·60 8·4 ± 0·60 108·3	0·73 ± 0·07 0·79 ± 0·08 92·4	0·97 ± 0·07 0·94 ± 0·1 103·0	0·19 ± 0·01 0·15 ± 0·01 126·6	– – –
14·3 ± 0·79 15·0 ± 0·95 95·0	17·3 ± 0·52 21·6 ± 0·72 80·0	0·86 ± 0·05 0·76 ± 0·06 113·0	0·59 ± 0·05 0·9 ± 0·03 64·8	0·29 ± 0·03 0·26 ± 0·04 111·0	0·93 ± 0·14 1·05 ± 0·1 88·5
13·4 ± 0·2 14·1 ± 0·8 95·0	12·2 ± 1·6 18·4 ± 0·70 66·3	0·58 ± 0·10 1·04 ± 0·12 55·7	0·55 ± 0·01 1·10 ± 0·07 50·0	0·44 ± 0·07 0·61 ± 0·10 72·0	– – –
12·8 ± 1·0 14·0 ± 0·33 91·0	9·8 ± 0·70 12·8 ± 1·0 76·5	0·64 ± 0·04 1·1 ± 0·10 58·1	0·66 ± 0·02 0·85 ± 0·05 77·0	0·25 ± 0·01 0·38 65·7	– – –

The rats which received 30 mg $CaNa_2$ (CDTA) excreted in the urine during the day from 28·4 to 30·7 per cent of ^{59}Fe administered, while rats receiving $CaNa_2$ (EDTA) excreted approximately the same amount (from 30·1 to 30·8 per cent, cf. Table 5).

The radioactivity of the tissues and organs of animals of both groups in each experiment was also materially the same (Table 6).

Similar results were obtained when the preparations were used at the 20 mg dose.

Thus, at short periods after ^{59}Fe injection $CaNa_2$ (CDTA) proved as effective as $CaNa_2$ (EDTA).

The purpose of the following series of experiments was to investigate the possibility of reducing the intake of ^{59}Fe through the gastro-intestinal tract by means of adsorbents. A commercial preparation of pectin, obtained from sunflowers, was used as the adsorbent.

The pectins are a group of substances which contain a chain of galacturonic acid radicals. The carboxyl groups of these radicals are free and can form compounds with metals.

As far as its physical properties are concerned pectin is a hydrophylic colloid with great adsorptive capacity.

Work by a number of writers has shown that pectin reduces absorption

of lead from the gastro-intestinal tract (Murer and Grandall, 1942; and others).

Our investigations have established that administration of pectin reduces absorption in the gastro-intestinal tract and deposition in the skeleton of radiostrontium. It might be assumed that the preparation would prove effective also in regard to other metals entering the gastro-intestinal tract.

TABLE 5. *Effect of $CaNa_2$ (CDTA) and $CaNa_2$ (EDTA) on Excretion of ^{59}Fe in Rat Urine*

No. of experiment	Number of animals in the experiment	Dose of compound (mg)	^{59}Fe content of urine in 1 day (percentage of amount administered)	
			$CaNa_2$ (CDTA)	$CaNa_2$ (EDTA)
1	10	30	30·7 ± 0·77	30·8 ± 1·2
2	10	30	28·4 ± 1·7	30·1 ± 1·7
3	10	20	24·9 ± 0·5	24·1 ± 1·7
4	10	20	29·7 ± 1·0	30·0 ± 0·8

The experiments of this series were carried out as follows. In each experiment 15–18 rats were used. Each animal received orally 20 μc $^{59}FeCl_3$; 5–6 rats received orally 1·5 ml 2 per cent solution of pectin 1–2 min before administration of the ^{59}Fe, and another 5–6 animals the same dose 1 hr before. The control rats were given physiological saline before receiving the ^{59}Fe. The ^{59}Fe solution contained a rather large amount of stable carrier: 0·67, 0·87 mg and only in one experiment 0·019 mg. The animals were killed 2–3 days after ^{59}Fe administration and the ^{59}Fe content of the blood, liver, spleen and lungs measured.

It can be seen from the results obtained that, when ^{59}Fe enters the gastro-intestinal tract, the amount retained in the body and its distribution depend on the amount of carrier in the preparation (Table 7).

When the quantity of carrier is large very little ^{59}Fe is retained in the body. Thus, after 2–3 days from 1 to 1·7 per cent of the amount administered can be found in the liver and up to 5 per cent in the blood.

When the amount of carrier is small (cf. Table 6, experiment No. 3) retention in the liver is somewhat greater at these periods, while a considerable quantity of ^{59}Fe can already be found in the blood (up to 21 per cent of the amount administered). The ^{59}Fe content of the spleen and lungs in all the experiments at these periods was negligible—measured in hundredths and tenths of 1 per cent of the administered amount.

The results obtained (cf. Table 7) show that when the amount of stable iron in the $^{59}FeCl_3$ solution is small the presence of pectin reduces the amount of radioactive iron which passes through the wall of the gastro-intestinal tract.

As can be seen from Table 7, the blood of rats, which received pectin before ^{59}Fe with a low stable iron content, contained only half the amount of

TABLE 6. *Effect of $CaNa_2$ (CDTA) and $CaNa_2$ (EDTA) on Distribution of ^{59}Fe in Rat Tissues and Organs a Day after Injection*

No. of experiment	Number of animals	Dose of compound	Liver	Spleen	Kidneys	Lungs	Blood
			as percentage of amount of ^{59}Fe received by the animal				
1	10	$CaNa_2$ (CDTA) 30 mg	25·1 ± 0·75	1·29 ± 0·2	1·07 ± 0·05	0·45 ± 0·09	2·6 ± 0·24
		$CaNa_2$ (EDTA) 30 mg	27·7 ± 1·2	1·17 ± 0·1	1·05 ± 0·6	0·38 ± 0·07	2·8 ± 0·16
2	10	$CaNa_2$ (CDTA) 20 mg	19·2 ± 0·57	1·1 ± 0·14	1·0 ± 0·0	0·6 ± 0·1	2·0 ± 0·20
		$CaNa_2$ (EDTA) 20 mg	22·0 ± 1·4	1·1 ± 0·16	1·04 ± 0·05	0·58 ± 0·02	2·0 ± 0·19

TABLE 7. *Content of ^{59}Fe of Rat Tissues and Organs*

No. of experiment	Number of animals in the experiment	Amount of ^{59}Fe (mg) in administered volume of solution	Group of rats	Organs: liver	spleen	lungs	blood	When killed
				in whole organ as a percentage of total received by the animal				
1	15	0·67	Experimental					
			1) Pectin at 1–2 minutes before ^{59}Fe administration	0·9 ± 0·05	0·05 ± 0·001	0·06 ± 0·002	2·0 ± 0·03	After 3 days
			2) Pectin at 1 hr before ^{59}Fe administration	1·4 ± 0·05	0·13 ± 0·02	0·09 ± 0·003	5·0 ± 0·4	
			Control	1·3 ± 0·04	0·21 ± 0·008	0·12 ± 0·007	4·4 ± 0·3	
2	18	0·87	Experimental					
			1) Pectin at 1–2 minutes before ^{59}Fe administration	1·7 ± 0·3	0·07 ± 0·01	0·15 ± 0·002	2·0 ± 0·2	Ditto
			2) Pectin at 1 hr before ^{59}Fe administration	1·0 ± 0·1	0·07 ± 0·01	0·07 ± 0·011	1·7 ± 0·1	
			Control	0·9 ± 0·1	0·06 ± 0·01	0·06 ± 0·001	1·5 ± 0·11	
3	15	0·019	Experimental					
			1) Pectin at 1–2 minutes before ^{59}Fe administration	1·5 ± 0·2	0·4 ± 0·01	0·20 ± 0·03	–	Ditto
			2) Pectin at 1 hr before ^{59}Fe administration	2·1 ± 0·03	0·4 ± 0·04	0·21 ± 0·03	9·5 ± 0·4	
			Control	4·0 ± 0·4	0·4 ± 0·03	0·30 ± 0·02	21·0 ± 1·4	

the isotope as did the blood of the control animals. Other investigations showed that the tissue content of ^{59}Fe in the experimental rats was less than that of the control animals.

Little radioactive iron is found in tissues and organs after the oral administration of an $^{59}FeCl_3$ solution containing a large quantity of stable iron. This is due to the low uptake of inorganic iron compounds in the gastro-intestinal tract and the great dilution of the radioactive isotope in the administered solution.

Under these conditions pectin has virtually no effect on retention of ^{59}Fe in the body. The ^{59}Fe content of tissues and organs of rats receiving pectin is similar to that of the control animals.

Thus, the experiments have shown that when the amount of stable iron is low, pectin limits uptake of ^{59}Fe by the gastro-intestinal tract.

Conclusions

1. $CaNa_2$ (CDTA) accelerates excretion of ^{59}Fe and reduces its deposition in tissues and organs.
2. $CaNa_2$(CDTA) is effective in increasing excretion and reducing the amount of ^{59}Fe deposited in the tissues if administered during very early periods after its intake.
3. $CaNa_2$(CDTA) is as effective in respect of ^{59}Fe as $CaNa_2$(EDTA).
4. Pectin reduces uptake of ^{59}Fe by the gastro-intestinal tract when the orally administered solution contains little stable iron.

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STUDIES OF RABBIT ORGANS AFTER INTRAVENOUS INJECTIONS OF PECTIN SOLS

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THE NEED for a substitute for blood has been recognized for some time. Many substitutes for whole blood have been proposed for use in the treatment of shock, nephrosis and other diseases for which transfusions are indicated. Early experience with whole blood revealed that it was necessary to cross match the blood of the donor with that of the recipient. This inconvenience, together with that of finding suitable donors at the time of the emergency requiring the need of a transfusion, led to the search for substitutes or a method for storing typed blood. A further point in favor of substitutes for blood is the high cost of treatment with blood or plasma as well as the frequent incidence of jaundice noted after the use of plasma during the war.

Citrated whole blood has been used widely for many years and still remains a desirable means of transfusion in certain cases; it has, however, a poor storage life. Liquid plasma is only slightly more stable, whereas dried plasma can be stored indefinitely. The preparation of dried plasma is expensive, and massive transfusion treatment by this means is almost financially prohibitive. For these reasons several substitutes for plasma have been proposed. Pectin is one of those suggested.

The use of isotonic pectin sols as a substitute for plasma has been studied by many workers[1] using solutions prepared by different pro-

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1. (a) Hartman, F. W.; Schelling, V.; Harkins, H. N., and Bush, B.: Pectin Solution As a Blood Substitute, Ann. Surg. **114**:212-225 (Aug.) 1941. (b) Hartman, F. W.; Schelling, V.; Bush, B., and Warren, K. W.: The Relative Value of Pectin Solution in Shock, J. A. M. A. **121**:1337-1341 (April 24) 1943. (c) Hueper, W. C.: Experimental Studies in Cardiovascular Pathology: VI. Pectin Atheromatosis and Thesaurosis in Rabbits and in Dogs, Arch. Path.

(Footnote continued on next page)

cedures. Hartman and others[1a,b] have advocated a concentration of 0.75 to 1.5 per cent pectin. They prefer the 0.75 per cent pectin sol sterilized by heating for fifteen to eighteen hours in an Arnold sterilizer and shaking three times during this period. They adjust the p_H to 7 by adding a phosphate buffer just before intravenous use. Hueper[1c] studied the effectiveness of pectin sols, using autoclaved and unautoclaved 1.2 per cent pectin sols which he buffered to p_H 6.5 to 7.0 with "lime water or phosphate buffer solution." Kozoll,[1d,e] Meyer,[1f] and Popper[1g] used a 1.5 per cent pectin sol buffered to about p_H 7.2 with phosphate or lactate.[2] Figueroa and Lavieri[1h] used a 1 per cent pectin sol buffered to p_H 6.0 to 6.5 with a 5 per cent solution of disodium acid phosphate. Jacobson and Smyth[1i] used a 0.75 per cent pectin sol furnished by Dr. F. W. Hartman. In our earlier work we[1j] used an autoclaved unbuffered 5 per cent pectin sol.

Clinical studies[3] showed that autoclaved buffered pectin sols give favorable results in the treatment of patients in shock as well as patients not in shock.

Hueper[1c] and Popper and others[1g] observed pathologic changes in the livers of animals and human beings after the intravenous administration of massive amounts of pectin sols. However, chemical analyses failed to show any appreciable amounts of pectin retained in the affected organs.

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2. The phosphate buffer is a 5 or 10 per cent solution of disodium acid phosphate. The lactate buffer consists of a mixture of glycine, sodium lactate and sodium hydroxide.

3. Hartman and others.[1a,b] Kozoll and others.[1d,e] Meyer and others.[1f] Popper and others.[1g] Figueroa and Lavieri.[1h]

Many lesions were noted by Hueper in all rabbits given the unautoclaved buffered pectin sol. However, when he used an autoclaved buffered pectin sol, he observed a few lesions in just 1 of the 6 rabbits so treated.

The purpose of the present paper is to discuss the effect on rabbit tissues of massive volumes of intravenously injected pectin sols. The pectin sols were prepared to contain 1.5 per cent pectin N. F. dispersed in isotonic sodium chloride solution. The sols were autoclaved at 15 pounds pressure (250 F.) for thirty minutes and were filtered sparkling clear. They were administered with and without added buffer. The apparent molecular weight of the pectin in the sols used was between 35,000 and 45,000.

Comparisons of the weight of the heart, kidneys, liver, lungs and spleen are given, and histopathologic tissue studies were made of the kidney, liver and spleen of each rabbit. These experiments were extensive enough to determine whether the pathologic changes noted by Hueper and Popper are permanent or transitory as intimated by Popper. The tissues were also studied histologically when stained with ruthenium red before and after treatment with ammonium oxalate.

Ruthenium red, although primarily a stain for pectic material in plant tissues, as discussed by Branfoot [4] and Norman,[5] has been used in histologic studies of human and animal tissues of subjects previously treated intravenously with pectin sols.[1c,g] The use of ruthenium red necessitates the use of pectic-dissolving agents to differentiate the pectic substances staining with ruthenium red. In the present study 0.5 per cent ammonium oxalate in 3 per cent ammonia was used to dissolve the pectic material, as suggested by Mehta.[6]

PART I. TREATMENT OF THE RABBITS

Pectin sols were injected into the marginal ear vein of rabbits of both sexes ranging in weight at the start of treatment from 1.28 to 2.15 Kg. The 36 rabbits used in this study were New Zealand Whites and Himalayans raised in the vicinity of Corona, Calif., on Albers whole family ration.

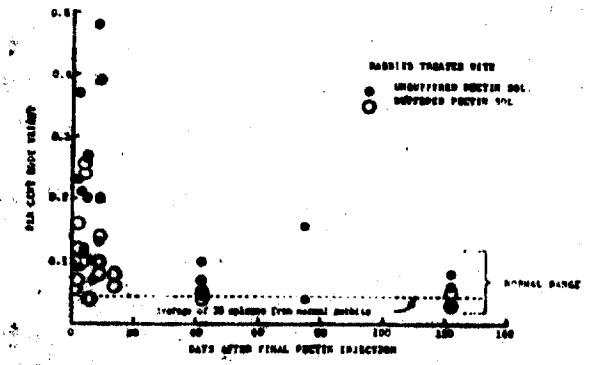
Two groups of rabbits were given injections of autoclaved pectin sols. One group (15 rabbits) was given unbuffered autoclaved 1.5 per cent pectin sols (p_H 3.7 to 3.9), and the other group (18 rabbits) was given buffered autoclaved 1.5 per cent pectin sols (p_H 6.0 to 7.5). The sols

4. Branfoot, M. H.: A Critical and Historical Study of Pectic Substances [illegible] Food Investigation, Special Report Series, no. 33, London, His Majesty's [illegible] Office, 1929.

5. Norman, A. G.: The Biochemistry of Cellulose, Polyuronides, Lignin, [illegible], Clarendon Press, 1937.

were buffered with the sodium lactate mixture just before being used. Three rabbits were used as controls. Each rabbit receiving pectin was given a total of 100 cc. of the pectin sol per kilogram of original body weight in doses of 30 cc. per day. On completion of the injections the animals were killed at intervals varying from one to one hundred and twenty-two days after the last injection, i. e., one, two, four, five, six, nine, fourteen, forty-two and one hundred and twenty-two days.

A summary of the weights of the various organs in terms of the per cent of body weight at the time the animal was killed in relation to the p_H of the pectin sol injected and the time interval after the last injection is given in the accompanying table. The chart gives the weight of the



Change in weight of rabbit spleens following injection of pectin.

TABLE 1.—*Summary of Data in Relation to p_H of Pectin Sol*

Pectin Sol p_H	Days After Last Injection	Number of Rabbits in Group	Per Cent of Body Weight				
			Liver	Kidneys	Spleen	Heart	Lungs
Controls	...	3	[illegible]	[illegible]	[illegible]	[illegible]	0.47
2.7 to 3.[illegible]	2	3	3.05	0.73	[illegible]	[illegible]	[illegible]
	5	3	3.64	0.81	[illegible]	[illegible]	0.64
	9	3	[illegible]	0.65	0.34	0.27	[illegible]
	42	3	[illegible]	0.61	[illegible]	[illegible]	[illegible]
	122	3	3.05	0.54	[illegible]	0.19	0.37
4.0 to 7.8	1	3	3.19	0.67	0.67	[illegible]	[illegible]
	3	3	[illegible]	[illegible]	0.15	[illegible]	0.41
	4	3	[illegible]	[illegible]	0.15	[illegible]	[illegible]
	6	3	[illegible]	0.67	[illegible]	[illegible]	0.45
	9	3	[illegible]	1.01	[illegible]	[illegible]	0.46
	14	3	[illegible]	[illegible]	0.47	[illegible]	[illegible]
	42	3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	120	3	[illegible]	[illegible]	[illegible]	[illegible]	0.38
Normal	Previous data	As shown	[illegible]	[illegible]	[illegible]	[illegible]	...
	Shelton*	Not given	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

* Data are from "The Storage of Water by Various Tissues of the Body" by H. Shelton, Arch. Int. Med. 40: 140-52, 1927, cited on page 906 in Macleod's "Physiology in Modern Medicine," ed. 5, St. Louis, C. V. Mosby Co., 1930.

spleens following injection of pectin. It includes the spleens of the rabbits reported on in the present study as well as those of 9 rabbits previously studied by us.

PART II. HISTOPATHOLOGIC STUDIES OF TISSUE

The organs obtained at autopsy were embedded in paraffin, and several sections of each organ were cut 8 microns thick.

The discussion of the microscopic examination of the tissue sections will be considered under two headings: (*A*) routine staining with hematoxylin-eosin and (*B*) special staining with ruthenium red. Section B will be further broken down to consider the tissue sections before and after treatment with ammonium oxalate.[6]

Section A. Hematoxylin-Eosin.—When unbuffered pectin sols were used, pathologic lesions were noted in the spleens of all animals autopsied within nine days after the last injection of pectin. These were similar to the splenic lesions noted by Hueper [1c] and by Popper.[1g] The spleens showed many giant cells and some fibrosis. Five of the 18 animals treated with pectin sols buffered to a p_H of 6.0 to 7.5 had lesions of the spleen. However, the lesions were not as pronounced as those seen in the unbuffered group and appeared only in animals autopsied during the first four days after the final injection of pectin.

Renal lesions were observed in 2 of the animals treated with the unbuffered pectin sols. These lesions were similar to those described by Hueper,[1c] with the exception that no swollen glomerular endothelial cells were seen. The kidneys of the animals treated with the buffered pectin sols were normal.

One animal of each treated group had a slight periportal lymphocyte infiltration, which was more pronounced in the animal receiving the unbuffered pectin sol. The hepatic lesions described by Hueper [1c] and by Popper [1g] were not found in these tissues.

The lesions caused by treatment with pectin sol appeared to be transitory, as they were not seen beyond the ninth day after the last injection of unbuffered pectin sol or beyond the fourth day after the last injection of buffered pectin sol and did not recur in one hundred and twenty-two days, when the study ended.

Section B. Ruthenium Red.—Duplicate slides of the tissues were studied after they were stained with ruthenium red. The ruthenium red solution was prepared by adding 6.2 mg. of the ruthenium salt to 20 cc. of carbon dioxide-free distilled water, which was shaken occasionally while standing at room temperature for two to three hours. It was then

6. Detailed tables describing the microscopic examination of these tissues have been omitted from this manuscript because of their space requirement. Investigators interested may secure copies of these tables from the authors.

centrifuged at 2,000 revolutions per minute for fifteen minutes. It was found by experiments on solubility that different lots of ruthenium red contain varying amounts of insoluble material. Since the salt we used contained 35.5 per cent insoluble material, the supernatant fluid of the mixture described is approximately a 1:5,000 solution of ruthenium red. This is the solution which was used to stain the sectioned animal tissues after they had been hydrated and buffered to p_H 7.0.

Further studies of duplicate tissue sections were made by treating them with ammonium oxalate before staining with ruthenium red. After the tissues had been treated for forty-eight hours in ammonium oxalate they were washed in carbon dioxide–free water, placed in a buffer with a p_H of 7.0, stained, dehydrated and mounted in the usual manner.

To determine if the treatment with ammonium oxalate was necessary to dissolve the amorphous deposits in the tissues, sections of the kidneys, liver and spleen from 3 rabbits were soaked in carbon dioxide–free water for sixty-five hours before they were stained with ruthenium red. Microscopic examination of these tissues showed them to be the same as the control tissues which had some red-stained material in the pulp of the spleen.

In a comparison of tissues stained with hematoxylin and eosin and the tissues stained with ruthenium red it was noted that eight of the tissues showing lesions when stained with hematoxylin and eosin did not contain any foreign material which would stain with ruthenium red. On the other hand, there were thirteen tissues stained with ruthenium red that did not show any lesions when hematoxylin-eosin stain was used. It was also found that the treatment with ammonium oxalate partially removed the material from the tissues which were originally stained with ruthenium red.

An example of the picture shown by the tissues more intensely stained with ruthenium red and not treated with ammonium oxalate is as follows for 3 rabbits receiving the unbuffered pectin sol:

Rabbit No. 114: The glomeruli of the kidney contained small red-stained plugs of hyaline-appearing homogeneous material in the capillary loops and in Bowman's spaces. No other abnormality was seen. The splenic pulp showed large branching sheets and masses of red-stained material in the sinuses. Some similar material was seen in the cytoplasm of giant cells in the pulp.

Rabbit No. 115: The glomeruli contained irregular tubellike plugs of red-stained material in the capillary loops and in Bowman's spaces. The spleen showed irregular sheets of red-stained material in the sinuses of the pulp. None was seen in the giant cells.

Rabbit No. 116: The glomeruli showed many red-stained plugs in the capillary loops. In the spleen almost every available crevice of the pulp was filled by red-stained material. None was seen in the giant cells.

The data reveal that staining with ruthenium red showed deposits of foreign material in the livers of 4 animals and in the kidneys and spleens of 8 animals treated with autoclaved unbuffered pectin sols. The four liver sections and six of the kidney sections showed material stained with ruthenium red, which was not evident by the hematoxylin-eosin method. However, the abnormalities of the spleens noted after staining with ruthenium red were seen in all but 1 animal when hematoxylin-eosin stain was used.

The group of animals treated with the autoclave buffered 1.5 per cent pectin sol gives a different picture from that just noted. No foreign material was found in the kidneys or liver, but four spleens were stained with ruthenium red, three of which also showed abnormalities in the hematoxylin-eosin-stained tissue sections.

These experiments indicate that rabbits treated with autoclaved unbuffered 1.5 per cent pectin sols are subject to a deposit of foreign material in the kidneys and spleen. This foreign material stains with ruthenium red and may be removed completely or partially by treatment with ammonium oxalate. Whether or not the material deposited and the lesions seen in the kidney are enough to impair its normal function was not determined. The material deposited in the spleens was sufficient to cause temporary splenomegaly (see chart).

Previous analytic work on human organs[14] after massive (7 to 9 liters) intravenous injections of pectin sol failed to reveal any appreciable amount of pectic material present in the kidneys or spleen, even in organs which showed foreign material that stained with ruthenium red. We believe that this deposit may have been an acid-insoluble pectic substance and therefore was not extracted from the tissue in the slurry used for analysis. However, since in our animals these deposits soon disappeared it is possible that they are disintegrated by the natural enzymes of the tissue.

It is of interest to note that in no case in which buffered pectin was used were deposits present that could be stained with ruthenium red in any organ of the animals killed later than the fourth day after the final injection of pectin, whereas the treatment with unbuffered pectin caused ruthenium red–stained deposits in all but 3 animals of that group.

SUMMARY

The effects of massive volumes of intravenously injected 1.5 per cent autoclaved pectin sols, buffered and unbuffered, on rabbit organs have been studied. The pectin sols were administered daily in 30 cc. portions until a total of 100 cc. per kilogram of body weight had been given to each animal.

Unbuffered pectin sols (p_H 3.7 to 3.9) were given to one group of 15 animals. Pectin sols buffered with sodium lactate mixture (p_H 6.0 to 7.5) were given to a second group of 18 animals. Two or 3 animals from each group were killed at intervals varying from one to one hundred and twenty-two days after the final injection of pectin.

The weights of the livers, kidneys, spleens, hearts and lungs were recorded for the treated animals. A new set of values for the average per cent of the body weight for these organs from normal rabbits is given.

Tissue sections of the kidney, liver and spleen of each animal were examined for lesions and pectic deposits.

The splenomegaly and lesions caused by administration of pectin sol were transitory and were not present in any animal treated with the buffered pectin sol and autopsied later than four days after the final injection. However, they did persist slightly longer in the animals treated with the unbuffered pectin sols.

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NUTRITION REPORT INTERNATIONAL

EFFECT OF PECTIN AND ALGIN UPON PROTEIN UTILIZATION, DIGESTIBILITY OF NUTRIENTS AND ENERGY IN YOUNG RATS.

by

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ABSTRACT

Pectin, algin, galacturonate and mannuronate were substituted for 5 or 10% of starch in a standard diet for weanling rats in order to compare their effect on the digestibility of nutrients and energy and on the utilization of protein . Pectin remained almost completely unabsorbed and reduced food intake, weight gain, protein-, lipid- and calcium absorption. Its contribution to the digestible energy was negative. Slow setting pectin - 5% esterified - induced higher utilization of the digested dietary protein than the standard diet. On the other hand medium-rapid-setting pectin - 6% esterified did not induce the same effect. The energy of the pectin was wasted in the feces. Galacturonate also reduced food intake and weight gain, but in contrast with pectin : it was mostly absorbed without impairing protein digestion, its digestible energy was positive. Protein utilization attained a very low level, signifying protein waste due to lack of available energy. Algin heavily reduced food intake and weight gain, but the absorption of protein and lipids was only slightly impaired; yet, calcium absorption was drastically depressed. One-third of the algin was eliminated in the feces, so that its digestible energy remained positive. Protein utilization exceeded that of galacturonate, implying partial utilization of the algin in the energy metabolism. Mannuronate resembled galacturonate in every respect, its value for protein utilization was the lowest among all the test substances.

INTRODUCTION

Pectin - a mixture of polygalacturonic acids partially esterified with methanol - and algin - a mixture of sodium polymannuronates - are not utilized efficiently by monogastric animals (1,2); nevertheless, they undergo degrees of degradation during their passage through the alimentary canal and also affect the absorption and utilization of nutrients. In mature rats pectin is decomposed readily by microorganisms in the colon. The degrees of decomposition depends on the composition of the diet, its retention time in the intestine and the adjustment of the animal to it (3).

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a. Prof. G. Zimmermann who was the initiator of this work died on August 21, 1969.

Another factor is the degree of esterification : pectin N.F. (9-11% methoxyl) was decomposed to a far greater extent than pectin L.M. (3-5% methoxyl) (4). The main decomposition products are lower fatty acids, such as acetic and formic acid (3), and CO_2 (5). Most investigators deny the possibility of enzymatic digestion in the small intestine; however, in order to explain various absorption effects, a certain degree of alkaline degradation in the small intestine must be assumed. This could explain an increased content of uronic acids in blood, liver and urine (4,6,7).

Galacturonic acid, the compound most likely to be split off and absorbed in the small intestine, was proved to be absorbed as explained (8), but its utilization has been questioned. The blood-sugar level was not raised and most of the galacturonate was excreted in the urine or transformed to 2,5-dicarboxyfuran (9). Gilmore's findings (4) of small increases in furfural-yielding substances in the liver and kidneys of pectin-fed young rats indicate absorption of galacturonate, but do not provide proof of its utilization; they are probably transitional effects preceding excretion. The participation of galacturonic acid in the detoxification mechanism like the one of glucuronic acid, as assumed by early workers (7), has never been substantiated.

Algin was studied by Nilson with several species of animals (10) Amounts of up to 10% in their diets produced no significant differences in performance, but at higher levels growth was retarded. Varying fractions of the algin were recovered in the feces. Diemair et al. considered the digestive enzymes as capable of attacking algin (11). Contrarily Matsuura et al. found that algin and mannuronate were digested very slightly and retarded growth strongly (2).

As for the effect of pectin and algin on the digestion and absorption of other nutrients and non-nutritive substances, the following conclusions were reported : Interference with the digestion of protein was observed (1), and its extent in mature rats was measured by Gideksen (5). He reported also a slight decrease in the digestible energy of the basal ration. Pectin reduced absorption of lead, stronium and other toxic materials by forming complexes in the alimentary canal (12) . Likewise, it depressed the cholesterol level in the blood and liver (13) by reducing the absorption of dietary cholesterol and of bile acids secreted into the intestines (14). Calcium absorption in humans was not affected by a daily dose of 8 grams of algin (15).

The object of this study was to compare the effect of pectin, algin and their monomers on the absorption and utilization of protein, fat and calcium in young rats. Their effect on the digestible energy was investigated too.

MATERIALS AND METHODS

Test substances. Two types of pure (medical) citrus pectin[b] were used : slow setting, 55% esterified (subsequently referred to as pectin-S) and medium-rapid setting, 65% esterified (pectin-M). The algin was commercially-pure sodium alginate, food grade. The cellulose was Alphacel[c]. Galacturonate was prepared enzymatically : 300 g pectin were stirred slowly into 3000 ml of water and 200 ml of N NaOH and 15 g of "Pectinase-Takamin"[d] were added. The slurry (pH 3.8) was stored under toluene (2 cm depth) for 12 days at 24°C, then the digest was filtered through Celite, neutralized with $Ca(OH)_2$ to pH 6.5 and concentrated under reduced pressure. The thick syrup was dried at 105°C to yield brown crystals of sodium-calcium galacturonate. Mannuronate was prepared by acid hydrolysis : 200 g algin were wetted with ethanol and dispersed in 3000 ml of 88% formic acid (18). The mixture was refluxed for 12 hours, filtered and concentrated under reduced pressure. The thick syrup was dissolved in half a liter of boiling ethanol and chilled overnight. The precipitate was dried at 105°C to yield brown hygroscopic crystals of sodium mannuronate.

The moisture, ash and calcium contents of the test substances and their gross energy values are listed in Table I .

TABLE I. Analysis of Test Substances

TEST SUBSTANCE	MOISTURE	A S H	CALCIUM	GROSS ENERGY
	g/100 g	g/100 g	g/100 g	Kcal / g
Pectin-S	9.3	5.9	0.125	3.2
Pectin-M	8.0	6.6	0.078	3.2
Algin	15.5	25.7	0.156	2.0
Cellulose	7.8	0.1	-	3.9
Galacturonate	-	20.4	4.21	3.0
Mannuronate	-	41.2	-	2.0

Diets. The composition of the standard diet was (in grams per 100 g); casein (vitamin free)[e] 10; soybean oil 8; Cellulose 2; corn starch 75; vitamins 1 (19); mineral-mix USP XVI 4 (19). The moisture content was 10.5%, gross energy 4.5 Kcal/g. The experimental diets were prepared by substituting 5 or 10% of the test substances for equivalent amounts (by dry weight) of starch. This low-protein diet was chosen because it is known for its high protein utilization (20). Any test substance containing little available energy would alter the ratio of protein to energy and lead to reduced protein utilization.

b. Purchased from Yachin, Tel-Aviv, Israel.
c. Nutritional Biochemical Corp. Cleveland, Ohio .
d. Miles Chemical Corp. Elkhart, Indiana.

Animals. Albino rats of the Charles River C.D. strain, 21 days old, were formed into groups of 6 animals of the same mean weight. Each group comprised 3 males and 3 females, since in our previous experiments with animals of this age and of this duration no differences between the sexes have been found concerning food intake and utilisation. The later investigations of the separate figures for males and females confirmed the assumption. They were housed in individual screened cages and fed ad lib. for 10 days. Relative humidity was held at 55±5% and temperature at 24°C. At the end of the trials the weights of the animals, the consumed food and the air-dried feces were determined individually.

Analytical methods. The food and feces of each group were assayed for moisture content, nitrogen, ash and calcium according to AOAC (17); the energy content was determined in a ballistic bomb calorimeter[e]. Results were analysed statistically according to Snedecor (19).

RESULTS AND DISCUSSION

Food intake and weight gain. The test substances - being neither nutrients nor ballast - were compared to starch and to cellulose. Every substance unsuitable for complete utilization should depress the metabolizable energy level of the food and lead to reduced utilization of protein as measured by the Protein Efficiency Ratio (Table II).

TABLE II. Effect of Test Substances on Weight Gain and Protein Utilisation

LEVEL OF TEST SUBSTANCE IN DIET		RELATIVE FOOD INTAKE[1] %	RELATIVE WEIGHT GAIN[1] %	PER[2]
Control		100 (110 g)	100 (36.8 g)	3.2 ± 0.10[3]
Pectin-S	10%	82.5[4]	71.5[4]	2.75± 0.12
Pectin-N	10%	70.5[4]	52.0[4]	2.3 ± 0.14[4]
Algin	10%	70.2[4]	62.2[4]	2.85± 0.16
Galacturonate	10%	87.5[4]	68.5[4]	2.5 ± 0.09[5]
Mannuronate	10%	69.3[4]	35.4[4]	1.7 ± 0.22[4]
Cellulose	10%	116.5[4]	101.5	2.8 ± 0.27
Pectin-S	5%	97.0	108.5	3.6 ± 0.20
Galacturonate	5%	99.3	84.0[5]	2.7 ± 0.26
Cellulose	5%	102.0	103.5	3.25± 0.05

e. Gallenkamp & Co. London E.C. 2 , England.

[1]Mean for 6 animals, for entire period of 10 days, expressed as percentage of control (actual weight in parentheses)
[2]PER (Protein Efficiency Ratio) = weight gain (g) per gram total protein intake
[3]Mean for 6 animals ± S.E.
[4]$P < 0.01$
[5]$P < 0.05$

At the 5% level all food intakes resembled the control, but at 10% all test substances except cellulose reduced the intake, particularly algin, pectin-M and mannuronate. The growth was not proportional to food intake, indicating different effects on protein utilization. Pectin-M, galacturonate and mannuronate caused poor protein utilization, indicating that they are not inert ballast like cellulose, but subject the organism to specific stresses. The nature of this stress should be sought in the digestive or metabolic processes of these substances.

Digestibility and absorption. The substances in question are decomposed in the digestive system by various processes - enzymatic, bacterial and chemical - yielding a diversity of products, some of them absorbed and some not. The degree of digestion as estimated by the classical procedure does not represent absorption. Therefore it was estimated by a different procedure. The difference in feces level between control and experimental groups was determined. This excess contained all the undigested test substance, its unabsorbed non-volatile decomposition products, and any other nutrients whose absorption had been impeded by the test substance. The results are shown in Table III.

TABLE III. Digestibility of test substances and protein, and utilization of digested protein (DPER)[1]

DIET		EXCESS OF FECES[2,4]	INTAKE OF TEST SUBSTANCE[2]	APPARENT DIGESTIBILITY OF PROTEIN[3]	DPER[1,3]
		g	g	%	
Control(10% starch)		-	(10-9)	92.5	3.45
Pectin-S	10%	10.2[5]	9.0	79.5	3.45
Pectin-M	10%	8.6[5]	7.7	79	2.9
Algin	10%	1.7	7.7	88.5	3.2
Galacturonate	10%	4.7[5]	9.6	86	2.9
Mannuronate	10%	3.0	6.8	88.5	1.9
Cellulose	10%	12.4[5]	12.7	90	3.1
Pectin-S	5%	5.0[5]	5.3	85.5	4.2
Galacturonate	5%	1.4	5.4	89.0	3.0
Cellulose	5%	7.0[5]	5.6	92.0	3.55

The moisture contents of the food and feces were adjusted to 10% for the calculations.

[1] DPER (Digestible protein efficiency ratio) : weight gain (g) per gram digested protein.

[2] Mean for 6 animals for experimental period of 10 days.

[3] Pooled mean for 6 animals for 10 days.

[4] Excess of feces above normal percentage of food, as determined from control.

[5] $P < 0.01$.

Undigested protein was assayed independently. The excess of undigested protein was determined in accordance to the excess of feces. It accounted mostly for 10% of the excess of feces; hence, the difference between the excess of feces and the ingested amounts of the test substance gives an estimate of its absorption.

The excess of feces by 100% or over the ingested amount of test substance in the diets containing pectins or cellulose, signified that they were not absorbed at all. Yet galacturonate, algin and mannuronate increased the normal percentage of feces only by 25-45% of the ingested weight signified that these substances were largely digested or absorbed. The coefficient of protein digestion was reduced significantly by pectin even at the 5% level and somewhat less by galacturonate, but none of the other substances interfered significantly with protein digestion.

However, when the utilization of the digested protein was investigated by defining a new parameter DPER, (gram gain per gram digested protein), analogous to PER, remarkable differences were observed between pectin and the other test substances (Table III). Galacturonate and mannuronate, although mostly absorbed, drastically reduced the utilization of the digested protein. Apparently they were not used for energy production, but rather imposed an additional stress on the energy metabolism for the excretion of copious amounts of dark-brown urine. On the other hand, pectin-S which had not been absorbed and had reduced protein digestibility, did not impair its utilization. Even at the 10% level the DPER-value corresponded to the control and at the 5% level exceeded it. These results corroborates Gudaman, who fed a 12% apple pectin diet to adult rats and found decreased digestibility of protein, but increased nitrogen retention (5). Pectin-M differred from pectin-S in this respect and resembled galacturonate, although it was not absorbed and did not have to be excreted. Its stress effect was evidently different from that of mannuronates. Algin did not show any noticeable effect on protein utilization, unlike its monomer mannuronate. Its absorbed part did not imply any stress on the energy metabolism, but must have been partly suitable for energy utilization.

Digestible energy. The energy content of the feces revealed another phenomenon in the digestive process. Since cellulose alone was capable to raise the energy content of 30 Kcal/g of the normal feces to the level of 3.7 Kcal/g, whereas the caloric value of all the other test substances were equal to or less than the energy content of the normal feces. Higher energy values of the feces must indicate undigested proteins or lipids because carbohydrates alone were unable to account for the increase of the fecal energy content.

It has been shown previously that only the pectins and 10% galacturonate had impeded protein digestion, and even these not enough to account for the high energy of the feces. It may be concluded that all the test substances at the 10% level, and the pectins in particular, reduced the absorption of fat (Table IV). Verification of this hypothesis requires further investigations.

TABLE IV. Effect of test substances in diet on digestible energy.

DIET		GROSS ENERGY[1] OF FECES	COEFFICIENT OF DIGESTIBLE ENERGY[1]	SPECIFIC DIGESTIBLE ENERGY[1,2]
		kcal/g	%	kcal/g
Control (10% starch)		3.0	96.7	+ 3.6
Pectin-S	10%	4.1	84.1	- 1.65
Pectin-N	10%	4.2	84.8	- 1.6
Algin	10%	3.45	94.1	+ 1.05
Galacturonate	10%	3.35	92.5	+ 1.15
Mannuronate	10%	3.6	92.5	+ 0.15
Cellulose	10%	3.7	87.9	+ 0.15
Pectin-S	5%	3.4	92.4	- 1.3
Galacturonate	5%	3.25	95.4	+ 1.0
Cellulose	5%	3.7	93.2	- 0.1

[1] Pooled mean for 6 animals

[2] Specific contribution per gram test substance, to digestible energy of basal diet. (For explanation, see text).

A better criterion for the effect on the digestible energy was derived as follows : The digestible energy of the basal diet was calculated from the starch control diet by assuming the value of 3.6 Kcal/g for starch (corrected for 10% moisture). The digestible energy values for the experimented diets were found by difference between their gross energy and the energy of the feces. The difference between the digestible energy of the basal diet and that of the experimental diets, determined as described above, was divided by the weight of the ingested test substance. This provided the specific contribution of one gram of test substance to the digestible energy of the basal diet and may be interpreted as its apparent digestible energy under the conditions of the experiment (TableIV). The specific contribution of the pectins was negative, even at 5% in contrast to Gödeken who found a digestible energy of 95% for pectin (5). The partially digested algin contributed one half of its gross energy and galacturonate one third. Mannuronate balanced the loss due to undigested materials with its own digestible energy. Cellulose, as expected, made no contribution at all.

Retention of food calcium. Although both pectin and algin were known to form precipitates with calcium and other bivalent metals, no detrimental effect on the absorption of food calcium had been reported (1, 4, 15, 20). Our own findings which are shown in Table V do not confirm these reports.

TABLE V. Apparent retention of calcium .

DIET	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible][1]	[illegible]
	%	%	%
Control	[illegible]	[illegible]	[illegible]
Pectin-S	[illegible]	[illegible]	[illegible]
Pectin-H	[illegible]	[illegible]	[illegible]
Algin	[illegible]	[illegible]	[illegible]
Calcium-alginate	[illegible]	[illegible]	[illegible]
Cellulose	[illegible]	[illegible]	[illegible]
Pectin	[illegible]	[illegible]	[illegible]
Calcium-pectate	[illegible]	[illegible]	[illegible]

[1] Mean for 6 animals

The calcium in the water was not included in the calculations, since its amount was negligible compared with the food calcium. Calcium retention was lowest for the three groups fed low calcium, i.e. little more than one-half of the control and some [illegible] in comparison to the intake of the control and some [illegible] in the [illegible] deficiency due to malabsorption should not be [illegible] critical fecal calcium [illegible] no changes in calcium [illegible] Pectin L.M. For 2 weeks, [illegible] appropriate location for studying the effect of calcium [illegible].

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Growth Inhibitory Effect of Certain Polysaccharides for Chickens

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IT IS well-known that the growth of chickens is inhibited if their diet contains either linseed meal (Kratzer and Williams, 1948; MacGregor and McGinnis, 1948), or ground carobs (Kratzer and Williams, 1951; Bornstein *et al.*, 1963), or guar meal (Borcher and Ackerson, 1950; Vohra and Kratzer, 1964). In each case, the feces of the chickens were extremely sticky and tended to paste up the vent. Linseed meal loses its growth-inhibitory properties if it is given a preliminary water treatment; or if the pyridoxine content of the diet is increased (Kratzer and Williams, 1948; MacGregor and McGinnis, 1948). Similar treatment of ground carobs

gave either very slight but significant (Kratzer and Williams, 1951), or no growth improvement (Bornstein *et al.*, 1963) of chicks. Our unpublished studies indicate that guar meal is not improved either by treatment with water or by the addition of pyridoxine.

Linseed meal, carob and guar seeds are excellent sources of polysaccharides which are classified as gums and mucilages. An obvious question in considering these materials is whether this growth depressing effect is due to the high levels of polysaccharides which they contain.

A part of the growth inhibitory properties of linseed meal has been attributed to its mucilage content (Mani *et al.*, 1949; Clagett *et al.*, 1955) even though the latter workers doubted this in another report (Schlamb *et al.*, 1955). A depression in the growth of rats has been observed when their diets contained more than 5% agar agar or Irish moss (Nilson and Schaller, 1941). The digestibility of agar agar, pectin and guar gum is reported to be 21%, 19% and 76% respectively, and no significant depression in the growth of rats was observed when the diets contained these polysaccharides at levels of 15%, 10% and 6%, respectively (Booth *et al.*, 1963). Ershoff and Wells (1962) found no significant depression in the growth of rats which were fed 10% pectin, guar gum, locust bean gum or carrageenan. Carrageenin appears to interfere with the digestion of proteins by pepsin under certain conditions (Vaughan *et al.*, 1962). The present work which was reported in abstract form (Kratzer and Vohra, 1963) is a study of the effects of feeding polysaccharides on the growth of chickens.

EXPERIMENTAL

Commercially prepared samples of the following polysaccharides were used in this study, guar gum,[1] locust (or carob) gum,[1] gum arabic,[1] gum ghatti, gum tragacanth,[1] gum karaya, pectin, carrageenin, cellulose,[3] methylcellulose, sodium salt of carboxymethylcellulose, agar agar, dextrin, dextran and polygalacturonic acid.[2]

Dried okra powder was prepared by homogenizing fresh okra in a blender and an excess of methanol was added to the homogenate. The mixture was stirred and filtered on a Büchner funnel under vacuum. The solid residue was dried in a current of air at room temperature, and ground.

To obtain mucilage, linseed oil meal was soaked in an excess of water overnight, stirred and filtered through muslin. The volume was reduced by leaving the mucilage in shallow pans in a draft of air. The polysaccharide was precipitated from the mucilage solution by the addition of methanol. It was filtered and dried in a current of air at room temperature.

Caramel was prepared by the heating of sugar crystals (sucrose) in an iron crucible over a burner.

Psyllium husk and dried kelp were obtained from commercial sources.

The polysaccharides were added to the stock diet (Table 1) at the required level without making any adjustment in its composition (Table 1) at the required level without making any adjustment in its composition. Day-old, Arbor-Acres broiler type chicks were weighed and distributed into groups of approximately equal average weight. They were housed in electrically heated batteries in which water and feed were available *ad libitum*. The chicks were weighed twice every week as groups and individually at the termination of the experiment.

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[1] Samples through courtesy of Stein, Hall and Co., Inc.

[2] Through courtesy of Dr. M. A. Joslyn.

[3] Solka-floc, Brown Co., Berlin, New Hampshire.

TABLE 1.—*Composition of the stock diet*

Ingredient	Inclusion, gm./kg.
Ground milo	175
Ground corn	157
Ground barley	200
Bran, wheat	150
Soybean meal, 44% protein	120
Fish meal	75
Meat and bone meal, 50% protein	12
Whey, dried	25
Milk, skimmed, dried, 35% protein	25
Alfalfa meal	40
Limestone, ground	15
Salt*	5
Vitamins†	1

* Contains 0.025 gm. manganese sulfate (70% feed grade).

† Supplies: riboflavin, 1.1 mg.; niacin, 1.1 mg.; Ca-pantothenate, 1.1 mg.; choline chloride, 5.5 mg.; sulfaquinoxaline, 125 mg.; vitamin A, dry (20,000 I.U./gm.), 375 mg.; vitamin D_3, dry (4,500 I.C.U./gm.), 250 mg.; and folic acid, 33.3 μg.; and bran to make 1 gm.

A slurry of 120 gm. guar gum in 1500 ml. of water was reacted overnight at 37°C. with 100 ml. of a solution containing 6 gm. of either Cellulase-100[4], or the isolated enzyme from the sprouted guar beans (Vohra and Kratzer, 1964). The mucilage broke down in this process and a fluid was obtained which was reduced to about half its volume in shallow pans in a current of air at room temperature. This concentrated solution was mixed with 5880 gm. of stock diet and the diet was fed as such without any further drying.

Pectin (240 gm.) was stirred in 2 liters of water to give a thick, viscous mass which was reacted with 4.8 gm. of pectinase in 100 ml. water at 37°C. for about 12 hours. The viscous mass was stirred frequently and yielded a fluid solution which was concentrated to about 800 ml. in shallow pans in a draft of air and mixed wtih 5760 gm. stock diet to feed the chicks. It was not dried further.

The relative growth value compares the final average weight of the chickens fed the experimental diet with the average weight of chickens fed the stock (control) diet in the same experiment. For the comparison, the relative weight of the group fed the control diet is adjusted to 100.

[4] Through courtesy of Miles Chemical Co.

TABLE 2.—*Relative growth (R.G.) and gm. feed/gm. gain (F/G) of chickens fed various polysaccharides in their diets*

Experiment Series	1	2	3	4	5	
Duration of trial, days	20	21	20	21	20	
Polysaccharide and its level	R. G.	R. G.	R. G.	R. G.	R. G.	F/G
Control	100	100	100	100	100	1.73
Cellulose, 2%	101	92				
Guar gum, 2%	67.4	64		61		
Guar gum, 1%	84.4	80		72.2	79.1	1.79
Guar gum, 0.5%				78.6	92	1.75
Guar gum, 0.25%				89.1	98.4	1.72
Locust gum, 2%	73	73.6				
Locust gum, 1%			96.8			
Locust gum, 0.5%			87.8			
Locust gum, 0.25%			96.5			
Gum tragacanth, 2%	65.8	66				
Gum arabic, 2%	96.5	88.6				
Dextran, 2%	94.8					
Dextrin 2%		103				
Pectin, 4%	88.6	77.5		89.5		
Pectin, 2%	92.4	86.5				
Kelp, 4%			99.5			
Kelp, 2%			99.5			
Actual gain of control, gm.	256	330	332	294	297	

TABLE 3.—*Relative growth (R.G.) and mg. pancreas/100 gm. body weight (P/B.W.), gm. feed/gm. gain (F/G), of chickens fed various polysaccharides in their diets*

Experiment Series	6		7		
Duration of trial, days	20		20		
Polysaccharide, and its level	R. G.	P/B.W.	R. G.	P/B.W.	F/G
Control	100	375	100	342	1.54
Cellulose, 2%	96.8				
Okra, dried, 2%	87.6	470	81.8	462	1.84
Carrageenin, 2%	75	532	75.4	355	1.91
Linseed mucilage, 2%	95.4	370	97.4	—	1.84
Psyllium husk, 2%	87.4	435	81.8	456	1.92
Methylcellulose, 2%	103				
Carboxymethylcellulose, (Na), 2%	96.5	360	95.8	—	1.88
Caramel, 2%	100				
Actual gain, gm.	339		320		
Control			100	474	1.78
Kelp, 2%			114	506	1.59
Agar agar, 2%			110	415	1.72
Gum karaya, 2%			72	432	1.67
Gum ghatti, 2%			104.5	427	1.81
Actual gain, gm.			280		
Control			100	410	1.78
Gum ghatti, 2%			95		1.81
Gum karaya, 2%			70	500	1.92
Agar agar, 2%			102		1.87
Actual gain, gm.			315		

RESULTS AND DISCUSSION

The relative growth of the chickens fed the diet diluted with 2% of cellulose was 92 to 101% of the controls (Table 2 and 3). When the diets contain 2% guar gum, the relative growth of the chickens was 61 to 67.4% of the controls. The depression in growth was probably not due to the dilution of the energy content of the diet by the addition of 2% gum because the addition of 2% cellulose which also diluted the energy of the diet to the same extent, caused little or no growth depression. The addition of the following polysaccharides at a level of 2% to the chicken diets caused a definite depression in growth of chickens: locust gum, gum tragacanth, gum karaya, dried okra, carrageenin, and psyllium husk (Tables 2 and 3). Pectin exhibited a marked depression in chick growth at a level of 4%. At a level of 2%, the growth of chickens on diets containing pectin, gum arabic, dextran, dextrin, gum ghatti, linseed mucilage, methylcellulose, carboxymethylcellulose, caramel, agar agar or kelp was of the same order as for control diets or those containing 2% cellulose.

As low as 1% guar gum in chicken diets caused a definite growth depression (Table 2). As the level of guar gum was decreased to 0.5 or 0.25%, the growth inhibition was also proportionately reduced. However, in case of locust gum, a definite growth inhibition of chicks occurred only at a 2% level.

Relation to composition. The variation in growth depressing effect of various polysaccharides prompted consideration of a possible common constituent responsible for this effect. Most of the data on the chemical constitution of these polysaccharides have been taken from the reviews of Smith and Montgomery (1959).

Guar gum and locust gum are neutral

polysaccharides containing 33-36%, and 14-25% D-galactose; and 64-67% and 75-86% D-mannose, respectively. Guar gum was far more growth inhibitory than locust gum at a level of 1% of the diet. D-galacturonic acid content of gum karaya and okra mucilage is 43% and 6-8%. It is also present in gum tragacanth, linseed mucilage, psyllium husk and pectin but the exact amounts are not known. D-glucuronic acid is present to an extent of 16% in gum arabic; and 12% in gum ghatti. D-galactose content of the gums is as follows: arabic, 52%; ghatti, 27%; karaya, 14%; okra mucilage, 79-80%. Linseed mucilage contains L-galactose. D-galactose is also present in gum tragacanth, agar agar and carrageenin. About 8% D-mannose is present in gum ghatti. Gum tragacanth and psyllium contain L-arabinose which is estimated to be 19% in gum arabic, 41% in gum ghatti, 3-14% in okra mucilage and 12% in linseed mucilage. L-rhamnose is present in gum arabic and its content in gum karaya and linseed mucilage is 15% and 29%, respectively. However, no prediction is as yet possible about the growth inhibitory properties of polysaccharides from their chemical constitution.

The growth inhibition is probably not due to the mucilage character of the polysaccharides in general because not all of them have exhibited growth inhibitory properties for chickens in this study. If those polysaccharides which do exhibit growth depression are allowed to be reacted with suitable enzymes, the reaction products cause no depression in the growth of chickens (Table 4). Guar gum (2%) when fed along with an enzyme capable of hydrolyzing it, at a level of 0.1%, overcame the growth inhibition of chickens to some extent. However, the growth of chickens fed guar gum which has been hydrolyzed with the enzyme was of the same order as on control diets. The enzyme was either isolated from the sprouted guar beans or a commercial preparation, Cellulase-100, capable of splitting polysaccharides. Growth inhibition due to 4% pectin was completely overcome if it was first re-

TABLE 4.—*Effect of enzymes on the relative growth (R.G.), mg. pancreas/100 gm. body weight (P/B.W.), and gm. feed/gm. gain (F/G) of chickens fed various polysaccharides*

Experiment Series	8			9		
Treatment and level	R. G.	P/B.W.	F/G	R. G.	P/B.W.	F/G
Control	100	474	1.78	100	410	1.78
Guar gum, 2%	69.4	510	1.58	61.6	620	2.35
Guar gm. 2% +0.1% guar enzyme	79	508	2.17	76.5	474	2.32
Control+0.1% guar enzyme	117	376	1.63	102		1.86
Guar gum (2%) reacted with guar enzyme	102.5	386	1.60	95	378	2.0
Control+0.1% Cellulase-100				100		1.90
Guar gum 2%+Cellulase-100, 0.1%				86	356	2.02
Actual gain, gm. (Experimental period)	280 (20 days)			315 (20 days)		
Control	100			100		
Pectin, 4%	61.5			69.2		1.94
Pectin, 4%+0.08% pectinase	84			83		1.97
Pectin, (4%) reacted with pectinase	100			102		1.89
Control+0.08% pectinase	95.5			98.8		1.75
Actual gain, gm. (Experimental period)	294 (21 days)			297 (20 days)		
Control	100					
Pectin, 4%	53.5					
Polygalacturonic acid, 4%	89.8					
Actual gain, gm. (Experimental period)	327 (20 days)					

acted with the enzyme pectinase. When pectin and the enzyme were fed together, the growth inhibition was only partially overcome. The presence of methoxy groups in pectin was essential for its growth inhibition. This is borne out by the fact that polygalacturonic acid when fed at a level of 4% gave a relative growth of about 90% of the controls and the methoxy derivative of this (pectin) had a value of about 54%.

No information, as yet, is available about the exact sequence in which the various sugars are linked in the various polysaccharides. But it appears that as the frequency of branching increases in the molecule, the growth inhibitory properties also increase. Guar gum has D-galactose on every alternate D-mannose molecule in contrast to carob gum which has D-galactose on every 3rd or 4th mannose molecule of the straight chain part of the polysaccharide. Guar gum is more growth inhibitory than locust (carob) gum. Gum arabic has fewer branches and more of the component sugars are arranged as a straight chain. It has little growth inhibitory properties for chicks. In contrast to this, gum tragacanth has many more sugars branching from the straight chain of the polysaccharide and has definite growth inhibitory properties.

The ratio of feed intake/gain for controls and the diet containing 2% guar gum was of the same order (Table 2), but tended to be higher for okra, carrageenin, linseed mucilage, psyllium husk and carboxymethycellulose (Table 3). Not all of these substances were growth inhibitory.

In general, the pancreas weight per 100 gm. body weight (Table 4) tended to be higher for those polysaccharides which had a growth inhibitory property. Chickens fed psyllium husk had an accumulation of mucilage in the proventriculous which gave it an abnormal appearance of a sausage similar to ones observed by O'Dell *et al.* (1959). The incidence of this gross abnormality was only in chickens fed growth-inhibitory polysaccharides but was never as serious as for psyllium husk.

SUMMARY

Several naturally occurring polysaccharides depressed the growth of chickens when fed in a nutritionally balanced diet containing soybean meal, cereals, and fish meal. The growth of chickens was inhibited about 25% to 30% by the inclusion of guar gum, locust gum, gum tragacanth, gum karaya, or carrageenin at levels of 2%; or 4% of pectin in their diets. Dried okra or psyllium husk caused a depression in growth of about 15% at a level of 2% inclusion, and cellulose, methylcellulose, carboxymethylcellulose, dextrin, dextran, linseed mucilage, caramel, gum ghatti, agar agar and kelp appeared to be without any deleterious properties. The growth depressing properties of pectin and guar gum were overcome by their treatment with enzymes capable of hydrolyzing them, namely, pectinase and cellulase or a preparation from the sprouted guar beans.

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Comparative Effects of Pectin N. F. Administration on the Cholesterol-Fed Rabbit, Guinea Pig, Hamster and Rat.* (27739)

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Previous findings indicate that citrus and apple pectin when incorporated at a 5% level in the diet largely counteracted the increase in plasma and liver cholesterol and liver total lipids induced by cholesterol feeding in the rat(1). The activity of pectin in this regard was correlated, at least in part, with its methoxyl content since pectic substances with a methoxyl content of 5.0% or lower were devoid of activity in contrast to the marked activity exhibited by those with a methoxyl content of 10.1% or higher(2). Data are presented here on the comparative effects of pectin of relatively high methoxyl content (*i.e.*, 10.7% on a moisture-ash-free basis) on plasma and liver cholesterol and liver total lipid levels in the cholesterol-fed rabbit, guinea pig, hamster and rat.

Procedure. Male rabbits, guinea pigs, hamsters and rats were selected at the weights indicated in Table II and each species was divided into 3 comparable groups fed respectively a purified basal ration, the basal ration + 1% cholesterol, and the basal ration + 1% cholesterol + 5% pectin N.F. The composition of the diets is indicated in Table I The animals were placed in metal cages with raised screen bottoms (the rabbits in individual cages; the other species, 2 animals per cage) and were provided the test diets and water *ad libitum*. The guinea pigs were killed after 4 weeks of feeding and the other species after six. At time of autopsy the animals were anesthetized with sodium pentobarbital, and blood was withdrawn from the aorta into a heparinized syringe. Livers were excised, blotted to remove excess blood, weighed, and stored in a freezer until analyzed. Lipid was extracted from the livers by the method of Thompson *et al.*(3), and cholesterol was determined on liver and plasma by the method of Nieft and Deuel(4). Total lipids were determined gravimetrically on an aliquot of the liver extract.

Results. A significant difference in response was observed between the rat in contrast to the rabbit, guinea pig and hamster in respect to the effects of pectin feeding on plasma and liver cholesterol and liver total lipids following cholesterol administration. Citrus pectin with a methoxyl content of 10.7% when fed at a 5% level in the diet largely counteracted the increment in plasma and liver cholesterol and liver total lipids induced by cholesterol feeding in the rat but was without significant effect in the cholesterol-fed rabbit, guinea pig and hamster. With the exception of rabbits fed the basal + cholesterol ration, all cholesterol-fed animals gained weight during the experimental period. The increment in plasma cholesterol levels following cholesterol feeding was highest in the rabbit and least in the rat. Liver cholesterol levels after cholesterol feeding were highest in the hamster. Results are summarized in Table II.

No data are available to account for the differences in response between the various species. Since the 3 species in which pectin was without significant effect were herbivorous whereas the rat in which it was active is not, it is possible that the "anti-cholesterol" effect of pectin is confined to non-herbivorous species. Of possible pertinence in this regard is the observation of Keys *et al.*(5) that citrus pectin when fed at a level of 15 g per day caused a slight but statistically significant reduction in serum cholesterol of another non-herbivorous species, namely man. Further studies are indicated to determine to what extent differences in intestinal physiology or in the digestion or absorption of pectin between herbivorous and non-herbivirous species contributed to the diverse results.

Summary. Citrus pectin with a methoxyl content of 10.7% when fed at a 5% level in the diet largely counteracted the increment

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TABLE I. Composition of Experimental Diets.

Ingredients	Rabbit			Guinea pig			Hamster			Rat		
	A_1	A_2	A_3	B_1	B_2	B_3	C_1	C_2	C_3	D_1	D_2	D_3
Magnesium oxide				.5	.5	.5						
Potassium acetate				2.5	2.5	2.5						
Cholesterol		1	1		1	1		1	1		1	1
Salt mixture*	5	5	5	5	5	5	5	5	5	5	5	5
Casein†	25	25	25	30	30	30	24	24	24	24	24	24
Sucrose	45	44	39	37	36	31	61	60	55	61	60	55
Pectin N.F.‡			5			5			5			5
Cellulose§	10	10	10	10	10	10						
Agar				5	5	5						
Crisco	10	10	10									
Cottonseed oil	5	5	5	10	10	10	10	10	10	10	10	10

To each kg of diets A_1, A_2 and A_3, and B_1, B_2 and B_3 were added the following vitamins: thiamine hydrochloride, 16 mg; riboflavin, 16 mg; pyridoxine hydrochloride, 16 mg; calcium pantothenate, 40 mg; nicotinic acid, 200 mg; biotin, 1 mg; folic acid, 10 mg; vit. B_{12}, 100 μg; 2-methyl-1,4 naphthoquinone, 5 mg; para-aminobenzoic acid, 100 mg; ascorbic acid, 1.5 g; choline chloride, 2 g; vit. A, 5000 U.S.P. units; vit. D_2, 500 U.S.P. units; and alpha-tocopherol acetate, 100 mg. To each kg of diet A_2, A_3 and A_3 were added 200 mg of inositol; and to each kg of diets B_2, B_3 and B_3 were added 2 g of inositol. The vitamins were added in place of an equal amount of sucrose. Each guinea pig also received daily (6 times/wk) an oral supplement of 10 mg ascorbic acid in 0.1 ml water.

To each kg of diets C_1, C_2 and C_3, and D_1, D_2 and D_3 were added the following vitamins: thiamine hydrochloride, 20 mg; riboflavin, 20 mg; pyridoxine hydrochloride, 20 mg; calcium pantothenate, 60 mg; nicotinic acid, 100 mg; ascorbic acid, 200 mg; biotin, 4 mg; folic acid, 10 mg; para-aminobenzoic acid, 400 mg; inositol, 800 mg; 2-methyl-1,4 naphthoquinone, 5 mg; vit. B_{12}, 150 μg; vit. A, 5000 U.S.P. units; vit. D_2, 500 U.S.P. units; and alpha-tocopherol acetate, 100 mg. The vitamins were added in place of an equal amount of sucrose.

* Hubbell, Mendel and Wakeman Salt Mixture, General Biochemicals, Inc., Chagrin Falls, Ohio.

† Vitamin-Free Test Casein, General Biochemicals, Inc., Chagrin Falls, Ohio.

‡ Citrus pectin N.F. (with a methoxyl content of 10.7% on a moisture-ash-free basis). This material was kindly provided by Dr. Glenn H. Joseph and Mr. Willard E. Baier of Sunkist Growers, Ontario, Calif.

§ Cellulose (Solka Floc BW 200), Brown Co., Boston, Mass.

TABLE II. Comparative Effects of Pectin Feeding on Plasma and Liver Cholesterol and Liver Total Lipid Levels in Cholesterol-Fed Rabbit, Guinea Pig, Hamster and Rat.*†

Group	Body wt, g		Plasma cholesterol, mg/100 ml‡		Liver wt, g‡	Liver total lipid, %‡	Liver cholesterol, mg/g‡	
	Initial	Final	Free	Total			Free	Total
Rabbit								
Diet A_1	2140	2463	42.3 ± 8.3	159 ± 30	72.0 ± 3.6	4.2 ± .3	2.18 ±.09	3.4 ± .4
" A_2	2179	1655	610 ± 77	1363 ±244	54.6 ± 8.4	7.0 ±1.0	5.77 ±.52	21.1 ± 6.2
" A_3	2172	2408	512 ± 75	1563 ±192	79.3 ± 4.1	11.0 ± .8	5.08 ±.52	31.8 ± 5.2
Guinea pig								
Diet B_1	295	494	10.6 ± 1.0	53 ± 4	21.9 ± 1.4	4.1 ± .1	2.09 ±.06	2.3 ± .1
" B_2	296	399	149 ± 11	398 ± 24	28.3 ± 1.1	13.9 ± .7	6.59 ±.35	27.6 ± 2.6
" B_3	294	387	129 ± 11	326 ± 27	28.3 ± 1.2	14.4 ± .9	6.55 ±.40	25.9 ± 3.3
Hamster								
Diet C_1	39	97	39.6 ± 2.6	132 ± 7	5.5 ± .2	3.9 ± .1	2.06 ±.07	2.3 ± .1
" C_2	39	89	140 ± 10	495 ± 32	7.3 ± .2	13.9 ± .3	6.36 ±.30	66.3 ± 1.8
" C_3	39	96	130 ± 10	435 ± 39	7.4 ± .3	14.6 ± .5	6.36 ±.20	71.6 ± 3.3
Rat								
Diet D_1	44	284	19.8 ± .9	95 ± 4	10.6 ± .2	4.5 ± .1	1.83 ±.03	2.3 ± .1
" D_2	45	294	26.6 ± 2.2	145 ± 8	13.3 ± .5	12.6 ± .9	4.06 ±.25	31.1 ± 3.4
" D_3	45	293	20.0 ± 1.6	104 ± 7	12.5 ± .5	7.3 ± .4	2.84 ±.16	10.3 ± 1.8

* Each dietary group of rabbits consisted of 6 animals; each dietary group of guinea pigs, hamsters and rats (Holtzman strain) consisted of 10 animals.

† Guinea pigs were sacrificed after 4 wk of feeding; rabbits, hamsters and rats after 6 wk.

‡ Including stand. error of mean.

in plasma and liver cholesterol and liver total lipids induced by cholesterol feeding in the rat but was without significant effect in the cholesterol-fed rabbit, guinea pig and hamster.

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On the Fate of Ingested Pectin*

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THE gelatinizing substance in fruit was discovered in 1825 by Braconnot, who named it Pectin (1).

Pectin is a hemicellulose or vegetable mucilage. According to Banes (2) and Link (2) and Schneider (2), it consists entirely of a long chain of galacturonic acid units, joined 1, 4, some of which are esterified with methyl alcohol. To simplify, starch, for example, is made up of glucose residues and pectin of galactose residues. When the monosaccaride galactose is properly oxidized, it yields galacturonic acid. Or, starch consists of glucose units and pectin of galacturonic acid units (2).

Since pectin is being used in the treatment of certain gastro-intestinal disturbances, notably nonspecific diarrhea in infants, it is proper to inquire; what is the fate of ingested pectin? It has been postulated that pectin adsorbs toxic substances. If it is decomposed in the intestine, its value as an adsorbent is untenable. It has also been postulated that pectin is decomposed into galacturonic acid which is absorbed and serves a detoxicating role in the liver by forming uronates with phenolic substances. If pectin is not split into galacturonic acid in the intestine, this hypothesis is untenable; or, if galacturonic acid, or some form of uronic acid that may be produced in the intestine is not absorbable, then the hypothesis is also untenable. Nickel pectin is also being used. If pectin is decomposed in the intestine, for example in the colon, the pectin would serve only as a carrier of nickel to the colon, unless the nickel prevents the pectin from being decomposed. Hence it is of considerable import to know what actually happens to ingested pectin.

A review of the literature up to 1929 on the digestion of hemicellulose and pectin may be found in the report of McCance and Lawrence (3). Though a systematic study of the fate of pectin had not been made by 1929, the work of Schneider (1912) is important. He prepared from apple marc a pectin, which according to his analysis yielded 35.9 per cent pentosan and 45.8 per cent galactosan. He fed the pectin with a mixed diet very low in cellulose to several human subjects, and found the "coefficient of digestibility" for the pentosans to be 88.7 per cent, and for the galactosans 76.8 per cent. He also found that intestinal bacteria decompose the hemicellulose of apple marc.

The following reports indicate indirectly that pectin may be decomposed in the intestine and may have some nutritional value.

Imhäuser (5) fed pectin preparations of low galactose content to dogs, and observed only slight and inconstant changes in the blood sugar. Fatty changes which occur in the livers of fasting dogs treated with phloridzin were not prevented by pectin. However, pectin apparently decreased the excretion of acetone bodies in the urine. Von and Friedrich (6) administered a considerable quantity of "Aplona" (prepared from apples), 100 gm. daily, to humans, and observed an increase in the formic acid content of the urine. This he explained by the partial decomposition in the intestine of pectin with the release and absorption of methyl alcohol, which is oxidized in the body to formic acid. Baker and Martin (7) found that in nature bacterial decomposition of pectins and hemicelluloses continues to the stage of fatty acid, hydrogen and methane. However, Arnold (8) fed nickel pectinate to young rats for 8 weeks and found that the growth curves were not influenced.

Only one report indicating that galacturonic acid may result from the decomposition of pectin in the intestine is available. Manville and associates (9), using rabbits, obtained some evidence indicating that uronic acid from pectin can be utilized in detoxifying menthol. The total average excretion of uronic acid by the pectin fed rabbits was 25 per cent greater than that of the rabbits fed menthol only. Unfortunately only two rabbits were used in the control, menthol, and menthol plus pectin series.

The work of Manville and associates throws light on the report of Scudi, Ratish and Bullowa (10) stating that there is an increase in glucuronic acid excretion after giving sulfapyridine and that the increase parallels the quantity of the drug given. Yanovsky (11), dealing with hemicelluloses and their relation to animal nutrition, suggested that larger amounts of toxic substance should be eliminated if the organism is supplied with galacturonic acid in the form of pectin.

Our work was undertaken to determine the "coefficient of digestibility" of pectin. It constitutes essentially a repetition of Schneider's experiments referred to above. However, a pure pectin, modern chemical methods, and dogs as well as humans were utilized. In addition to normal subjects, human and canine subjects with ileostomies were studied. Moreover, the pectin was administered, in the feeding experiments, with a controlled diet and during fasting.

THE PECTIN USED

The pectin used in this study was pure citrus pectin obtained from the Research Laboratories of the California Fruit Growers Exchange. It was essentially free of such impurities as pentoses, pentosans, color and other materials that are frequently found in other pectins.

Chemical Analysis: Three quantitative tests were

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used to analyze the pectin and to determine the pectin present in the feces or ileostomy material after the feeding of pectin. One was to determine the uronic acid by the Link method (12). The second consisted of the determination of furfural which is released by uronic acids (13). The third was to determine pectic acid by the calcium pectate method (14).

The pectin we used in these studies had the following characteristics: By the Link method 18.2 per cent CO_2 equivalent to 72.8 per cent uronic anhydride was liberated. By the A. O. A. C. method for the determination of furfural, one gram yielded 0.275 gm. of phloroglucide equivalent to 0.14528 gm. of furfural. (Uronic acids as well as pentoses yield furfural, hence this factor is not characteristic of all pectins, but must be determined for the particular pectin sample employed). The methoxyl content was 9.5 per cent and jelly grade 180. By the calcium pectate method one gram yielded 1.1 gm. of pectic acid as calcium pectate, on the basis of ash and moisture free pectin. When five grams of pectin were added to 100 gm. of feces, 98 per cent could be recovered by the uronic acid method, 94 per cent by the furfural method, and 97.6 per cent by the pectic acid method.

EXPERIMENTS ON DOGS

Normal dogs: Four dogs, weighing from 25 to 35 pounds, were placed, for the first absorption period, on a mixed diet low in crude fiber (as a control), consisting daily of 200 cc. of milk, 200 gm. of hamburger, and 100 gm. of liver. During the second period, 20 gm. of pectin were added daily. For a third period, the dogs were given pectin alone, 20 gm. dissolved in 500 cc. of water being given by stomach-tube. Each absorption period was seven days in length. Specimens of feces produced by the dogs during each period were pooled (more accurate separation was facilitated by the dye method) and dried, and pectin or its degradation products determined quantitatively as furfural, uronic and pectic acids.

Ileostomy dogs: Two of the dogs used in the above experiments were subjected to a two-stage ileostomy operation and then used in the following absorption study: As a control, one pound of beef (round steak) was fed for the first absorption period, while for the second, 20 gm. of pectin were mixed with the beef which had been cut into small pieces. Material from the ileostomy was collected with an ileostomy bag over 24 hours, and then

TABLE I

Showing pectin recovered from dog feces during a seven day absorption period as estimated by three chemical methods

Dog No.	Exper. No.	Feces Weight for Experimental Period	Recovery of Pectin by Furfural Method		Recovery of Pectin by Uronic Acid Method		Recovery of Pectin by Calcium Pectate Method	
		Grams	Grams	Per Cent	Grams	Per Cent	Grams	Per Cent
20 gm. of pectin per day given with a mixed diet								
I			16.24*	[illegible]				
	[illegible]	[illegible]	13.30	[illegible]	14.00	10.00		
	[illegible]	[illegible]	11.36	8.12	17.40	12.42	5.18	3.66
	[illegible]	[illegible]	[illegible]	9.06	14.14	10.10	7.70	5.50
II								
	[illegible]	[illegible]	12.68	[illegible]	20.23	14.45		
	[illegible]	[illegible]	12.05	[illegible]	15.12	10.80	10.00	7.14
	[illegible]	[illegible]	13.44	9.60	10.64	7.60	8.12	5.80
III			[illegible]	[illegible]				
	[illegible]		[illegible]	[illegible]	4.33	3.09		
		[illegible]**	[illegible]	6.35	1.51	1.08	0.00	0.00
		[illegible]**	[illegible]	5.32	5.60	4.00	1.40	1.00
IV								
	[illegible]	[illegible]	[illegible]	[illegible]	20.80	14.84		
	[illegible]	[illegible]	[illegible]	9.00	12.22	[illegible]	2.19	1.56
	[illegible]	[illegible]	[illegible]	[illegible]	12.95	9.25	2.96	2.11
Average			[illegible]	[illegible]	12.41	[illegible]	4.69	3.35
20 gm. pectin per day given during fasting								
I			[illegible]	91.23	123.80	88.48	120.80	86.30
	[illegible]		[illegible]	96.00	135.00	96.02	121.30	86.70
[illegible]		[illegible]	[illegible]	[illegible]	105.30	75.20	95.00	67.80
			[illegible]	86.60	118.70	84.60	85.60	61.20
III		[illegible]**	[illegible]	[illegible]	37.40	[illegible]	32.50	23.06
		[illegible]**	[illegible]	[illegible]	43.75	31.22	30.50	21.80
[illegible]		[illegible]**	3.15	2.25	4.27	3.05	1.80	1.29
		[illegible]**	5.39	3.85	6.07	4.33	2.94	2.10
Average			71.69	51.22	71.78	51.20	61.[illegible]	43.73

*Calculation: The gm. of pectin was fed, and is represented by 20.3 gm. of furfural. However, 2.345 gm. of furfural returned, or 11[illegible]%, or [illegible] gm. of pectin.

**Gave only one specimen during experimental period.

analyzed by means of the three chemical tests mentioned above.

HUMAN EXPERIMENTS

Normal subjects: Six individuals were placed for the first absorption period, on a basal diet low in crude fiber (as a control). During the second period fifty grams of pectin was added daily. For a third period, fifty grams of pectin was taken alone. The pectin was made up in gelatin capsules, and the periods were three days in length. Aliquots of feces (carmine marker) produced during each period were treated as in the dog experiments.

The basal diet consisted of the following foods and weights: Breakfast—pineapple juice, 200 gm.; eggs, 100 gm.; bacon, 30 gm.; white bread, 50 gm.; butter, 14 gm.; coffee, as much as desired. Lunch—spaghetti, 56 gm.; ketchup, 20 gm.; lamb chops, 113 gm.; white bread, 50 gm.; butter, 14 gm.; coffee, as much as desired. Dinner—beef steak, 300 gm.; potatoes, 120 gm.; white bread, 50 gm.; milk, 240 gm.; apple pie, 133 gm.; butter, 14 gm.

Ileostomy subjects: Two patients, who had been subjected to ileostomy operations because of long-standing ulcerative colitis, were used. For the control absorption period they were placed on a low crude fiber diet very much as described above, while for the test period, 20 gm. of pectin were given in capsules with breakfast. Ileostomy bags here too permitted us to collect the intestinal excreta. A 24 hour study was carried out in one case, a 12 hour in the other. The ileostomy material was subjected to analysis by the three chemical methods mentioned, as in the dog experiments.

RESULTS

When the normal dogs were fed the basal diet alone, no pectic acid was found in the feces, but a total of from 0.37 to 0.42 gm. of furfural and from 0.72 to 0.90 gm. of uronic acid was obtained from the different dogs during the seven day test period. The quantity of furfural and uronic acid obtained during the control period was subtracted from the total obtained during the period when the pectin was added to the control diet.

Table I shows the quantity of pectin recovered from the dog feces as determined by the three chemical methods. When the pectin was added to the mixed diet, furfural estimation indicated a recovery of 8.62, uronic acid estimation 8.86, and pectic acid 3.36 per cent (averages of data obtained from the four dogs). Averages of the data obtained when pectin was given during fasting are as follows: furfural estimation 51.22, uronic acid 51.20, pectic acid 43.78 per cent.

Analysis of the feces representing the period of the control diet in our normal human experiments gave the following values: furfural 0.58-1.80 gm., uronic acid 3.25-6.27 gm., and pectic acid 1.04-1.61 gm. As in the dog work, the control values given here were subtracted from the total obtained during the period when pectin was added to the basal diet.

Table II shows the quantity of pectin recovered in human feces as determined by the three chemical methods. When pectin was added to a mixed diet furfural estimation indicated a recovery of 7.44, uronic acid estimation 8.70, and pectic acid estimation 1.15 per cent (average of data obtained from the six individuals). Averages of the data obtained when pectin was given during fasting are as follows: furfural 12.21, uronic acid 13.75 and pectic acid 4.16 per cent.

In calculating the recovery of pectin in the ileostomy experiments (Table IV), the values obtained in the control and pectin periods were treated as in the other experiments. In the case of the dogs the average recovery was as follows: by the furfural method 88.9,

TABLE II

Showing pectin recovered from human feces during a three day absorption period as estimated by three chemical methods

Subject	Weight of Feces	Recovery by Furfural Estimation		Recovery by Uronic Acid Estimation		Recovery by Pectic Acid Estimation	
	Grams	Grams	Per Cent	Grams	Per Cent	Grams	Per Cent
			50 gm. of pectin per day with basal diet				
1	196	12.35	8.23	13.05	8.70	[illegible]	2.21
2	212	10.96	7.30	12.15	8.10	[illegible]	[illegible]
3	165	10.85	7.23	13.95	9.30	[illegible]	0.85
4	290	18.90	12.60	20.90	[illegible]	[illegible]	1.12
5	220	2.76	1.84	7.05	4.70	[illegible]	0.82
6	253	11.18	7.45	11.25	[illegible]	[illegible]	[illegible]
Average		11.16	7.44	13.06	8.70	[illegible]	[illegible]
			50 gm. of pectin per day alone				
1	90	22.35	14.90	[illegible]	[illegible]	[illegible]	3.27
2	88	27.60	18.40	28.58	19.05	[illegible]	2.52
3	165	16.68	11.12	18.96	12.64	[illegible]	[illegible]
4	125	15.51	[illegible]	17.70	[illegible]	[illegible]	[illegible]
5	142	12.75	[illegible]	18.60	2.40	[illegible]	[illegible]
6	165	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Average		18.31	12.21	[illegible]		[illegible]	[illegible]

TABLE III

Showing average recovery of pectin from human and dog experiments as evidenced by the three chemical methods

Experiments	Recovery by Furfural Estimation		Recovery by Uronic Acid Estimation		Recovery by Pectic Acid Estimation	
	Grams	Per Cent	Grams	Per Cent	Grams	Per Cent
			Pectin with a mixed diet			
Human*	11.16	7.44	13.06	8.70	1.73	1.15
Dog**	11.93	8.62	12.41	8.86	4.69	3.26
			Pectin by itself			
Human	18.31	12.21	50.62	13.75	6.25	4.16
Dog	71.69	51.22	71.78	51.20	61.30	43.78

*Three day absorption periods and 150 gm. of pectin.
**Seven day absorption periods and 140 gm. of pectin.

by the uronic acid method 88.32, and by the pectic acid method 84.85 per cent. In the case of the humans, the 24 hour study gave average returns as follows: by the furfural method 94.27, by the uronic acid 96.25, and by the pectic acid 96.5 per cent. The 12 hour study gave somewhat smaller values: by the furfural method 72.5, by the uronic acid 80.0, and by the pectic acid 76.0 per cent.

DISCUSSION

When one adds pectin to a mixed diet, in the case of the normal dogs, examination of the feces reveals that practically 90 per cent disappears. When given during fasting, about 50 per cent disappeared. In this latter study, dogs I and II defecated frequently, and recovery of pectin ranged from 76 to 96 per cent, whereas with dogs III and IV, which defecated only once during the seven day period, the amount of pectin recovered ranged from about 2 to 30 per cent. Essentially the same results have been communicated to us by Doctors L. A. Crandall and H. K. Murer, who have used an apple pectin. In addition, the results indicate that the decomposition is carried further when pectin is added to a mixed diet than when given alone, for only about a third of the amount which may be recovered in the feces in the former case may be obtained as pectic acid, while in the latter practically all the pectin recovered may be obtained as pectic acid. Decomposition may be furthered also if the pectin fed is retained in the colon for longer periods.

When one adds pectin to a mixed diet for normal humans, 10 per cent may be recovered from the feces, whereas when given alone, about 15 per cent is recovered. In the former case, only about 1 per cent could be recovered as pectic acid, and in the latter about four per cent. As in the dog experiments, the results indicate that the decomposition of pectin is

TABLE IV

Showing pectin recovery from dog and human ileostomy material when added to a low crude fiber diet

Subject	Experiment	Weight of Material	Recovery of Pectin by Furfural Method		Recovery of Pectin by Uronic Acid Method		Recovery of Pectin by Pectic Acid Method	
		Grams	Grams	Per Cent	Grams	Per Cent	Grams	Per Cent
			Dog experiments: 20 gm. of pectin with one pound of beef					
A	1	335	18.50	92.50	18.46	92.30	17.00	85.00
	2	360	17.52	87.60	17.10	85.50	16.60	83.00
B	1	320	17.44	87.20	16.80	84.00	16.82	84.10
	2	240	17.66	88.30	18.30	91.50	17.46	87.30
Average		296	17.78	88.90	17.66	88.32	16.97	84.85
			Human experiments: 20 gm. of pectin with a low crude fiber diet					
Mr. E.	1	640	19.11	96.55	19.00	95.00	19.00	95.00
	2	560	18.40	92.00	19.50	97.50	19.00	95.00
	Average	600	18.75	94.27	19.25	96.25	19.00	95.50
Mr. S.	1*	320	14.00	70.00	15.60	78.00	14.40	72.00
	2*	480	15.00	75.00	16.40	82.00	16.00	80.00
	Average	400	14.50	72.50	16.00	80.00	15.20	76.00

*12 hour study

carried further when pectin is added to a mixed diet than when given alone.

The results obtained from the normal human experiments did not differ from those of the normal dogs when pectin was added to a mixed diet. However, when fed alone the recovery from human feces was much less than for the dogs, being about 15 and 50 per cent respectively (Table III). It is also evident then that more pectin is decomposed in the alimentary tract of the fasting man than of the fasting dog.

Recovery of pectin from ileostomy material, obtained from dogs, ranged from 84 to 89 per cent. Human ileostomy material in the 24 hour study gave a pectin recovery which ranged from 94 to 97 per cent, while in the 12 hours study it ranged from 72 to 80 per cent. Since the addition of pectin to the diet may have slowed up the passage of food through the upper gastro-intestinal tract, the smaller values, observed in the 12 hour study may be explained to be due to the possibility that all of the diet was not represented by the material obtained. It is clear that most, if not all, of the pectin decomposed during passage in the alimentary tract is decomposed in the colon.

The human subjects complained of colonic flatulence while taking the pectin. The little pectin escaping decomposition increased the bulk of the feces. The feces passed by the dogs when pectin was fed alone or without meat and milk was like very firm jelly in consistency.

Since under ordinary conditions pectin is for the most part decomposed in the alimentary tract, it is not likely that it produces its effect in diseased conditions by serving as an adsorbent. It may operate in the small intestine by retarding propulsive rate. This possibility is only an impression and must be checked by special experimentation. Since pectin is decomposed in the intestine, the problem now is to ascertain (1) the products of decomposition, (2) their effect on the flora, (3) whether or which of the products are absorbed, and (4) whether, if absorbed, do they play a significant rôle in nutrition and detoxification.

While this work was under way a preliminary report by Kertesz (15) appeared which indicates that he has obtained results on the decomposition of pectin in the alimentary tract of dogs and humans similar to those we have observed.

SUMMARY

In the dog, when 140 gm. of pectin was fed with a mixed diet over a period of seven days, an average of 90 per cent of the pectin was decomposed; when fed during fasting an average of only 50 per cent was decomposed. During fasting decomposition of the pectin depended on the rate of passage through the gastro-intestinal tract, for in the dogs which defecated frequently the recovery of pectin ranged from about 75 to 96 per cent, while in the dogs which defecated but once during the absorption period the recovery ranged from 2 to 30 per cent. In other words, decomposition may be furthered if the pectin fed is retained for longer periods. In addition, the results indicate that the decomposition is carried further when pectin is added to a mixed diet than when given alone.

The results obtained with humans, when fed similar quantities of pectin with a mixed diet, did not differ from those of the dog, for here too decomposition of about 90 per cent took place. However, when pectin was fed alone the recovery from human feces averaged less than in the case of the dog, indicating that the fasting man is capable of decomposing more pectin than the fasting dog.

Our ileostomy experiments show both in the human and the dog that break down occurs chiefly in the colon and not in the upper intestine, and that bacterial enzymes are involved rather than the enzymes of the animal organism.

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A STUDY OF THE METABOLISM OF INGESTED PECTIN

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The use of an apple diet and pectin in the treatment of diarrhea raises the question of the fate of pectin in the alimentary tract and the body tissues.[1] How the apple diet or pectin exerts a favorable influence has not been elucidated. It is certain that ingested pectin is decomposed in the alimentary tract of the young adult, since only 10 per cent or less can be recovered from the feces.[2] When pectin is given to a fasting subject, however, less is decomposed; the amount decomposed is influenced, apparently, by the rate of passage through the alimentary tract and perhaps by whether the nitrogen, which is necessary to sustain the organism or organisms involved,[3] is present. The results of experiments on ileostomized human and canine subjects[2c] and the failure of the enzymes in the digestive juices to hydrolyze pectin[4] show that the decomposition of pectin occurs primarily in the colon. Since the secretion of the colon of the dog (and the cat) contains no significant concentration of enzymes,[5] and since we have found that the colonic secretion of the dog does not hydrolyze pectin, the decomposition of pectin in the colon must be due to micro-organisms.

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These observations led to a search in the literature for evidence regarding the decomposition products of pectin. When pectin is incubated with a dilute suspension of feces, it is decomposed,[6] with the production of acid.[7] The character of the acids has not been determined. According to Schneider,[2a] reducing substances are not formed when the hemicellulose of "apple marc" is decomposed by a fecal suspension. When partially purified pectin is fermented anaerobically with Granulobacter pectinovorum, butyric acid, a small amount of acetic acid and traces of carbon dioxide are found; and when it is fermented aerobically with Pectinobacter amylophilum, the chief products are carbon dioxide and water and about 2.5 per cent acetic and formic acids.[8] These results probably cannot be applied to the fermentation of pure pectin, since certain organisms which have been reported as fermenting pectin do not ferment pure pectin.[9] The pectinases of molds decompose pectin into galacturonic acid and methyl alcohol,[10] but Saunders and Kertesz[11] reported that the intestinal micro-organisms which hydrolyze pectin also ferment galacturonic acid. It has been concluded on the basis of meager evidence that galacturonic acid is formed and absorbed after the ingestion of pectin,[12] that pectin is metabolized and stored as glycogen[13] and that sufficient methyl alcohol is produced and absorbed to increase the output of formic acid in the urine.[14] However, Imhauser[15] reported that the decomposition products of pectin were not excreted and did not affect the blood sugar and the fatty changes in the liver of phloridzinized dogs, though some decrease in the urinary excretion of acetone bodies was observed.

On the basis of the foregoing observations, it was decided to examine the urine after the ingestion of pectin for galacturonic acid and to

6. Schneider,[2a] Kertesz.[4]

7. Werch, S. C.; Day, A. A.; Jung, R., and Ivy, A. C.: On the Role of Intestinal Bacteria in the Decmoposition of Pectin, Proc. Soc. Exper. Biol. & Med. **46**:569, 1941.

8. Makrinow, A. A.: Die aerobe Pektinstoffgärung, Zentralbl. f. Bakt. (Abt. 2) **85**:339, 1932.

9. McCoy, E., and Peterson, W. H.: The Use of Pectin in Fermentation Tests, J. Infect. Dis. **43**:475, 1928.

10. Kertesz, Z. I.: The Determination of Pectin Methoxylase Activity, J. Biol. Chem. **121**:589, 1937.

11. Saunders, E., and Kertesz, Z. I.: Note on the Fermentation of Pectin and Galacturonic Acid by Intestinal Bacteria, J. Nutrition **20**:289, 1940.

12. Manville, I. A.; Bradway, E. M., and McMinis, A. S.: Pectin as a Detoxication Mechanism, Am. J. Digest. Dis. & Nutrition **3**:570, 1936.

13. Fürth, V. O., and Engel, V.: Verwertung der Pentosane in Tierorganisms, Biochem. Ztschr. **237**:159, 1931.

14. Voit, K., and Friedrich, H.: Ueber das Auftreten von Ameisensäure im Harn bei der Apfeldiät, Klin. Wchnschr. **14**: 1792, 1935.

15. Imhauser, K.: Ueber das Verhalten der Pectine im Stoffwechsel, Arch. f. exper. Path. u. Pharmakol. **167**:589, 1932.

analyze both the urine and the feces for volatile fatty acids and reducing substances, it being kept in mind that these substances might be completely absorbed in the colon. Such determinations should supply data on most of the possible decomposition products of pectin. Of the volatile fatty acids, formic acid is of particular interest, since methyl alcohol is a possible product of its decomposition. Our attention was directed in large measure to the determination of galacturonic acid in the urine. Since pectin is composed of galacturonic acid, the acid is very likely a product of the decomposition of pectin; and if it is absorbed by the colon, it may appear as a galacturonate in the urine. However, before it could serve as a detoxifying agent by forming galacturonates in the liver, as was suggested by Manville, Bradway and McMinis,[15] it must be absorbed. Since it has not been shown whether it is absorbed from the small intestine or the colon, we have undertaken to obtain evidence on that point also.

METHODS

Analysis of Feces and Urine.—Six male medical students were kept on a diet low in crude fiber during a control absorption period. After the control diet had been consumed for four days, 30 Gm. of pectin in gelatin capsules was added to the diet of each subject for four days. Aliquots of feces and urine for analysis were obtained from material secured during the third and fourth days of each period. In the tables, specimen "a" represents material secured on the third day and specimen "b" that secured on the fourth day.

The pectin fed was the pure citrus variety which was used and described in our previous work[2c] and was obtained from the research laboratory of the California Fruit Growers Exchange. This product is essentially free of such impurities as pentoses, pentosans, pigment and other materials frequently found in other pectins.

The control diet consisted of the following: breakfast—200 Gm. of pineapple juice, 2 eggs, 30 Gm. of bacon, 50 Gm. of white bread and 14 Gm. of butter; dinner—90 Gm. of lamb chop, 100 Gm. of potato, 50 Gm. of white bread and 14 Gm. of butter, and supper—200 Gm. of beef steak, 120 Gm. of potato, 50 Gm. of butter and 100 Gm. of ice cream. Coffee was allowed in any quantity at all of the meals.

Ten gram aliquots of thoroughly mixed feces (wet weight), obtained immediately after passage, were used. The material was broken up in a solution containing 20 cc. of normal sulfuric acid and 70 cc. of distilled water, stored in stoppered, sterile bottles, sealed with paraffin and placed in a refrigerator until needed. During the collection of twenty-four hour specimens of urine, 1 cc. of twenty times normal sulfuric acid was added for every 500 cc. of urine. At the end of the period, 100 cc. was measured out and stored in the same way as were the samples of feces. Fresh specimens of urine were measured for volume; the time interval of passage was noted, and they were stored in 100 cc. amounts, as previously described, with 10 cc. of normal sulfuric acid, which was added to prevent bacterial action.[16]

Fresh urine was used for the estimation of galacturonic acid and free reducing substances, and the procedure was carried out the day the specimen was secured. Determinations for total hydrolyzable reducing substances and volatile acids were

16. Friedemann, T. E.: Metabolism of Pathogenic Bacteria: I. Bacteriological and Chemical Methods, J. Bact. 35:527, 1938.

made on material which had been hydrolyzed by heating in a boiling water bath for six hours with two times normal sulfuric acid. Determinations for volatile acids and total hydrolyzable reducing substances were carried out on both feces and urine, while those for galacturonic acid and free reducing substances were made only on urine. Values for uronic acid in feces were obtained by the Link method,[17] which we have used in previous studies.[20] By this method the recovery of pectin added to the feces is practically complete, as is the recovery of added galacturonic acid.

Galacturonic acid was estimated by the Kapp[18] method, reducing substances by the method of Hagedorn and Jensen[19] and the Somogyi modification[20] of the Shafer-Hartman method and volatile fatty acids by the Friedemann[21] method.

Absorption of Galacturonic Acid by the Ileum and Colon.—The galacturonic acid used was obtained, like the pectin, from the research department of the California Fruit Growers Exchange and is an enzymatic product of citrus pectin.[22] In analysis by the Link method[17] for determination of uronic acids, 21.8 per cent carbon dioxide, which is equivalent to 87.2 per cent uronic anhydride, was liberated. By the method of the Association of Official Agricultural Chemists for the determination of furfural,[23] 1 Gm. of the substance yielded 0.300 Gm. of phloroglucide.

Experiments were carried out on both human and canine subjects. Isolated loops of ileum (6 dogs) and colon (6 dogs) were studied in animals anesthetized with pentobarbital sodium. Three dogs with Thiery-Vella loops of the ileum and 1 dog with a similar loop of the colon were studied without anesthesia. Two human subjects with ileostomies were also studied without anesthesia. In all the experiments a 5 per cent solution of galacturonic acid having a Δ of -0.56 C. (isotonic and isosmotic with blood) and a p_H of 2.5 was used.

In the experiments with the anesthetized dogs the absorption period was two hours. For the unanesthetized dogs with loops of the ileum the absorption period was one hour, while for the dog with the loop of the colon it was two hours. In all of the experiments with dogs the loops were washed out with Ringer's solution before the galacturonic acid was introduced, and at the end of the absorption period the loop was drained and washed twice again, each time with 20 cc. of Ringer's solution. The latter washings were added to the fluid obtained from draining and marked for assay. The volume of the fluid drained and the final volume, after the washings were added, were noted.

The human subjects came to the laboratory in the morning without having had breakfast or drunk water. Since they had last eaten the evening before, the

17. Dickson, A. D.; [illegible], H., and Link, K. P.: The Determination of Uronic Acids, J. Am. Chem. Soc. [illegible]:775, 1930.

18. Kapp, E. M.: Microestimation of Uronic Acids, J. Biol. Chem. 134:143, 1940.

19. Hagedorn, H. E., and Jensen, B. N.: Zur Mikrobestimmung des Blutzuckers mittels Ferricyanid, Biochem. Ztschr. [illegible]:46, 1923.

20. Somogyi, M.: A Method for the Preparation of Blood Filtrates for the Determination of Sugar, J. Biol. Chem. [illegible]:655, 1930.

21. Friedemann, T. E.: The Identification and Quantitative Determination of Volatile Alcohols and Acids, J. Biol. Chem. [illegible]

22. Mottern, H. H., and Cole, [illegible]: Enzymatic Preparation of d-Galacturonic Acid, J. Am. Chem. Soc. [illegible]:701, 1939. [illegible] and Yamada, P. M.: Note on the Preparation of [illegible] 2973, 1939.

23. Official and Tentative Methods of Analysis, Association of Official Agricultural Chemists, ed. 4, Washington, D. C.: The Association [illegible]

bowel was practically empty, but a control test was performed first. They were given 200 cc. of distilled water to drink, and the value for the assay of the material in the ileostomy bags, obtained at the end of two hours, was subtracted from that for the assay carried out on material obtained at the end of a similar period after 200 cc. of the acid had been consumed.

All the assays included analyses by the previously mentioned methods of Link[17] and the Association of Official Agricultural Chemists.[28]

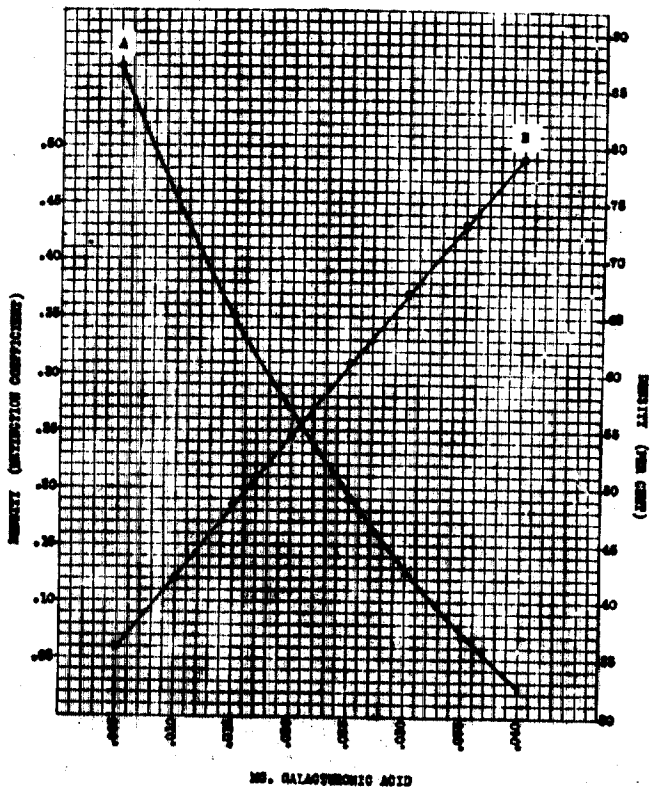

FIGURE 1.

Percentage (*A*) and extinction coefficient (*B*) curves for the recovery of galacturonic acid added to human urine.

RESULTS

Applicability of the Kapp Method for the Quantitative Recovery of Galacturonic Acid.—Since Kapp[18] did not include data about the quantitative recovery of pure galacturonic acid added to human urine, it was necessary for us to obtain such data and to test the method in our hands.

Normal human urine was assayed for galacturonic acid, and none was found. Pure galacturonic acid was added to the urine in quantities ranging from 5 to 40 micrograms for each 3.5 cc. An average recovery of 96.2 per cent of the added galacturonic acid was obtained. The averages of the data obtained in this work are given in table 1, and the corrected recovery curve is presented in the figure. (Kapp's article should be consulted for the details used in the calculations.)

Excretion of Galacturonic Acid and Reducing Substances in the Urine and Feces With and Without Pectin in the Diet.—The data on galacturonic acid in the urine and those on reducing substances in both the urine and the feces are recorded in table 2. No galacturonic acid was found in the urine of the subjects during either of the two absorption periods; no trace of color developed with the reagent. It should

TABLE 1.—*Recovery of Galacturonic Acid Added to Water and to Human Urine*

Galacturonic Acid Added to Water and Urine, Micrograms	Water				Human Urine			
	Stufenphotometer Reading		Recovery		Stufenphotometer Reading		Recovery	
	Percentage	Logarithm	Amount	Percentage	Percentage	Logarithm	Amount	Percentage
5	87	0.06	5	100	88.0	0.055	4.5	90.0
10	76	0.12	10	100	77.0	0.115	9.5	95.0
15	65	0.185	15	100	67.0	0.175	14.5	97.0
20	57	0.245	20	100	57.5	0.24	19.5	97.5
25	50	0.32	25	100	51.0	0.29	24.0	97.5
30	[illegible]	0.37	30	100	45.0	0.340	28.0	93.0
35	37	0.43	35	100	40.0	0.40	32.5	93.0
40	[illegible]	0.49	40	100	34.5	0.455	35.0	94.0
Average recovery				100				96.2

be recalled, however, that the Kapp method quantitatively determines galacturonic acid only in quantities ranging from 5 to 40 micrograms per 3.5 cc. of urine. If the twenty-four hour excretion of urine consisted of 1,200 cc. of urine and it contained 7 micrograms of galacturonic acid per 3.5 cc., to be measurable 2.4 mg. of galacturonic acid would need to be excreted. It should be added that the method employed is of such a nature that galacturonic acid is detected in the form of galacturonate. The total amount of reducing substances after hydrolysis while the subject consumed the diet that ranged from 380 to 1,240 mg. per hundred grams of feces had averaged 740 mg., while during the period that pectin was consumed the range was 220 to 930 mg. per hundred grams and the average was 570 mg.

The data show that as a result of the ingestion of 30 gm. of pectin daily with a mixed diet no detectable increase in galacturonic acid and reducing substances in the urine occurred. This raises the question whether galacturonic acid is absorbed from the small intestine and the colon. This question will be considered later.

No significant difference was observed in the urinary excretion of free reducing substances. When calculated on the basis of 100 cc. samples and twenty-four hour volumes, the averages were 51.6 and 521 mg., respectively, during the period that the basal diet was consumed and 50 and 516 mg. during that in which pectin was consumed. The total amount of reducing substances in the urine after hydrolysis averaged

TABLE 2.—*Excretion of Galacturonic Acid and Reducing Substances in the Urine and Feces of Subjects Consuming the Basal Diet With and Without the Addition of Pectin*

		Urine							Feces,
				Free Reducing Substances		Total of Reducing Substances After Hydrolysis		Hydrolyzable Reducing Substances in 24 Hour Specimen, Mg.	Total of Reducing Substances After Hydrolysis, Mg. per 100 Gm.
Subject	Specimen Designation	Volume of 24 Hour Specimen, Cc	Galacturonic Acid in 24 Hour Specimen, Mg.	Mg. per 100 Cc.	Mg. in 24 Hour Specimen	Mg. per 100 Cc.	Mg. in 24 Hour Specimen		
				Basal Diet					
1	a	1,340	0	..	...	382	5,119	.....	380
	b	1,500	0	..	...	...	.....	.....	570
2	a	1,190	0	..	...	274	3,261	.....	1,240
	b	1,170	0	..	...	253	2,900	.....	750
3	a	1,200	0	46	557	336	4,036	3,509	560
	b	1,130	0	49	538	282	3,187	2,624	780
4	a	980	0	51	500	292	2,862	2,362	820
	b	870	0	56	495	250	2,217	1,722	740
5	a	1,120	0	44	498	264	2,957	2,464	680
	b	1,080	0	46	497	282	3,046	2,549	720
6	a	900	0	58	522	336	3,024	2,502	820
	b	880	0	63	554	340	2,992	2,438	780
Average			0	51.6	521	300	3,245	2,522	740
				Pectin and Basal Diet					
1	a	1,273	0	..	...	361	4,596	.....	750
	b	1,254	0	..	...	350	4,389	.....	930
2	a	1,023	0	..	...	376	3,846	.....	680
	b	1,080	0	..	...	333	3,596	.....	220
3	a	1,300	0	41	533	308	4,189	3,631	680
	b	1,200	0	49	588	318	3,716	3,128	680
4	a	950	0	58	554	343	3,000	2,500	760
	b	800	0	61	495	292	2,336	1,848	720
5	a	1,220	0	41	500	238	2,904	2,404	680
	b	980	0	49	480	300	2,940	2,460	720
6	a	910	0	58	510	306	2,967	2,457	840
	b	940	0	58	498	318	2,989	2,491	860
Average			0	50.0	516	313	3,456	2,615	770

300 mg. per hundred cubic centimeters and 3,245 mg. per twenty-four hour volume while the subjects were on the basal diet and 313 mg. per hundred cubic centimeters and 3,456 mg. per twenty-four hour specimen while they consumed the pectin-containing diet. The amount of hydrolyzable reducing substances, on the basis of twenty-four hour excretion, was calculated by subtracting the value for free reducing substances from that for the total amount of reducing substances after hydrolysis. The

average value while the basal diet was consumed was 2,522 mg. and that for the period when the pectin-containing diet was used was 2,615 mg.

Excretion of Volatile Acids in the Urine With and Without Pectin in the Diet. Table 3 provides the data on the excretion of volatile acids in the urine with and without pectin in the diet. The values for formic acid, in terms of cubic centimeters of tenth normal alkali per twenty-four

TABLE 3.—*Excretion of Volatile Acids in the Urine of Subjects Consuming the Basal Diet With and Without the Addition of Pectin*

Subject	Specimen Designation	Volume of 24 Hour Specimen, Cc.	First Distillation, Cc. of N/100 Alkali: Per 100 Cc.	First Distillation, Cc. of N/100 Alkali: Per 24 Hour Specimen	Second Distillation, Cc. of N/100 Alkali: Per 100 Cc.	Second Distillation, Cc. of N/100 Alkali: Per 24 Hour Specimen	Formic Acid,* Cc. of N/10 Alkali per 24 Hour Specimen	Acetic Acid, Cc. of N/10 Alkali per 24 Hour Specimen
				Basal Diet				
1	a	1,340	71.2	954	39.6	531	42	63
	b	1,500	50.8	852	51.2	765	8	77
2	a	1,190	93.4	1,111	68.6	821	29	82
	b	1,150	32.6	381	26.0	294	9	29
3	a	1,210	29.8	361	16.6	206	15	21
	b	1,130	45.6	515	27.6	316	20	32
4	a	980	26.4	259	17.2	167	9	17
	b	870	42.8	372	21.2	185	19	18
5	a	1,120	41.6	466	35.2	394	7	39
	b	1,080	54.0	583	44.8	483	10	48
6	a	900	50.8	459	40.0	360	10	36
	b	880	61.6	546	54.0	475	7	48
Average			50.[illegible]	572	36.8	416	15	42
				Pectin and Basic Diet				
1	a	1,275	51.0	649	42.7	547	10	55
	b	1,254	60.3	752	53.4	690	6	60
2	a	1,023	74.8	767	65.2	665	10	67
	b	1,080	93.4	1,007	55.0	594	41	59
3	a	1,360	28.0	381	20.0	272	11	27
	b	1,200	40.0	480	31.4	372	11	37
4	a	950	35.2	333	23.2	219	11	22
	b	800	36.4	288	22.4	176	11	18
5	a	1,220	54.8	669	48.8	594	7	59
	b	980	59.6	584	48.8	478	8	49
6	a	900	64.8	580	59.6	542	5	54
	b	940	61.2	575	52.0	489	9	49
Average			55.0	590	43.7	470	12	47

* The values for formic acid are not absolute, since the analytic method used does not exclude all of any pyruvic acid which may be present.

hour specimen, were obtained by subtracting the results on the second distillation from those of the first distillation. The value for acetic acid is also indicated in terms of cubic centimeters of tenth normal alkali per twenty-four hour specimen. The values obtained from the second distillation were used. The average values for the excretion of formic acid and acetic acid in the samples from the 6 subjects while they were on the basal diet were 15 and 42 cc., respectively, while average values of 12 and 47 cc. were recorded for the period during which they consumed the test diet containing pectin.

It should be pointed out that the values for formic acid are not absolute since the analytic method used does not exclude all of any pyruvic acid which may be present.[21]

The results show that the presence of pectin in the diet does not significantly increase the excretion of formic and acetic acids in the urine.

Excretion of Volatile Acids in the Feces With and Without Pectin in the Diet.—The values obtained for the excretion of volatile acids in the feces before and after pectin was added to it are shown in table 4. The results for the excretion of formic and acetic acids presented in this table were calculated in the manner previously indicated for the excretion

TABLE 4.—*Excretion of Volatile Acids in the Feces of Subjects Consuming the Basal Diet With and Without the Addition of Pectin*

Subject	Specimen Designation	First Distillation, Cc. of N/100 Alkali per 100 Gm.		Second Distillation, Cc. of N/100 Alkali Per 100 Gm.		Formic Acid,* Cc. of N/10 Alkali per 100 Gm.		Acetic Acid, Cc. of N/10 Alkali per 100 Gm.	
		Basal Diet	Pectin and Basal Diet	Basal Diet	Pectin and Basal Diet	Basal Diet	Pectin and Basal Diet	Basal Diet	Pectin and Basal Diet
1	a	1,920	1,920	1,840	1,800	8	12	184	180
	b	2,040	1,920	1,920	1,400	12	12	192	140
2	a	1,320	1,520	1,240	1,440	8	8	124	114
	b	1,240	1,560	1,160	1,400	8	16	116	140
3	a	1,200	880	1,160	840	4	4	116	84
	b	720	1,240	600	1,200	12	4	60	120
4	a	1,260	1,800	1,200	1,720	6	8	120	172
	b	960	1,680	920	1,560	4	12	92	156
5	a	1,024	900	992	836	3	6	99	84
	b	896	984	832	920	6	6	83	84
6	a	952	992	892	908	6	8	89	91
	b	1,020	1,048	956	992	6	6	96	99
Average		1,218	1,305	1,164	1,251	7	8.5	114	127

* The values for formic acid are not absolute, since the analytic method used does not exclude all of any pyruvic acid which may be present

of these substances in the urine, but they are given in terms of cubic centimeters of tenth normal alkali per hundred grams of feces. For the periods in which the basal diet and the pectin-containing diet were consumed, the average excretion of formic acid were 7 and 8.5 cc. of tenth normal alkali, respectively, and for the excretion of acetic acid the values were 114 and 127 cc.

These results indicate a tendency toward an increase in the excretion of volatile acids in the feces after pectin is ingested, but the tendency is not sufficiently pronounced to be definitely significant. It should be explained that these results hold only for the addition of pectin to a mixed diet and do not necessarily apply to the administration of pectin alone or to the effect of administration of pectin in the presence of [illegible] or in the absence of protein from the diet, conditions which affect the flora of the colon.

Absorption of Galacturonic Acid from the Small Intestine and the Colon.—If galacturonic acid is absorbed from the small or the large intestine it should appear in the urine as a galacturonate, unless it is oxidized in the tissues. Our failure to find measurable quantities of galacturonic acid in the urine caused us to ascertain whether it is absorbed.

The recovery of galacturonic acid from the ileum and that from the colon after a two hour absorption period in the case of the anesthetized animals were practically the same (table 5). Recovery from the ileum was 93 per cent by both the phloroglucide and the uronic acid method, while recovery from the colon was 91 per cent by the phloroglucide method and 94 per cent by the uronic acid method. In these experiments

TABLE 5.—*Recovery of Galacturonic Acid from the Ileums and from the Colons of Anesthetized Dogs*

Experiment	Loop	Volume of Fluid Introduced, Cc.	Volume of Fluid Drained, Cc.	Final Volume, Cc.	Recovery of Galacturonic Acid: By Phloroglucide Method, Grams	By Phloroglucide Method, Percentage	By Uronic Acid Method, Grams	By Uronic Acid Method, Percentage
1	Ileum	50	56	96	0.665	88	0.595	92
2	Ileum	50	46	92	0.695	93	0.515	95
3	Ileum	50	52	90	0.720	96	0.515	94
4	Ileum	50	60	96	0.700	93	0.510	94
5	Ileum	50	52	90	0.675	90	0.495	91
6	Ileum	50	56	92	0.700	93	0.525	96
Average					0.692	93	0.511	93
1	Colon	50	48	84	0.705	94	0.520	95
2	Colon	50	46	80	0.690	92	0.530	97
3	Colon	50	48	82	0.665	88	0.490	90
4	Colon	50	50	90	0.695	89	0.510	94
5	Colon	50	50	86	0.670	89	0.490	90
6	Colon	50	46	80	0.710	95	0.520	95
Average					0.684	91	0.510	94

the volume of fluid drained at the end of the test period was practically the same as that which was introduced. Recovery of galacturonic acid from the ileum and that from the colon in the case of the unanesthetized dogs with Thiry-Vella loops were also much the same (table 6). Recovery from the ileum was 90 per cent by the phloroglucide method and 94 per cent by the uronic acid method. The volume of fluid drained from the ileum in these experiments was greater in every instance than that introduced, while the volume of fluid drained from the colon was smaller than that introduced.

From the loops of human subjects who had undergone ileostomy the average recovery of galacturonic acid was 91 per cent by the phloroglucide method and 95 per cent by the uronic acid method. The volumes of fluid in the ileostomy bags obtained in all of the control experiments

were less than those obtained in all of the test experiments. The control volumes (table 7) for experiments 1a and 1b were 50 and 60 cc., respectively, while for experiments 2a and 2b they were 85 and 140 cc., respectively.

TABLE 6.—*Recovery of Galacturonic Acid from the Ileums of Three Unanesthetized Dogs and from the Colon of One*

Dog	Experiment	Location of Thiery-Vella Loop	Volume of Fluid Introduced, Cc.	Volume of Fluid Drained, Cc.	Final Volume, Cc.	Recovery of Galacturonic Acid: By Phloroglucide Method, Grams	By Phloroglucide Method, Percentage	By Uronic Acid Method, Grams	By Uronic Acid Method, Percentage
1	a	Ileum	25	35	90	0.315	84	0.250	92
	b	Ileum	25	40	75	0.340	90	0.265	96
	c	Ileum	25	32	70	0.350	93	0.260	95
Average						0.335	90	0.256	95
2	a	Ileum	50	65	100	0.650	87	0.520	96
	b	Ileum	50	60	95	0.665	89	0.520	96
	c	Ileum	50	58	95	0.665	89	0.500	92
Average						0.660	88	0.513	95
3	a	Ileum	40	50	88	0.520	88	0.405	93
	b	Ileum	40	55	90	0.540	90	0.425	97
	c	Ileum	40	60	100	0.550	92	0.410	94
Average						0.540	90	0.410	94
Average for the 3 dogs						.....	89	.....	94
1	a	Colon	50	30	65	0.630	86	0.485	89
	b	Colon	50	35	70	0.665	89	0.505	93
	c	Colon	50	35	60	0.690	92	0.515	95
	d	Colon	50	30	70	0.630	84	0.490	90
	e	Colon	50	30	65	0.620	82	0.470	86
	f	Colon	50	40	80	0.680	91	0.530	97
Average						0.685	87	0.500	92

TABLE 7.—*Recovery of Galacturonic Acid from Human Subjects Who Had Undergone Ileostomy*

Subject	Experiment	Volume of Fluid Given, Cc.	Volume of Fluid Recovered, Cc.	Recovery of Galacturonic Acid: By Phloroglucide Method, Grams	By Phloroglucide Method, Percentage	By Uronic Acid Method, Grams	By Uronic Acid Method, Percentage
1	a	200	340	2.85	95	2.05	95
	b	200	410	2.75	91	2.10	95
2	a	200	360	2.65	89	2.08	92
	b	200	600	2.80	94	2.10	96
Average				2.76	91	2.08	95

Since in all of the experiments the average values for recovery were practically 90 per cent or more and since the difference between these values and 100 per cent recovery may be accounted for by the errors inherent in the methods employed, we concluded that if galacturonic acid was absorbed at all, the amount was very small.

COMMENT

The results show that the addition of 30 Gm. of pectin to a mixed diet did not significantly increase the fecal and urinary excretion of volatile acids, particularly formic and acetic acids. Such volatile acids as are produced during the decomposition of pectin in the colon are to a large extent oxidized either in the colon or in the tissues. Our data on the excretion of volatile acids in the urine confirm the unpublished data of Dr. T. E. Friedemann, whose method we used. He observed, as did we, that the excretion of acetic acid varies considerably in different subjects, while the excretion of formic acid is more constant.

The formation of methyl alcohol from pectin in the colon is possible. If methyl alcohol is found in appreciable quantities, most of it must be exhaled or otherwise disposed of, since appreciable increase in the excretion of formic acid in the urine was not observed when we administered pure citrus pectin. Voit and Friedrich[14] fed a pectin preparation called aplona and observed a slight increase in the excretion of formic acid in the urine. Apparently, they performed only 1 experiment on 1 subject, and when our values for the excretion of formic acid are converted into milligrams and compared with their results, the small increase observed by them falls within the range of daily variation observed for our subjects. The difference in results, however, may be due to the difference in the product used.

Our results show that galacturonic acid is not absorbed in significant amounts from the small intestine of the canine or the human subject or from the colon of the dog. This fact and our failure to find galacturonic acid in the urine in the presence of pectin in the diet shows that pectin, by providing galacturonic acid, cannot favor the detoxicatory function of the liver, by which certain phenolic compounds are known to be conjugated with glucuronic acid. Malyoth[15] stated, without giving data or methods, that a high percentage of galacturonic acid given to animals can be recovered in the feces and that galacturonic acid given parenterally is found unconjugated in the urine.

Our results, as well as those of Kertesz, Walker and McCoy,[24] strongly indicate that one must study the local effects of apple and pectin in the alimentary tract to discover how their favorable effect on diarrhea is exerted. The cellulose and lignin in apples probably act as absorbing colloids, or mechanically, since they are not appreciably decomposed. Pectin, which is decomposed only in the colon, could act similarly in the small intestine but not in the colon, unless, as in the fasting dog, a considerable amount of the pectin is not decomposed. It is possible that the decomposition of pectin produces [illegible]

24. Kertesz, [illegible] I.; Walker, M. S., and McCoy, C. M.: The Effect of Feeding Apple Sauce on Induced Diarrhea in Dogs, Am. J. Diseases Dis. [illegible], [illegible]

medium for the organisms causing diarrhea. The offending organisms may actually be destroyed by the acids produced, or when the diet of the patient is chiefly apple or pectin, they may be starved out or be overgrown by organisms which use pectin as a source of food.

CONCLUSIONS

The decomposition of citrus pectin in the colon in young men on a mixed diet does not significantly change the excretion of formic acid, acetic acid, which are volatile acids, and reducing substances in the urine and the feces.

Pectin is not excreted in the urine as galacturonates in quantities which can be measured by the micromethod of Kapp.

Galacturonic acid is not absorbed in appreciable quantities from the human and canine small intestine and colon. Hence, the favorable effects of a diet containing apple or pectin in the treatment of diarrhea cannot be ascribed to an improvement of the detoxicatory function of the liver as a result of the absorption of galacturonic acid. The favorable effects, according to our evidence, are exerted exogenously in the lumen of the bowel.

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THE DECOMPOSITION OF PECTIN AND GALACTURONIC ACID BY INTESTINAL BACTERIA

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Interest in the fate of ingested pectin has been instigated by the use of the apple diet in the treatment of diarrhea.[1]

Ingested pectin has been shown to be decomposed in the human and dog alimentary tracts, since only 10% or less can be recovered in the feces.[2,3,4] When given to a fasting subject, however, less is decomposed; the amount depending apparently on the rate of alimentary passage, and perhaps on the lack of nitrogen necessary to sustain the organism or organisms involved.[4,5] The results of experiments on iliostomized and canine subjects,[4] and the failure of the enzymes in the digestive juices to hydrolyze pectin,[6] show that the decomposition of pectin occurs primarily in the colon. Since the secretions of the colon of the dog and cat contain no significant concentration of enzymes,[7] the decomposition of pectin in the colon must be due to microorganisms. This deduction has been made in a previous study[4] but lacks the support of a thorough bacteriological analysis.

The bacterial fermentation of pectin in nature, associated with the retting process, is well known. We have interested ourselves in the process, because similar decomposition may take place in the animal organism. Since extensive reviews on this subject may be found in the literature, we will deal with it here only if it may bear on pectin decomposition in the gastro-intestinal tract.

Schneider[2] was one of the first to study the digestion of pectin by fecal bacteria. He found that mixtures of intestinal bacteria from fresh human feces destroy the hemicellulose of apple marc. Upon determining the amount of reducing sugar present, he found that 66.4% of the sugar-reacting radicals were destroyed. Results obtained with a pure culture of Bacillus coli communis indicated that this organism was neither the chief nor an important factor. Kertesz[8] made suspensions of human and dog feces and incubated large aliquots with pectin (50 cc. of suspension with 25 cc. of 1.25% pectin solution). He also found decomposition to take place, and that it even occurred in the presence of toluol. He used the calcium pectate method to determine decomposition. Werch, Day, Jung and Ivy[8] also found that suspensions of human and canine feces ferment pectin. They evaluated the breakdown by determination of pH and titratable acidity, pectate formation and relative viscosity. Coles[9] reported that pectin was not decomposed by the colon-typhoid group of organisms commonly

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1. American Medical Association Council on Foods, J.A.M.A. **109:** 1636, 1937; Malyoth, G.: Klin. Wchnschr. **13:** 51, 1934.
2. Schneider, E. C.: Am. J. Physiol. **30:** 258, 1912.
3. Ehrlich, F., Imhauser, K., and Voit, K.: Schl. Gesel. f. Vaterlande. **103:** 104, 1930-31.
4. Werch, S. C., and Ivy, A. C.: Proc. Soc. Exper. Biol. & Med. **44:** 366, 1940; Am. J. Digest. Dis. & Nutrition **8:** 101, 1941.
5. Murer, H. K., and Crandall, L. A.: Am. J. Physiol. **129:** 427, 1940.
6. Kertesz, Z. I.: J. Nutrition **20:** 289, 1940.
7. Wright, R. D., Flarey, H. W., and Jennings, M. A.: Quart. J. Exper. Physiol. **28:** 207, 1938.
8. Proc. Soc. Exper. Biol. & Med. **46:** 569, 1941.
9. Plant Physiol. **1:** 379, 1926.

found in feces. He concluded that only bacteria occurring in the soil are capable of attacking pectin. Baker and Martin[10] studied the iodophile microflora of the cecum of the rabbit with special regard to the disintegration of cell wall plant substances, and described a number of the bacteria involved. The related work of McCoy and Peterson[11] is also of interest. They found that laboratory cultures of retting organisms may not ferment pure pectin under artificial conditions, and that pectin must be of known purity in order to avoid false reactions.

The possible products of bacterial decomposition of pectin have also been considered briefly. Schneider[2] concluded that reducing sugars are not formed when the hemicellulose of apple marc is decomposed by a fecal suspension. Fecal decomposition of pectin was found to be associated with the production of acid by Werch, Day, Jung and Ivy.[8] Makrinov[12] reported that, when partially purified pectin was fermented anaerobically with Granulobactor pectinovorum, butyric acid, a small amount of acetic acid and traces of carbon dioxide were found; and when fermented aerobically with Pectinobacter amylophilum the chief products are carbon dioxide and water, and about 2.5% acetic and formic acids. Kertesz[9] found that pectinases of molds decomposed pectin into galacturonic acid and methyl alcohol. The production of galacturonic acid from pectin in the alimentary tract was presumed to occur on the basis of meager evidence by Manville, Bradway and McMinn.[13] Voit and Friedrich[14] concluded that sufficient methyl alcohol is produced from "aplons" in the intestine of man and absorbed to increase the formic acid output in the urine. We were unable to confirm this when 30 gm. of pure citrus pectin was fed daily to human subjects on a mixed diet. No increase in volatile acids and galacturonic acid in the urine and feces was observed.

Our previous work[8] dealt with the preparation of a sterile pectin-broth medium, and showed that suspensions of human and canine feces are capable of digesting pure citrus pectin with the production of acid. Here we wish to present our work on the isolation and identification of the responsible bacteria, the enzymes involved, the products of decomposition, and more thoroughly the various pectin mediums used.

GENERAL METHODS

Pectin-containing medium.—The pectin used was the pure citrus variety employed in our previous work, and was obtained from the research department of the California Fruit Growers Exchange. This product is essentially free of such impurities as pentoses, pentosans, pigment and other materials frequently found in other pectins.

Before much work was done it became evident that a liquid, pectin-containing medium was necessary, and had to conform to the following requirements (a) that the pectin be unchanged, (b) that the *p*H of the medium be readily set and adjusted within the range where pectin is relatively stable for the period of incubation, and (c) that it be easily sterilized.

Heat or steam distillation resulted in decomposition of pectin, with acid production, lowered viscosity, and reduction of calcium pectate formation. Chemical sterilization would also alter its properties, besides complicating any chemical analysis, and thus was not [illegible] sufficient.

10. Zentralbl. f. Bakt. (Abt. 2) 96: 18, 1935.
11. J. Infect. Dis. 43: 475, 1928.
12. Zentralbl. f. Bakt. (Abt. 2) 85: 339, 1932.
13. Am. J. Digest. Dis. & Nutrition 3: 570, 1936.
14. Klin. Wchnschr. 14: 1790, 1935.

Pectin powder could not be sterilized after 2 weeks of desiccation. Berkefeld filtration proved satisfactory when certain precautions were observed. These precautions were included in a previous paper.[8] Lately we have found it better to make a water solution of pectin and to adjust the *p*H with normal alkali. Contamination is minimized by lengthening the arm of the suction flask, to accommodate a large quantity of cotton filter, and by adding a rubber diaphragm to its neck. This diaphragm is placed between the rubber stopper and mantle, after its center is pierced by the metal end of the candle. It is then folded over the neck of the suction flask and tied.

A culture medium containing more than 1% of pectin can not be filtered successfully. If a greater percentage of pectin is included, filtration does not occur or is prolonged to the point where contamination can be expected even after every precaution is observed. Before use the medium is best incubated for at least 48 hours, in the flask in which it is to be used, for contaminating organisms often grow very slowly.

In our recent work we have used a medium, which for brevity we have designated "pectone," made up to contain 1% each of pectin and peptone and 0.5% salt. To make up 2 liters of the medium, 20 gm. of pectin is dissolved in a liter of distilled water with the aid of an electric stirring machine, and then mixed with a liter of water solution of 20 gm. of peptone and 10 gm. of NaCl. This mixture is then adjusted to *p*H 7.2 with normal alkali and sterilized by Berkefeld filtration.

Other liquid mediums used included plain broth, dextrose (0.1% dextrose), pectone containing 1% and 0.1% dextrose, thioglycollate broth (Brewer), thioglycollate broth plus pectone added in equal volumes, and milk.

Various types of solid medium containing an indicator and pectin were tried in the isolation of the pectin fermenting organisms. These plate mediums included the following combinations: (a) an agar medium containing Andrade's indicator and 0.5% pectin. The pectin was made up separately in water (1%), adjusted to *p*H 7.2, and filtered through a Berkefeld filter, and equal parts added aseptically to sterile, melted agar made with sugar-free broth; (b) eosin-methylene blue agar plus pectin instead of lactose; (c) modified Endo's medium containing pectin instead of lactose, and water and peptone in place of broth.

Galacturonic acid-containing medium.—The pure galacturonic acid used was obtained from the research department of the California Fruit Growers Exchange, and is an enzymatic product of citrous pectin.[15,16]

The galacturonic acid containing medium was made like the pectone, except that the acid was substituted for the pectin. The final content of the acid, like the pectin, was 1%. This medium, for brevity, we designated "galactone."

Physical and chemical methods.—Hydrogen ion concentration and titratable acidity determinations were made in our study on the decomposition products of pectin, and in the isolation of the responsible bacteria. Titratable acidity was determined with 0.1 NaOH and phenolphthalein as indicator, using 10 cc. of the unknown fermentable material. Determinations of *p*H were made with the glass electrode.

Determination of viscosity may be considered of some importance in any work in which pectin is used, for the degree of viscosity is a rough measure

15. Mottern, H. H., and Cole, H. L.: J. Am. Chem. Soc. **61**: 2701, 1939.

16. Manville, I. A., Reithel, F. J., and Yamada, P. M.: J. Am. Chem. Soc. **61**: 2973, 1939.

of the amount of pectin present.[6] Since small changes are associated with long periods of incubation or sterilization (e.g., heat or steam), it can not by itself be significant when pectin decomposition is in consideration. We determined the relative viscosity of our fluids by observing the time necessary to pass through a 10 cc. pipette, to the lower end of which a capillary tube had been fused; distilled water passed through in 30 seconds at a room temperature of 30 C.

Calcium pectate formation depends on an intact pectin molecule or the presence of pectic acid. This gravimetric method[17] is sufficiently accurate to determine pectin breakdown beyond the pectic acid stage. Since some change in pectin structure is associated with the production of pectic acid from pectin, a more delicate measure is needed to evaluate such an alteration. We used this method in our study of the products of pectin decomposition, and in our work on the isolation of the bacteria concerned.

Galacturonic acid was determined when we studied the decomposition products of pectin, and was carried out by the Kapp method.[18] This method can be used with reasonable accuracy when small amounts of the acid are present (in a range of 5 to 40 micrograms in 3.5 cc. of fluid). This determination was made the day on which the designated incubation period terminated. This is also true of the other tests mentioned above.

Free reducing substances, reducing substances after hydrolysis and volatile acids were determined to ascertain the decomposition products of pectin and galacutonic acid. The first 2 determinations were made by the Somogyi modification[19] of the Shafer-Hartman method, while volatile acid determinations were carried out by the Friedemann method.[20]

Reducing substances after hydrolysis and volatile acids were carried out on fermented fluid which had its bacterial action stopped.[21] This was done by introducing 100 cc. of fermenting fluid in a narrow necked bottle containing 10 cc. of normal sulfuric acid. The bottle was then stoppered, sealed with paraffin, and placed in a refrigerator until needed. Hydrolysis was carried out by heating for 6 hours in a boiling water bath with 2 normal sulfuric acid.

RESULTS

Isolation and identification of active organisms. Attempts to isolate the active organisms were made on the solid mediums described above. This was done under aerobic and anaerobic conditions. The purpose in using such mediums was that we wished (a) to discourage the growth of all bacteria except those able to use pectin, and (b) to produce colored colonies by the acid formed from the decomposition of pectin. Pure cultures obtained in this manner were then inoculated into pectone, or a pectone-containing medium, which after a period of incubation was subjected to the pectate test. Since some reduction in pectate formation occurs even in uninoculated controls, controls are necessary for comparison. Reduction in pectate formation proved to be, in our hands, ample evidence of pectin decomposition.

For the purpose just indicated we did not always find it necessary to carry the pectate test to completion. When comparable volumes of fermenting fluids and controls were tested the state of the gel was sufficiently indicative to tell us whether partial or complete liquefaction (absence of gel) had taken place. In

17. Joseph, G. H.: Personal communication.
18. J. Biol. Chem. 134: 1[illegible]3, 1940.
19. J. Biol. Chem. 86: 655, 1930.
20. J. Biol. Chem. 123: 161, 1938.
21. J. Bact. 35: 527, 1938.

some instances the gel took on the appearance of a coagulum. Bacteria causing such gel were designated "coagulating organisms." The reaction of the fermenting fluids with a mixture of bacteria, as manifested by pH and titratable acidity, was indicative only in association with diminished pectate formation. A neutral or alkaline reaction could mean that a primary acid reaction had been neutralized by an overgrowth of alkali producing organisms, while an acid reaction could be caused by acid formation from the more readily available carbohydrate of the medium.

It was also considered of interest to determine if pectin-fermenting bacteria could be isolated from the various levels of the alimentary tract. Three dogs were used. They were fed nothing but pectin (20 gm. per day in 500 cc. of water, by stomach tube) for a week. At the end of this period they were anesthetized with nembutal, and operated to expose the levels of the intestinal tract. Material was obtained by first clamping off the loop of bowel in consideration, washing the exterior with alcohol, introducing 15 cc. of sterile water with a sterile syringe, which was then withdrawn after a few minutes and introduced into a small sterile flask. Direct smears and cultures, on aerobic blood agar plates and brewer's thioglycollate broth, were made at once from the water suspension. Table 1 contains a list of the active organisms isolated from such material, and shows their degree of activity in different pectin-containing mediums. These water suspensions were also subjected to Berkefeld filtration. Our work with such filtrates is discussed under the enzymes involved.

TABLE 1.—*Effect on Pectin of Bacteria Isolated from Dog's Intestine*

Bacterium			Medium			
Description	Strain No.	Source	Pectone	0.1% Dextrose Pectone	0.1% Dextrose Pectone	Thioglycollate Pectone
Spore-rod	1	Stomach	Coagulation*	30%**	10%	60%
Spore-rod	2	Ileum	Negative*	40%	Negative	
Streptococcus	3	Stomach	No growth	50%	10%	
Gram-positive yellow coccus	4	Ileum	Coagulation	60%	Coagulation	
Gram-positive oval diplococcus	5	Cecum	No growth		10%	
Gram-positive bacillus	6	Colon	Coagulation	40%		
Slender gram-positive bacillus	7	Rectum	No growth	Negative*	Negative*	80%
Slender gram-positive bacillus	8	Stomach	No growth	Negative*		90%
Slender gram-positive bacillus	9	Duodenum	No growth	10% moderate growth	90%	90%
Slender gram-positive bacillus	10	Jejunum	No growth	30%*		60%
B. coli	11	Colon	Coagulation			

The incubation period of all cultures was 2 weeks.
* Slight growth.
** Percentage of decomposition.

The first source of material was canine feces. Feces representing both a normal diet and one to which pectin had been added did not prove fruitful, even when the serial culture of suspensions in pectin-broth medium was carried out before plating. Feces from a dog fed pectin alone (by stomach tube) for a week proved fruitful. A single active organism was obtained. It appeared within 48 hours in a plain broth culture of the feces emulsion, and was isolated under anaerobic conditions from an Andrade medium containing lactose. Since this procedure proved successful, further steps towards the isolation of an active organism from feces were not taken.

Work carried out with combinations of various strains isolated in pure culture, with the purpose of studying possible synergistic action was unsuccessful except in one instance. The organisms

[illegible] were a gram-positive yellow coccus from the upper ileum and a gram-positive, oval diplococcus from the colon. Together these organisms were capable of decomposing pectin, within a given incubation period, to a greater extent than the sum of the two acting alone.

The possibility was suggested that acid formed from the more readily available carbohydrate in pectone might be the active agent in the decomposition of pectin. To test this supposition the following experiment was done: The spore-bearing rod isolated from the dog was inoculated into 200 cc. of 0.1% dextrose broth and incubated at 37 C. Determinations of *pH* were then made at frequent intervals to ascertain the point of greatest acidity. The lowest *pH* reached was 5.0, and occurred at the end of 24 hours, after which it slowly rose, at the end of 6 days it was still acid *pH* 6.2. Material at the end of the 24-hour incubation period was taken and used to inoculate pectone containing 0.5% phenol. At the end of 2 weeks of incubation the medium was subjected to the pectate test. No decomposition was observed.

We undertook to identify only the more active organisms.

Organism 1, the spore-bearing rod, appeared to belong to the Aerobacillus group. A Gram stained by Gram's method showed the organism to be a gram-negative, long, slender bacillus. However, some of the organism at times stained purple while the rest was pink. The purple staining portion appeared larger than the rest of the organism and often was seen separately as the small gram-positive body. One also [illegible] terminal enlargements, which took the spore stain of Moeller. Morphologically this organism looked like Bacillus cellulosae dissolvens of Khonvine. These spores were present even in dextrose broth, in which the organism grew readily. Of the sugars tested, dextrose, lactose, saccharose, mannitol, maltose and salicin were fermented with acid and gas production. Rhamnose was not affected. The organism was a microaerophile, growing very sparsely on surface medium. In dextrose broth it grew equally well at room temperature, at 37 C. and at 42 C. It had no effect on potato. There was slight hemolysis on blood agar plates. There was no growth in dextrose broth at *pH* 4.4, 8.0 or 8.5, but growth was profuse at *pH* 5.6 and good at *pH* 7.0. It also grew well in 2% urea broth. A few motile forms could be seen in a 24 hour dextrose broth culture. Brom-cresol-purple milk did not seem to be affected, although growth occurred. All cultural tests were observed for at least 2 weeks.

The slender gram-positive bacillus, no. 7, has been placed in the Lactobacillus group. It is also microaerophilic. Of the carbohydrates tested it fermented dextrose, lactose, saccharose, maltose and salicin. No gas was produced. Mannitol and rhamnose were not fermented. The organism grew well in dextrose broth at room temperature and 37 C., but slightly at 42 C. A slight positive reaction was obtained in 0.5% dextrose broth with methyl red. The Voges-Proskauer test was negative. The organism did not grow in plain infusion broth. Aerobically, it grew slightly, if at all, on surface medium. There was good growth in dextrose broth at *pH* 4.4 and 7.0, but no growth at *pH* 8.0 and 8.5. The organism did not grow in 2% urea broth. The organism was non-motile and no spores were observed.

The gram-positive coccus, no. 4, belonged to the Micrococcus group. When first isolated it produced a very pale lemon yellow pigment on plain agar, and appeared in clumps under the microscope. It grew well aerobically on all ordinary media. It fermented [illegible]

dextrose, saccharose, mannitol and lactose slowly (12 days) without gas. Acid was produced in brom-cresol-purple milk in 3 to 12 days. Gelatin was not liquefied in 6 weeks. Blood agar is not hemolyzed.

The gram-positive diplococcus, no. 5, belonged to the Enterococcus group. It fermented dextrose, lactose, maltose and salicin without gas production, but did not affect saccharose, mannitol or rhamnose. It grew well on dextrose broth, and moderately well in plain broth and on plain agar. A slight hemolysis was produced on blood agar. It was not bile soluble. Brom-cresol-purple milk was rendered acid, and coagulation followed. The organism grew well at 37 and 42 C., but little, if at all, at 15 C. In dextrose broth containing 6.5% NaCl, good growth was obtained in 24 hours. At *p*H 9.6, in dextrose broth, slight growth could be seen.

The enzyme involved.—Experiments were first done to determine if the animal enzymes present in the upper levels of the alimentary tract were capable of digesting pectin. Material obtained from 2 of the dogs fed on pectin for a week, as described above, was subjected to Berkefeld filtration, and the resulting filtrates were used for inoculation into pectone. Filtrates from washings of the stomach, duodenum, jejunum and 3 portions of the ileum did not cause the decomposition of pectin during a 2-week incubation period. In all of the animals studied the digestive tract above the cecum was pratically empty, while the large bowel had large quantities of pectin of gel-like consistency. A Berkefeld filtrate of washings (Ringer's solution) from a Thiery-Vella loop of the colon of a dog was also inactive. No activity was shown, moreover, by a Berkefeld filtrate of a saline (physiologically normal) suspension of mucosa scrapings of a dog colon.

Determinations of *p*H of the material before filtration were made to ascertain the effect of a pectin diet on the reaction of the digestive tract. The values given by this material are presented in table 2. The acid reaction of the different parts of the large bowel appears characteristic.

TABLE 2.—*H-ion Concentration of Washings from Dog's Alimentary Tract When Pectin Is Fed Alone*

Source of Washings	Dog. A (*p*H)	Dog. B (*p*H)
Stomach	4.1	4.4
Duodenum	7.4	7.2
Jejunum	7.3	7.2
Ileum (upper)	7.1	7.0
Ileum (middle)	6.8	6.9
Ileum (lower)	6.8	6.8
Cecum	5.1	5.4
Descending colon	5.3	5.0
Rectum	5.5	5.0

Our interest was next directed towards the possible action of filtrates of feces suspensions. In these experiments, 10 gm. of feces, from a dog fed pectin along with a general diet, was emulsified in approximately 150 cc. of distilled water, centrifuged, and the supernatant fluid subjected to Berkefeld filtration. This will be referred to as filtrate A. To determine if a filtrate from a small quantity of feces would also be active, feces procured at the end of a glass tube inserted into the rectum of a dog were suspended in 10 cc. of broth and filtered as above. This will be referred to as filtrate B. Three different quantities of filtrate A were introduced into 50 cc. of pectone: 0.1, 1.0 and 10 cc. Only 1.0 cc. of filtrate B was used. As a control, a loopful of the feces suspension was inoculated into another 50 cc. of medium. At the end of a 2 week incubation period, pectin decomposition had occurred in all instances. In another experiment, a filtrate like A was introduced in 10 cc. quantities into 50 cc. of pectone and incubated 3 to 6 days. Decomposition was as great at the end of 3 as at the end of

[illegible] days. Activity was lost after the filtrates were boiled.

From this study one might conclude that an exoenzyme was involved, yet this may not have been the case. It is possible that the enzyme was liberated only after the death of the organisms which produced it. In order to ascertain if pectin decomposition could be brought about by an autolysate of feces bacteria, the following experiment was

TABLE 3.—*Pectin Decomposition by Filtrates from Cultures of a Single Active Organism*

Age of Culture in Days	1 cc. of Culture in Thioglycollate Pectone	1 cc. of Filtrate in Pectone
1	No breakdown	No breakdown
2	" "	" "
3	" "	" "
4	50%	" "
5	[illegible]	60%
7	75%	50%
19*	40%**	20%

* When incubated in dextrose pectone 70% breakdown [illegible].
** In pectone.

done: Four serial dilution cultures of a feces suspension in pectin-broth were [illegible] made to stimulate the growth of the active bacteria. One part of the fourth dilution culture was filtered (Berkefeld), another was allowed to [illegible], while the third remained un[illegible]. The culture, the filtrate and the [illegible] were next mixed aseptically [illegible] pectone and incubated 3 days. Pectin liquefaction was observed in each mixture, showing that the active agent was present not only in the culture and filtrate but also in the autolysate.

Since we were able to deal with a single pectin fermenting bacterium, it was decided to ascertain if an exoenzyme could be found in a culture of such an organism. The spore-bearing rod (no. 1, table 1) was used. It was grown for varying lengths of time in thioglycollate broth, and the culture and its corresponding filtrate (Berkefeld) tested for pectin decomposition after incubation for 2 weeks with a pectin-containing medium. The results obtained are presented in table 3. These results show that the active agent was present in the filtrates, and also indicate that the age of the culture was associated with the degree of activity not only of itself but also of its corresponding filtrate. The optimum results were obtained when the cultures were 5 and 7 days of age.

The decomposition products of pectin and galacturonic acid.—Pectone and galactone were studied in the uninoculated state before and after incubation, and in the inoculated state after incubation at 37 C. The study included the following determinations: titratable acidity, *p*H, relative viscosity, calcium

TABLE 4.—*Experiments on Inoculated "Pectone" after Incubation*

Material Tested	Reaction of Medium (*p*H)		Titratable Acidity of 10 cc. of Material Without Aeration (cc. 0.1 normal alkali)		Relative Viscosity (seconds)	Pectin, Calculated from Calcium Pectate Yield (gm. per liter)	Production of Galacturonic Acid (mg. per liter)	Increase in Volatile Acids* (cc. normal alkali per liter)	
								Formic	Acetic
	After 2 Days	After Days	After 2 Days	After 7 Days	After 7 Days	After 2 Days	After 2 Days	After 2 Days	After 2 Days
[illegible]	7.2	7.2	[illegible]	0.2	60	11.[illegible]	0	0	1.[illegible]
	7.1	6.9	[illegible]	0.8	59	11.[illegible]	0	0	1.[illegible]
[illegible]	6.2	5.8	[illegible]	3.0	32	0	20.0	10.0	24.8
	6.4	6.2	9.7	3.2	31	0	17.1	3.0	28.6
Culture of nerobacillus	6.2	7.6	3.0	0.2	51	4.[illegible]	2.[illegible]	1.0	0.6
	6.0	7.2	3.2	0.3	50	5.[illegible]	2.[illegible]	2.0	1.0
Culture of micrococcus plus enterococcus	6.2	7.0	2.8	0.3	41	3.[illegible]	[illegible]	0.7	1.1
	5.6	6.5	4.1	1.0	42	3.[illegible]	7.[illegible]	3.2	7.0

* These values were obtained by subtraction from those given by the uninoculated medium.

pectate formation, free galacturonic acid, furfural, free and total reducing substances after hydrolysis, and volatile acids. The accompanying tables present the data obtained. This analysis may be considered fairly inclusive, yet it is not a complete study of the metabolism of the bacteria used. Work now being carried out has this as its purpose.

From the results presented in tables 4 and 5, one may observe that when pectone other [illegible] after incubation. The conditions, moreover, are comparable to the alkalinity and temperature present in the gastro-intestinal tract.

Table 4 also presents the data dealing with the bacterial fermentation of pectin by: (a) a suspension of dog feces (the animal having been fed pectin along with a general diet), (b) by the spore-bearing rod (no. 1) isolated from

TABLE 5.—*Experiments on Inoculated "Galactone" after Incubation*

Material Tested	Reaction of Medium (pH)		Titratable Acidity of 10 cc. of Material Without Aeration (cc. 0.1 normal alkali)		Decrease of Uronic Acid, Calculated from Yield of Furfural (millimols per liter)	Decrease of Reducing Substances, Calculated as Dextrose (millimols per liter)		Increase in Volatile Acids (cc. normal alkali per liter)	
						Free	Total after Hydrolysis	Formic	Acetic
	After 2 Days	After 7 Days	After 2 Days	After 7 Days	After 2 Days	After 2 Days	After 2 Days	After 2 Days	After 2 Days
Uninoculated medium: Before incubation	7.2	7.2	0.3	0.3	50.0	51.0	62	1.8	3.6
After incubation	7.1	6.8	0.8	1.0	49.0	49.3	62	2.48	4.4
Culture of aerobacillus*	5.5 5.8	6.0 6.4	3.0 2.8	1.5 1.2	3.6 5.0	4.8 3.0	10.9 8.0	0.8 1.4	1.8 0.2
Micrococcus plus enterococcus*	5.0	7.0	3.5	0.4	3.6	3.0	12.0	1.0	3.4
Feces suspension*	4.4	6.2	10.6	2.8	43.7	44.4	41.0	1.2	20.8

* These values were obtained by subtraction from those given by the uninoculated medium.

pectin or galacturonic acid is part of a buffered uninoculated culture medium and incubated at 37 C. slow decomposition takes place. This is demonstrated in the case of the galacturonic acid by the change of pH, titratable acidity, galacturonic acid, reducing power, and an increase in volatile acids; and in the case of the pectin, by a similar change in pH, titratable acidity, calcium pectate yield, and the production of acetic acid. A comparable observation is associated with the work of Henderson[22] who found that dextose is decomposed when incubated at 37 C. It is important to note that under these conditions no measurable amount of free galacturonic acid is present in the dog feces, and (c) by the 2 coccal organisms (nos. 4 and 5), which act synergistically. One may observe that decomposition was caused by all the agents used, and that the trend of the results is similar. Correlation exists between the pH and titratable acidity determinations; and acid reaction is characteristic after 2 days of incubation, and a neutral or relatively more alkaline reaction after 7 days of incubation (which possibly may be explained by a loss of CO_2). The decrease in calcium pectate yield is correlated by the decrease in relative viscosity, which depends on the amount of pectin or pectic acid. The production of formic and acetic acids in all the cases is also characteristic, as is the presence of free galacturonic acid.

22. J. Biol. Chem. 10: 3, 1911.

Table 4 contains the results on the fermentation of galacturonic acid by the mixed agents used in the experiments with pectin. The trend of these data is similar to that on pectin. Decomposition is evidenced by the decrease in pH and increase in titratable acidity (associated with an increase in volatile acids), and a decrease in reducing power and galacturonic acid (reduction in furfural yield).

Other similarities were observed in the fermentation pictures of pectin and galacturonic acid. The rate of fermentation by the feces mixture was rapid, both in the pectin and galacturonic acid-containing mediums, but the rate was slow when the isolated organisms were used. This was clearly shown by the amount of acetic acid formed. Also interesting is the fact that there was produced, on a molar basis, practically the same quantity of acetic acid from pectin as from galacturonic acid when the feces mixture was the fermenting agent.

According to Friedemann,[23] formic and acetic acids and ethyl alcohol are produced approximately in the proportions of 2 to 1 to 1 respectively, from a molecule of sugar. Assuming that galacturonic acid is metabolized in a similar manner, then the 44 millimols of this acid assumed in experiment 16, table 5, should yield 0.80×44 or 35 millimols of acetic; 30.8 were obtained. These quantities were practically duplicated in experiments 5 and 6, table 4, in which pectin was used. Therefore, one may conclude that there was the same general type of decomposition for [illegible]

Discussion

The possibility that the acid formed from the more readily available carbohydrate of our medium may be the active agent in the decomposition of pectin should be considered in discussing in vivo digestion of pectin. Large quantities of organic acids representing bacterial decomposition products are constantly present in the large bowel. Since we did not obtain breakdown with a sterile acid medium, in which the acid was a bacterial product, it is clear that living organisms must be the intermediary. This is further borne out by our results with isolated strains, the enzymes involved, our previous work and by the reports of other investigators.

Suspensions of feces representing the residue of a general diet to which pectin has been added, though they rapidly decompose pectin, are not a fruitful source for the isolation of pectin-fermenting bacteria. The difficulty associated with their isolation is probably due to their cultural properties. A diet of pectin alone affords a better opportunity for isolating from feces an organism capable of acting on pectin. Pectin-fermenting bacteria can also be isolated from various levels of the alimentary tract of a dog when the animal is placed on such a diet.

Since different bacteria are able to ferment pectin, as has been depicted in table 1, and are often found in the same animal they probably act together. Such synergistic action is illustrated by our in vitro experiments with strains 4 and 5.

The degree of activity, during a given incubation period, varies with the different strains isolated and with the medium, and very likely depends on the cultural characteristics of the organism. This is shown in tables 1, 4 and 5. The quantity of dextrose in the medium, and the oxygen tension are determining factors. Readily available carbohydrate, such as dextrose, favors growth. Thioglycollate broth, and anaerobic medium, favors the growth of organisms which require lowered oxygen tension. These requirements are met by the conditions [illegible]

23. J. Biol. Chem. 129: 787, 1939.

present in the colon, where pectin decomposition takes place, and consequently it is not unusual that the bacteria involved have properties which fit these conditions. All of the organisms studied readily utilize dextrose, as well as other sugars. Moreover, the fact that the spore-bearing rod belonging to the Aerobacillus group and the slender gram-positive bacillus belonging to the Lactobacillus group are microaerophilic, appears to be more than a coincidence. They would find the colon a favorable habitat.

Our observations on the enzyme, or enzymes, concerned indicate that the source is the bacteria which ferment pectin. The animal enzymes present in the alimentary tract did not digest pectin. These results are in accord with those reported by Kertesz.[6] Since autolysates of the bacteria contain the active agent, an endoenzyme is signified. Such an enzyme would be liberated on the death of the organisms, and its presence in Berkefeld filtrates could thereby be explained. Nevertheless, the production of an exoenzyme can not be ruled out. An exoenzyme as well as an endoenzyme may be involved. According to Waksman and Davidson,[24] the enzyme capable of hydrolyzing pectin, "is often considered to be a group consisting of three enzymes: pectosinase, which converts pectose to pectin; pectase, which coagulates pectin, and pectinase, which hydrolyzes pectin to reducing sugars of the pectose series. Pectinase can also apparently convert pectin to pectic acid, methyl alcohol and acetone." The intestinal organisms which we have found to cause coagulation probably possess the pectase, while the organisms which cause liquefaction possess the pectinase.[25]

24. Enzymes: Properties, Distribution, Methods and Applications, Baltimore, Williams and Wilkins, 1926, chapter X

The experiments on the decomposition products of pectin and galacturonic acid afford further evidence of the fermentation of pectin by fecal bacteria. This study also indicates that the bacteria which act on pectin also act on galacturonic acid. Definite similarities in the fermentation pictures of the two substances may be observed, and the same degree and type of fermentation can be concluded for both, as shown in tables 4 and 5. These tables also show that the products of pectin decomposition are the same as for galacturonic acid, except for the formation of this acid when pectin is used. The end products for both substances are formic and acetic acids and other substances in smaller quantities. These products will be considered more throughly in another report.

The production of galacturonic acid in the decomposition of pectin is of particular interest, since this substance has been supposed to be absorbed and utilized in the detoxification function of the liver.[16] Our study indicates, however, that the quantities formed are very small, or that breakdown occurs quickly. Since Werch and Ivy have shown[25] that galacturonic acid is not absorbed in appreciable quantities from the human and canine small intestine and colon, this is further evidence that any galacturonic acid produced as the result of decomposition of pectin in the alimentary tract is not rendered available in significant quantities for the formation of uronides in the liver.

SUMMARY AND CONCLUSIONS

Suspensions of dog feces representing the residue of a general diet to which pectin had been added, though they rapidly decomposed pectin, are not a

25. Werch, S. C. and Ivy, A. C.: Proc. Soc. Exper. Biol. & Med. 48: 9, 1941.

fruitful source for the isolation of pectin-decomposing bacteria. Feces from a diet of pectin alone is a better source of material. Pectin-decomposing bacteria can also be isolated from various levels of the canine alimentary tract.

A number of organisms capable of decomposing pectin were isolated. We undertook to identify only the more active. These belong to the following groups: Aerobacillus, Lactobacillus, Micrococcus and Enterococcus. Synergistic action was observed.

Berkefeld filtrates of the pectin decomposing organisms, as well as of feces suspensions, contained a heat labile, enzyme-like substance. The following organisms (table 1) produced a pectinase-like, or liquefying enzyme: strains 2, 3, 5, 7, 8, 9 and 10. The following produced a pectase-like or coagulating enzyme, in addition to a pectinase: strains 1, 4 and 6. Strain 11 produced only a pectase. The filtrates of feces suspensions produced only liquefaction.

The following strains were found to decompose galacturonic acid: No. 1 (Aerobacillus), and a mixture of nos. 4 & 5 (Micrococcus and Enterococcus). The decompositon products were chemically similar, insofar as they were studied. The chief end products found were formic and acetic acid.

When pectin was decomposed by the bacteria just mentioned, formic and acetic acids were also the chief products formed; in addition some galacturonic acid was produced. The quantity of galacturonic acid formed in the decomposition of pectin was either very small or breakdown occured rapidly. This is further proof that this acid is not rendered available in significant quantity for the formation of uronides in the liver and that the action of pectin is confined to the alimentary tract.