TABLE I.
Incidence of Alopecia on Various Purified Rations.

Addition to 100 g of basal	Total No. of animals	No. of cases of alopecia
100 mg inositol	9	6
5 mg *d,l* pantothenate	39	9
5 mg *d,l* pantothenate and 100 mg of inositol	12	0
5 mg *d,l* pantothenate following a 2-week depletion period	35	19

It seems, however, that the inositol effect was not a mere reflection of pantothenic acid effect because of the difference in the early picture of alopecia in the two deficiencies.

The fact that mice may be raised satisfactorily and rapidly to maturity on a highly purified diet is noteworthy. The only constituents of the ration about which there can be uncertainty as to chemical composition are the oils and the casein; all the other constituents were pure compounds. It will be of interest to determine whether the oils and casein can be replaced by pure fat-soluble vitamins and pure amino acids. Whether or not natural foodstuffs contain materials which increase slightly the rate of growth obtained with the purified diet has not been determined.

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Rôle of the Intestinal Bacteria in the Decomposition of Pectin.

S. C. WERCH, A. A. DAY, R. W. JUNG AND A. C. IVY.

From the Departments of Bacteriology, and Physiology and Pharmacology, Northwestern University Medical School, Chicago, Illinois.

Previous observations[1, 2] have shown that orally administered pectin is decomposed in the human and dog gastrointestinal tract, that the decomposition occurs in the colon, and that it is probably brought about by bacterial action. A thorough study of the part played by intestinal bacteria in pectin decomposition has not as yet been made. Schneider,[3] however, found that mixtures of intestinal bacteria destroy the hemocellulose of apple marc. He also observed that reducing sugars did not constitute part of the end products.

1 Werch, S. C., and Ivy, A. C., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 336; *Am. J. Dig. Dis.*, in press.

2 Kertesz, Z. I., *J. Nutrition*, 1940, **20**, 289.

3 Schneider, E. C., *Am. J. Physiol.*, 1912, **30**, 258.

The related work of McCoy and Peterson[4] 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 pectin used in this study was pure citrus pectin obtained from the Research Laboratories of the California Fruit Growers' Exchange. It is essentially free of such impurities as pentoses, pentosans, color and other materials frequently found in other pectins. Before much work was done it became evident that a sterile pectin-broth medium was obligatory, and had to conform to the following requirements: (a) that the pectin be unchanged, (b) that the pH 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 and chemical sterilization altered the properties of the pectin, and desiccation proved inefficient. Berkefeld filtration proved satisfactory, when certain precautions were observed in the care of the candle and pH of the medium. After being used, the candle is washed freely under tap water with a soft brush, and then rinsed in the reverse direction for 30 minutes (using gravity). Next it is placed for 10 minutes in a boiling water bath containing 5% $NaHCO_3$. After this it is rinsed with 3 liters of distilled water in the usual direction by means of suction. Lastly, slow drying is permitted by leaving it near a draft over night. The day it is to be used, the candle, mantle and suction flask are assembled, wrapped in a large muslin sheet and autoclaved for an hour. This procedure establishes a neutral or weakly positive surface charge on the candle, permitting a pectin solution of pH 7.2 (for example) to pass through, yet retaining the bacteria. The technic developed for the preparation of the pectin-broth medium conforms with our other requirements. Pectin is dissolved in a buffer solution of desired pH, and then added to a volume of sugar-free broth sufficient to make the desired dilution. The mixture may be adjusted for pH and then sterilized by filtration. Table I represents an analysis of such a medium, of pH 7.0 and containing 1% pectin, before and after filtration. The different values obtained after filtration indicate that only a small part of the pectin has been withheld by the candle. This is probably due to slow clogging of the pores of the candle.

With such media and suspension of human and dog feces, representing a normal diet and one to which pectin was added, as sources of bacteria, experiments were devised to study decomposition.

[4] McCoy, N., and Peterson, W. H., *J. Inf. Diseases*, 1928, **43**, 475.

TABLE I.

Berkefeld filtration	Phlorogluicide formation in g	Viscosity in time	(H)+ Potentiometer
Before	.250	3′ 33″	6.95
After	.220	3′ 5″	6.90

Possible decomposition was followed by physical and chemical examination of the inoculated media after incubation at designated intervals. Results were obtained by observation of gas formation, and determination of titratable acidity, pectate formation, viscosity and hydrogen ion concentration.

Gas production is illustrated by the following typical experiment: This made use of a sterile pectin-broth medium of pH 7.0 and containing 0.5% pectin, which was introduced in equal quantities in fermentation tubes. These were inoculated with a broth suspension of human and dog feces respectively, representing a diet which included pectin. After incubation for 48 hours, the average increase of gas formation in the pectin-broth tubes over the broth control tubes, in the case of dog feces (3 subjects) was 25%, and in the human (3 subjects), 13%. Statistical analysis of the data indicated a significant difference, and it was concluded that the source of the excess gas is from pectin decomposition.

Table II represents experiments in which feces from 4 dogs were used. Nos. 3 and 4 were on a daily ration of one can of commercial dog food (Pard) and 200 cc of milk, and Nos. 1 and 2 on the same diet to which 20 g of pectin were added daily. Inoculations were here made in Erlenmeyer flasks, containing either the pectin-broth medium or the plain broth control medium, diluted with a volume of buffer equal to that used in the pectin medium. The incubation temperature was 37°C and the periods were 24, 48, and 72 hours. Results were obtained by determination of titratable acidity, pectate formation, viscosity and pH. Titratable acidity was carried out with 0.1 N NaOH and phenolphthalein as indicator; pectate formation by the calcium pectate method;[5] viscosity by observing the time necessary to pass through a 10 cc pipette, to which a capillary tube had been fused; pH with a Coleman potentiometer.

Increase in titratable acidity and lower pH values indicate acid production, reduction in time, as a measure for viscosity, proves change in the physical character of the solution, while absence of pectate formation points to a breakdown beyond the gel stage. These changes may be observed by following the data in Table II. It

[5] Joseph, Glenn H., Corona, California, personal communication.

TABLE II.

	(H)+ Potentiometer	Titratable acidity cc of 0.1N NaOH	Viscosity in time	Pectate in g
		Control Before Incubation.		
Pectin*	7.2	2.0	1′ 30″	.82
Broth†	7.4	1.0	32″	.00
		Control After Incubation for 72 hr.		
Pectin	7.2	2.0	1′ 32″	.83
Broth	7.5	1.0	33″	.00

		Fermentation Periods in hr.											
Media	Dog	24	48	72	24	48	72	24	48	72	24	48	72
						Inoculated and Incubated.							
Pectin	1	6.5	6.0	6.3	2.5	4.5	4.0	1′ 18″	33″	34″	.78	.15	.00
Broth	1	7.2	7.45	7.9	1.0	0.5	0.3	33″	32″	32″	.00	.00	.00
Pectin	2	6.7	5.9	6.3	2.4	5.0	3.8	47″	32″	33″	.67	.12	.00
Broth	2	7.3	7.35	7.5	1.0	0.8	0.7	34″	31″	31″	.00	.00	.00
Pectin	3	6.9	6.6	6.5	2.0	2.5	3.5	1′ 06″	35″	32″	.81	.20	.00
Broth	3	7.25	7.35	8.1	1.0	1.0	0.5	33″	31″	32″	.00	.00	.00
Pectin	4	6.95	6.0	6.6	1.8	5.0	3.3	1′ 16″	32″	33″	.76	.24	.00
Broth	4	7.3	7.4	7.55	1.0	0.7	0.6	33″	31″	32″	.00	.00	.00

*Pectin-broth.
†Broth control.

should be noted that, in 3 of the 4 tests cited in the table, after 48 hours base was produced, which probably arose from the broth.

Work dealing with the isolation of responsible bacteria, determination of the exogenous or endogenous source of the enzyme, and the products of decomposition is being carried out.

Conclusion. The results show that pectin is decomposed by bacteria present in feces obtained from human and canine subjects on a normal diet and one after pectin had been added.

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Influence of Emotional Excitement on Insulin Content of Blood in Normals and Psychotic Patients.

E. GELLHORN, A. ALLEN AND J. FELDMAN.

*From the Departments of Physiology and Psychiatry, University of Illinois College of Medicine, Chicago, Ill.**

It was shown recently[1] that emotional excitement leads to a dis-

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1 Gellhorn, E., Cortell, R., and Feldman, J., *Science*, 1940, **92**, 288.