MICROORGANISMS IN THE CECAL CONTENTS OF RATS FED VARIOUS CARBOHYDRATES AND FATS^{1,2}

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The nutritional significance of intestinal microorganisms was indicated by early work (Cooper, 1914; Theiler *et al.*, 1915), and during recent years it has been established that all the vitamins of the B complex, as well as vitamin K, may be synthesized in the alimentary tract of animals as a result of the activity of these intestinal organisms (Wegner *et al.*, 1941; Day *et al.*, 1943; Elvehjem, 1946). In addition, information has been obtained concerning the effect of different carbohydrates on the intestinal synthesis of B vitamins (Elvehjem and Krehl, 1947). It is fairly well known now that irrespective of what B vitamin is investigated its requirement is reduced when relatively insoluble carbohydrates like dextrin or starch are used in the diet. Lactose sometimes favors the production of some of the B vitamins, but glucose, sucrose, or other readily assimilable sugars are without any effect in this respect (Fridericia *et al.*, 1927; Guerrant *et al.*, 1934*a,b*, 1935, 1937; Morgan *et al.*, 1938; Schweigert *et al.*, 1945; Luckey *et al.*, 1946; Sarma *et al.*, 1946; Teply *et al.*, 1947; Couch *et al.*, 1948). The effect of different carbohydrates in encouraging or suppressing a vitamin-synthesizing intestinal flora is not unexpected in view of the fact that a carbohydrate-rich diet stimulates an aciduric flora, whereas meat or a high protein diet favors a proteolytic flora and large numbers of coliform organisms.

As the cecum has been postulated to be the main site of vitamin synthesis by intestinal microorganisms (Moro, 1905; Tissier, 1908; Torrey, 1919; Cannon *et al.*, 1920; Porter and Rettger, 1940; Taylor *et al.*, 1942; Schweigert *et al.*, 1945), all microbiological work in the present study was done on cecal contents. Of the various carbohydrates studied, lactose and dextrin have been found to have the most marked effect in favoring an aciduric flora (Cannon, 1920; Porter and Rettger, 1940). Interesting differences in the types of different intestinal organisms of animals on various carbohydrates have also been found recently (Johansson *et al.*, 1947; Gall *et al.*, 1948*a,b*).

The effects of the fat component of the diet on the intestinal flora of animals have received little attention, but, as pointed out by Elvehjem and Krehl (1947), recent investigations clearly indicate the role of fat in modifying the requirement for some of the B vitamins, especially thiamine, riboflavin, niacin, biotin, pantothenic acid, and pyridoxine.

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The present investigation was undertaken to determine the effects of carbohydrates and fats upon the cecal flora of the rat. This work was done in an attempt to develop a satisfactory explanation for the different growth-promoting activities of diets containing (a) different carbohydrates and fats and (b) different levels of fats.

EXPERIMENTAL METHODS

All experiments were performed with a "parasite-free" strain of rats obtained from Sprague Dawley Company, Madison, Wisconsin; the rats were individually caged in the new type of coprophagy-preventing "tube cage" first developed by Geyer *et al.* (1947). This was done in an attempt to obliterate variations in the vitamin intake of the animals. The diet consisted of alcohol-extracted casein, 20 per cent; salt mixture IV, 4 per cent; fat (butterfat or corn oil), 10 per cent or 28 per cent; and carbohydrate (sucrose, lactose, or dextrin), 66 per cent with low fat diet or 48 per cent with high fat diet. The fat-soluble vitamins were incorporated into the fat, whereas the water-soluble vitamins were added to the main batch of basal ration during the mixing of casein, salt mixture, and carbohydrate. The normal level of vitamin supplement per 100-g ration was as follows:

Thiamine.....	0.20 mg
Riboflavin.....	0.30 mg
Pyridoxine.....	0.30 mg
Ca-pantothenate.....	1.50 mg
Choline hydrochloride.....	150.00 mg
Beta-carotene (90 per cent beta-, 10 per cent alpha-carotene).....	0.56 mg
Alpha-tocopherol.....	2.24 mg
Calciferol (crystalline, irradiated ergosterol).....	0.014 mg
2-Methyl-1,4-naphthoquinone.....	0.21 mg

The experiments were conducted for different periods varying from 2 to 6 weeks, after which the animals were killed and analyzed.

Samples of cecal contents for analysis were obtained by opening the abdomen of the animal under aseptic conditions, removing the cecum to a sterile petri dish, and squeezing out the total cecal contents with sterile, smooth forceps by a "milking process." In most cases a record was kept of the total cecal contents of the animal. After proper mixing, 0.5 g of the cecal contents were transferred to a sterile 50-ml water blank containing glass beads. After vigorous and thorough shaking of this original suspension (1×10^{-2}), it was further diluted to 10^{-4} and then serially by steps of 100 until a dilution of 10^{-8} was obtained. Quantitative inoculation was then made of the respective dilutions into various culture media.

An estimate of the total viable population of aerobic and anaerobic bacteria was made by using the BBL thioglycolate medium. In a few cases, the total aerobic plate count was determined by the use of a tryptone glucose yeast extract agar medium. For culturing anaerobes, the oat jar method was used, and a period of 48 to 60 hours at 37 C was allowed for incubation of the plates. Inocula

used in both aerobic and anaerobic plate counts ranged from 10^{-5} to 10^{-8} in duplicate.

Difco EMB agar was used as the plating medium for culturing coliform organisms, and the dilutions of inocula ranged from 10^{-3} to 10^{-7} . An incubation period of 48 to 60 hours at 37 C was allowed for the growth of these organisms.

The "SF" medium of Hajna and Perry (1943), which utilizes 0.5 g per liter of Na_3N , as well as a selective incubation temperature of 45 C for the inhibition of organisms other than enterococci (and other intestinal streptococci which grow at 45 C), was used for enumerating this group. The tube dilution count method was adopted in this case; five tubes were inoculated per dilution and the counts calculated by the most probable number method.

Shake tubes of carrot liver extract medium introduced by Foster *et al.* (1942) were used for counting "lactics," and the dilutions of inoculum ranged from 10^{-5} to 10^{-9} . This medium was found to be selective for this group in view of the fact that at such high dilutions no other organisms were found to be present. It was possible to base these counts on numbers of colonies developing in the tubes.

RESULTS AND DISCUSSION

Relation of carbohydrates and fats of the diet to the cecal flora of rats. Results of the analyses of the cecal flora of rats on diets containing one of the three carbohydrates, sucrose, lactose, or dextrin, and either of the fats, butterfat or corn oil, are presented in tables 1 to 3. It is apparent from these data that a considerable amount of variation exists in the size and the composition of the microbial population of the cecum in the different groups of animals, even when they are maintained under almost identical dietary and other environmental conditions. In no case was it possible to obtain an exact duplication of the results obtained with any group of rats on a particular diet. The same extent of variation is also observed in the individual animals belonging to a group kept under the same dietary and environmental conditions, and therefore in order to come to any logical conclusions one must consider large numbers of animals in each group. The results given in the tables are based in most cases on analyses of the cecal contents of a series of 4 to 6 rats in each group. However, it has been observed that, even when the numbers of the organisms in the ceca of different animals belonging to the same group varied to a great extent, the relative proportions of the different kinds of organisms in the cecum of each animal were approximately the same.

The effects of the various carbohydrates on the microbial flora of the cecum is apparent in these results. In all cases the lactic group of organisms dominated the other organisms; this result seems to support the reports by most other workers in this field. However, the ratio of this group of organisms to some other group like coliforms depended to a great extent on the carbohydrate of the diet.

In the study on the influence of various types of carbohydrates, much higher aerobic and anaerobic plate counts were found in the cecal contents of rats on

the lactose diet as compared to that of rats on sucrose or dextrin diets. Of the 10 groups of animals on the lactose diet, in only 4 cases did the total count of cecal contents fall below a billion per gram, whereas on a sucrose diet only 1 out of the 12 groups studied yielded total counts above 1 billion per gram. On the dextrin diet, the average total count of cecal contents of all the animals was much below 1 billion per gram. Moreover, when the comparison is made on the basis of total number per cecum, the result is even more striking. A glance at the column giving the weights of the total cecal contents reveals that the lactose-fed animals had more total cecal contents: on the average, two to three times more cecal contents were found in lactose-fed animals than in those fed a sucrose

TABLE 1

Cecal flora of rats on diets containing sucrose as the dietary carbohydrate
(Counts indicate number of organisms per gram of fresh cecal contents)

TIME OF EXPERIMENT (WEEKS)	VITAMIN LEVEL*	INCREASE IN WT (g)	NO. OF RATS	AVG WT OF THE CECUM (g)	AEROBIC PLATE COUNT	ANAEROBIC PLATE COUNT	COLIFORMS	LACTICS
Group I. Fat—Butterfat								
2	A	41	5	0.632	215,000,000	398,000,000	220,000	199,800,000
4	A	84	5	0.889	60,000,000	248,600,000	222,000	95,700,800
6	A	122	5	1.095	194,000,000	221,900,000	528,000	181,800,000
6	A	119	5	—	107,340,000†	—	690,000	130,000,000
6	B	150	5	—	6,146,000,000†	—	1,040,000	268,000,000
9	A	168	5	—	75,875,000	62,575,000	391,000	170,250,000
Group II. Fat—Corn oil								
2	A	452	5	0.487	260,700,000	473,800,000	1,030,000	183,200,000
4	A	709	5	0.785	161,125,000	335,000,000	164,250	229,500,000
6	A	106	4	0.996	342,750,000	456,250,000	383,000	1,173,000,000
6	A	110	5	—	92,730,000†	—	496,000	152,500,000
6	B	128	5	—	93,100,000†	—	1,572,000	245,800,000
9	A	149	4	—	157,000,000	224,250,000	570,000	432,000,000

* Vitamin level A is normal; vitamin level B is approximately twice normal.

† Tryptone glucose yeast extract agar medium was used for counting.

or dextrin diet. Thus the total numbers of organisms per cecum was higher in rats fed lactose than in those fed sucrose or dextrin.

There was also a significant difference in the aerobic to anaerobic plate count ratio in the flora caused by feeding the three carbohydrates. It was nearly 1:1 on a dextrin diet, less than 1:2 on the sucrose diet, and less than 1:4 on the lactose diets.

Not only did the total aerobic and anaerobic plate counts run high on the cecal contents of rats on a lactose diet, the numbers of coliforms and lactics were also very high. The effect of lactose in inducing a high aciduric flora is known from very early times, but its effect in maintaining a high coliform flora has not been reported by most of the early workers. The chief effect of a lactose

TABLE 2

Cecal flora of rats on diets containing lactose as the dietary carbohydrate
(Counts indicate number of organisms per gram of fresh cecal contents)

TIME OF EXPERIMENT (WEEKS)	VITAMIN LEVEL*	INCREASE IN WT (g)	NO. OF RATS	AVG WT OF THE CECUM (g)	AEROBIC PLATE COUNT	ANAEROBIC PLATE COUNT	COLIFORMS	LACTICS
Group I. Fat—Butterfat								
4	A	21	5	3.37	4,150,000,000	8,220,000,000	1,523,000,000	8,520,000,000
6	A	88	5	3.88	1,248,000,000	6,334,000,000	396,000,000	29,500,000,000
6	A	86	5	—	1,680,000,000†	—	270,000,000	12,530,000,000
6	A + biotin + folic acid	103	5	—	322,000,000†	—	192,683,500	4,550,000,000
6	B	98	5	—	870,000,000†	—	170,960,000	22,330,000,000
Group II. Fat—Corn oil								
4	A	14	5	3.07	5,910,000,000	11,510,000,000	1,800,000,000	22,380,000,000
6	A	72	5	3.27	2,418,000,000	10,870,000,000	130,650,000	12,340,000,000
6	A	80	5	—	358,900,000†	—	85,670,000	1,026,000,000
6	A + biotin + folic acid	81	4	—	1,588,000,000	—	367,000,000	2,830,000,000
6	B	82	4	—	123,000,000†	—	4,272,000	1,822,000,000

* Vitamin level A is normal; vitamin level B is approximately twice normal.

† Tryptone glucose yeast extract agar medium was used for counting.

TABLE 3

Cecal flora of rats on diets containing dextrin as the dietary carbohydrate
(Counts indicate numbers of organisms per gram of fresh cecal contents)

TIME OF EXPERIMENT (WEEKS)	VITAMIN LEVEL*	INCREASE IN WT (g)	NO. OF RATS	AVG WT OF THE CECUM (g)	AEROBIC PLATE COUNT	ANAEROBIC PLATE COUNT	COLIFORMS	LACTICS
Group I. Fat—Butterfat								
2	A	44	4	0.865	233,000,000	274,000,000	40,620,000	440,000,000
4	A	89	3	1.32	175,600,000	117,000,000	1,598,300	132,830,000
6	A	133	5	2.00	557,500,000	588,400,000	12,365,000	650,000,000
Group II. Fat—Corn oil								
2	A	43	4	0.91	215,250,000	222,700,000	7,705,000	459,250,000
4	A	86	4	1.35	183,400,000	195,000,000	5,672,000	180,000,000
6	A	132	4	1.48	177,000,000	182,500,000	7,230,000	250,000,000

* Vitamin level A is normal.

diet, and in general of a diet containing any carbohydrate, has been reported to be a suppression of the proteolytic organisms, including some coliforms, but in view of the fact that the latter are lactose fermenters, there is no reason why they should be suppressed. Kendall (1911-1912) and Cruikshank (1928), as a result of their *in vitro* experiments, suggested long ago that feeding a large amount of a lactose diet might first lead to an abundant growth of *Escherichia coli*, *Clostridium perfringens*, and other carbohydrate fermenters, and that the acid developed might offer a suitable medium for the growth of lactobacilli. However, this theory was strongly opposed by Rettger and Cheplin, who observed almost complete elimination of *E. coli* and *C. perfringens* under these conditions.

Dextrin also seems to encourage a large coliform flora, though its effect appears to be much less than that of lactose. The influence of dextrin in maintaining a high coliform count in chicks has also been reported by Evenson (1947) and by Johansson, Shapiro, and Sarles (1947). In contrast to the reports by many workers, the effect of dextrin in maintaining a high aciduric flora could not be demonstrated.

The counts of all types of organisms studied were very low in the ceca of animals fed sucrose compared to those of animals fed on the other two carbohydrates.

When a comparison is made on the basis of organisms present per gram of the cecal contents, no significant difference in the numbers of different kinds of bacteria appears to be present when the groups of animals receiving either butterfat or corn oil are compared, except for the fact that the enterococcus group was somewhat lower in animals on the butterfat diet. The values of the numbers of these organisms are not included in the tables as not enough experiments were performed to warrant definite conclusions. It was, however, observed that butterfat-fed rats, in almost all cases, had heavier ceca, and hence when a comparison is made on the basis of the number of organisms present per cecum, the butterfat groups had higher numbers of each kind of cecal organisms than those of the corn oil groups.

In view of the somewhat higher amounts of oleic acid in corn oil, which has been reported to have a growth-stimulating effect on *Lactobacillus casei* (Williams and Fieger, 1946), one might expect a higher number of lactic organisms in the cecal flora of rats receiving corn oil. No such effect was observed in any of the groups on corn oil with dextrin or lactose as the dietary carbohydrate, but on the sucrose diet there seemed to be slightly higher numbers of lactic organisms in corn-oil-fed animals. It is probable that on dextrin and lactose diets the effect of the oleic acid of corn oil, if there is any, is masked by the much stronger influence of the carbohydrates themselves. Reports by Evenson (1947) that the coliforms are suppressed by high butterfat diets could not be confirmed in the present study.

A tentative explanation of the difference in growth-promoting values of the three carbohydrates can be offered by considering the different types of organisms encouraged by them. However, it should be mentioned that the basic idea of such an interpretation lies in the correlation of the physiological behavior

of the different carbohydrates and fats (as measured by the growth of the animal) with the changes in numbers of the most important groups of organisms, some of which are considered to be vitamin synthesizers, whereas certain others are utilizers of the vitamins available to the animal. From the standpoint of vitamin economy of the animal host, the three most important groups of intestinal bacteria are the coliforms, anaerobes, and lactics. Many investigators are of the opinion that intestinal synthesis of the vitamins is carried on by the coliform organisms to the greatest extent, and so this group deserves to receive the most careful attention from the nutritionist's point of view. Anaerobic bacteria have been reported by Benesch (1945) to utilize some of the vitamins already synthesized by the coliforms; hence this group as well as the lactic group of organisms, which require almost all the known growth factors for their growth and multiplication, may be regarded as agents harmful to the animal host. Though Metchnikoff has described the lactobacillus group of bacteria as the promoter of intestinal hygiene, Mitchell (1927) has expressed the contrary opinion, in view of the finding that the rate of growth of rats on different carbohydrates varied inversely with the percentage of the aciduric group of bacteria in the cecum. She concluded that an aciduric type of intestinal flora in itself does not ensure a good general condition of the animal over a long period of time if a high percentage of lactose or other slowly absorbed carbohydrate has been used to support the aciduric flora. If the aciduric flora can be supported by smaller amounts of lactose, the growth of the animal might then remain normal.

It is not out of place to point out here that the main defect in the approach to the problem employed in our work lies in the fact that, through such a study, no information can be obtained on which vitamins are actually synthesized and in what quantities. In addition, it is not possible to determine the kinds or quantities of vitamins liberated in the cecum or finally absorbed by the host animal. It is also definite that all the different organisms of the coliform group are not equally efficient vitamin synthesizers. Furthermore, it is not fair to assume that all the lactics and anaerobes are harmful agents that utilize or destroy the vitamins synthesized by the coliforms. It is also possible that there might be other, unidentified bacteria which, though present in smaller numbers, might exert a strong influence on the nutrition of the animal. Thus a complete picture of what is going on in the intestinal tract of the animal can only be secured when the information obtained by a quantitative study of the different organisms is supplemented by cultural studies of the predominant organisms belonging to each group, followed by testing of their vitamin-synthesizing capacities.

The nearly identical influence of the dietary fats, butterfat and corn oil, on the growth of young rats on a sucrose diet was reflected in the composition of their cecal floras, which were found to be practically identical. On a dextrin diet, too, there was hardly any difference in the cecal flora when the dietary fat consisted of either butterfat or corn oil. Whether the slightly higher numbers of anaerobic organisms found in the ceca of lactose-corn-oil-fed animals are respon-

sible for the inferiority of this diet as compared to that containing butterfat, or whether some biochemical abnormalities associated with a lactose diet are counteracted to a greater extent by butterfat than by corn oil, can only be settled through future research.

Effects of the level of fat of a sucrose-containing diet on some of the cecal microorganisms. Comparatively few kinds of bacteria have the ability to split fat, and probably small numbers of those normally present in the intestinal tract possess this property. Therefore the addition of more fat to the diet is not expected to induce increases in numbers of intestinal bacteria, but on the contrary tends to suppress some of those already present. That this is what happens to the microorganisms of the cecum upon the addition of more fat in a sucrose diet is clearly seen from the results in table 4, which indicate that there is a decrease in the total numbers of organisms when the fat intake of the animal is

TABLE 4
Cecal flora of rats on sucrose diets containing different levels of corn oil
(All counts represent numbers per gram of fresh cecal contents)

EX- PERI- MENT NO.	LEVEL OF CORN OIL (%)	NO. OF RATS	AVG IN- CREASE IN GROWTH (g)	AEROBIC PLATE COUNT	ANAEROBIC PLATE COUNT	LACTICS	COLIFORMS
I	10	5	169	808,000,000	2,091,000,000	1,230,000,000	12,660,000
	28	5	156	420,000,000	1,474,500,000	872,000,000	926,000
II	10	5	188	873,500,000	2,238,250,000	1,660,000,000	99,323,000
	28	5	169	690,100,000	1,787,700,000	1,942,000,000	7,975,800
	28+	5	202	1,416,000,000	3,070,000,000	1,918,000,000	51,890,000
	"reticulogen" 35	5	140	592,000,000	1,265,200,000	1,320,000,000	46,998,000
III	10	5		3,110,000,000	—	3,840,000,000	11,700,000
	35	5		1,996,000,000	—	2,460,000,000	8,725,000

increased. The anaerobic plate count is also decreased in the same order. In view of the fact that analysis was made of only a few groups of organisms like coliforms and lactics, the results do not show conclusively which types of organisms were most affected. However, there is definite indication that the numbers of coliform organisms are decreased to a considerable extent by increased levels of fat in the diet. There is very little change in the numbers of the lactic group of organisms with increases in fat. The slight decrease observed is probably not statistically significant.

The results of this experiment confirm the report by Torrey (1919), who worked on dogs and observed that the numbers of *E. coli* were frequently reduced, that the streptococcus group was markedly reduced, but that the *Lactobacillus acidophilus* type was the least affected and often increased in relative numbers when the animals were fed a high fat diet. However, according to Mitchell

and Isbell (1942), contrary results were obtained in 1931 by Forti, who observed a decrease in the aciduric flora with an increase of fat in the diet.

Analysis of the group of rats on the 28 per cent corn oil sucrose diet supplemented with "reticulogen" (Lilly liver extract) at the rate of 0.1 unit per day, showed a significant increase in the aerobic plate count and in numbers of the coliform group of organisms, but not of lactics. It should be mentioned here that we gave a supplement of "reticulogen" to this group of rats in an attempt to remove the dietary imbalance, if there was any, resulting from the effects of high amounts of fat. We actually observed an increase in growth of the rats of this group by 33 grams over that of the control group receiving no supplement of "reticulogen." This strongly supports the view that diminished growth on a high corn oil diet is due to inhibition of the intestinal synthesis brought about by microorganisms, and that the action of "reticulogen" might be due to a stimulation of intestinal synthesis.

SUMMARY

For animals on a lactose diet the total aerobic and anaerobic plate counts, as well as the numbers of coliforms and lactics, were much higher than the corresponding counts or numbers of the same organisms in the cecal contents of animals on diets containing either dextrin or sucrose.

Compared to sucrose, dextrin was found to favor higher numbers of coliforms in the cecum.

The ratio of the aerobic to the anaerobic plate count was highest on a dextrin diet and lowest on a lactose diet.

The aerobic and the anaerobic plate counts, as well as the numbers of coliforms, were found to be decreased in the ceca of most of the rats fed sucrose diets containing a high level of corn oil.

Supplementation of a high corn oil sucrose diet with "reticulogen" tends to counteract the inhibitory action of corn oil upon the growth of certain cecal microorganisms.

REFERENCES

- BENESCH, R. 1945 Synthesis and destruction of nicotinic acid by the mixed caecal flora of man. *Lancet*, **248**, 718-719.
- CANNON, P. R., DRAGSTEDT, L. R., AND DRAGSTEDT, C. A. 1920 Intestinal obstruction. A study of the influence of the bacterial flora on the toxemia of acute obstruction. *J. Infectious Diseases*, **27**, 139-144.
- COOPER, E. A. 1914 On the protective and curative properties of certain foodstuffs against polyneuritis induced in birds by a diet of polished rice. Part II. *J. Hyg.*, **14**, 12-22.
- COUCH, J. R., CRAVENS, W. W., ELVEHJEM, C. A., AND HALPIN, J. G. 1948 Relation of carbohydrate to intestinal synthesis of biotin and hatchability in mature fowl. *J. Nutrition*, **35**, 57-72.
- CRUIKSHANK, R. 1928 The experimental transformation of the intestinal flora. *Brit. J. Exptl. Path.*, **9**, 318-326.
- DAY, H. G., WAKIM, K. G., KRIDER, MERLE M., AND O'BANION, E. E. 1943 Effects of cecectomy, succinylsulfathiazole, and *p*-aminobenzoic acid on vitamin K synthesis in the intestinal tract of rats. *J. Nutrition*, **26**, 585-600.

- ELVEHJEM, C. A. 1946 The role of intestinal bacteria in nutrition. *J. Am. Dietet. Assoc.*, **22**, 959-963.
- ELVEHJEM, C. A., AND KREHL, W. H. 1947 Imbalance and dietary interrelationships in nutrition. *J. Am. Med. Assoc.*, **135**, 279-287.
- EVENSON, ADELAIDE E. 1947 The intestinal flora of laboratory animals and its modification by diet and drugs. Ph.D. thesis, University of Wisconsin.
- FORTI, C. 1931 The action of edible fats on the intestinal flora. *Boll. soc. ital. biol. sper.*, **6**, 950-956, 956-958.
- FOSTER, E. M., GAREY, J. C., AND FRAZIER, W. C. 1942 The bacteriology of brick cheese. III. The bacteria involved in ripening. *J. Dairy Sci.*, **25**, 323-333.
- FRIDERICIA, L. S., FREUDENTHAL, P., GUDJONNSSON, S., JOHANSEN, G., AND SCHOUBYE, N. 1927 Refection, a transmissible change in the intestinal content, enabling rats to grow and thrive without vitamin B in the food. *J. Hyg.*, **27**, 70-102.
- GALL, LORRAINE S., FENTON, P. F., AND COWGILL, G. R. 1948a The nutrition of the mouse. II. Effect of diet on the bacterial flora of the intestine and the cecum. *J. Nutrition*, **35**, 13-25.
- GALL, LORRAINE S., ILLINGWORTH, B. A., COWGILL, G. R., AND FENTON, P. F. 1948b The nutrition of the mouse. III. Relation of diet to the synthetic activity of the predominating flora isolated from the small intestine and cecum. *J. Nutrition*, **35**, 27-38.
- GEYER, R. P., GEYER, BEVERLY R., DERSE, P. H., ZINKIN, T., ELVEHJEM, C. A., AND HART, E. B. 1947 Growth studies with rats kept under conditions which prevent coprophagy. *J. Nutrition*, **33**, 129-142.
- GUERRANT, N. B., AND DUTCHER, R. A. 1934a Effect of type of carbohydrate on vitamins B and G potency of feces voided by rats. *Proc. Soc. Exptl. Biol. Med.*, **31**, 796-800.
- GUERRANT, N. B., AND DUTCHER, R. A. 1934b Some effects of the composition of the diet on the vitamin B and the vitamin G requirement of the growing rat. *J. Nutrition*, **8**, 397-420.
- GUERRANT, N. B., DUTCHER, R. A., AND BROWN, R. A. 1937 Further studies concerning the formation of the B-vitamins in the digestive tract of the rat. *J. Nutrition*, **13**, 305-315.
- GUERRANT, N. B., DUTCHER, R. A., AND TOMEY, L. F. 1935 The effect of the type of carbohydrate on the synthesis of the B vitamins in the digestive tract of the rat. *J. Biol. Chem.*, **110**, 233-243.
- HAJNA, A. A., AND PERRY, C. A. 1943 Comparative study of presumptive and confirmative media for bacteria of the coliform group and for fecal streptococci. *Am. J. Pub. Health*, **33**, 550-556.
- JOHANSSON, K. R., SHAPIRO, S. K., AND SARLES, W. B. 1947 The flora of feces of hens as influenced by various carbohydrates in a biotin-deficient ration. *J. Bact.*, **54**, 35-36.
- KENDALL, A. I. 1911-1912 Certain fundamental principles relating to the activity of bacteria in the intestinal tract. Their relation to therapeutics. *J. Med. Research*, **25**, 117-187.
- LUCKEY, T. D., MOORE, P. R., ELVEHJEM, C. A., AND HART, E. B. 1946 Effect of diet on the response of chicks to folic acid. *Proc. Soc. Exptl. Biol. Med.*, **62**, 307-312.
- MITCHELL, H. K., AND ISBELL, E. R. 1942 Intestinal bacterial synthesis as a source of B vitamins for the rat. *Univ. Texas Pub.*, no. 4237, 125-134.
- MITCHELL, HELEN S. 1927 Comparative physiological values of five carbohydrates, based on growth and fecal analysis. *Am. J. Physiol.*, **79**, 537-541.
- MORGAN, AGNES F., COOK, B. B., AND DAVISON, H. G. 1938 Vitamin B₂ deficiencies as affected by dietary carbohydrate. *J. Nutrition*, **15**, 27-43.
- MORO, E. 1905 Morphologische und biologische Untersuchungen über die Darmbakterien des Säuglings. *Jahrb. Kinderheilk.*, **61**, 687-734, 870-899.
- PORTER, J. R., AND RETTGER, L. F. 1940 Influence of diet on the distribution of bacteria in the stomach, small intestine and cecum of the white rat. *J. Infectious Diseases*, **66**, 104-110.

- SARMA, P. S., SNELL, E. E., AND ELVEHJEM, C. A. 1946 The vitamin B₆ group. VIII. Biological assay of pyridoxal, pyridoxamine, and pyridoxine. *J. Biol. Chem.*, **165**, 55-63.
- SCHWEIGERT, B. S., MCINTIRE, J. M., HENDERSON, L. M., AND ELVEHJEM, C. A. 1945 Intestinal synthesis of B vitamins by the rat. *Arch. Biochem.*, **6**, 403-410.
- TAYLOR, A., PENNINGTON, D., AND THACKER, J. 1942 The vitamin requirements of cecectomized rats. *Univ. Texas Pub.*, no. 4237, 135-144.
- TEPLY, L. J., KREHL, W. A., AND ELVEHJEM, C. A. 1947 The intestinal synthesis of niacin and folic acid in the rat. *Am. J. Physiol.*, **148**, 91-97.
- THEILER, A., GREEN, H. H., AND VILJOEN, P. R. 1915 Contribution to the study of deficiency disease with special reference to the Lamziekte problem in South Africa. *So. Africa Dir. Vet. Research Rept.*, **3-4**, 7-68.
- TISSIER, H. 1908 Recherches sur la flore intestinale normale des enfants agés d'un an à cinq ans. *Ann. inst. Pasteur*, **22**, 189-208.
- TORREY, J. C. 1919 The regulation of the intestinal flora of dogs through diet. *J. Med. Research*, **39**, 415-447.
- WEGNER, M. I., BOOTH, A. N., ELVEHJEM, C. A., AND HART, E. B. 1941 Rumen synthesis of the vitamin B complex on natural rations. *Proc. Soc. Exptl. Biol. Med.*, **47**, 90-94.
- WILLIAMS, VIRGINIA R., AND FIEGER, E. A. 1946 Oleic acid as a growth stimulant for *Lactobacillus casei*. *J. Biol. Chem.*, **166**, 335-343.