EFFECT OF CECECTOMY ON THE FECAL FLORA OF WHITE RATS

Thesis for the Degree of M. S.
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THESIS

This is to certify that the

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EFFECT OF CECECTOMY ON THE FECAL FLORA OF WHITE RATS

By

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A THESIS

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INTRODUCTION

The influence of diet on the bacterial flora of the intestinal tract has been the subject of intensive research from the time Herter and Kendall (1909) first showed that a change in the diet from one dominating in proteins to that of milk and sugar is followed by a gradual but rapid substitution of an acidophilic non-proteolytic type of flora for a strongly proteolytic type. Later researches (Rettger and Horton, 1914; Hull and Rettger, 1917; Torrey, 1919; Porter and Rettger, 1940; Winblad, 1941; Mitchell and Isbell, 1942) have all confirmed this and established, beyond doubt, a relationship between the type of flora predominating in the intestinal tract and the type of food ingested.

Of late years, intestinal flora have come to be regarded as useful to the well being of the individual harboring them and one of the factors under consideration is their ability to synthesize accessory food substances. This view has changed the trend of research from the original line of search for harmful metabolic products, and normal flora of the intestinal tract have now come to be regarded as important symbionts rather than merely incidental, much less harmful.

Guerrant, Dutcher and Tamey (1935) observed cecal enlargements in white rats fed on diets rich in dextrinized corn-starch and indicated that the cecum of the rat is a seat of synthesis of B vitamins. These workers also discovered the presence, in the cecum of these rats, of large numbers of yeast cells and believed them to be the specific agents of elaboration of these vitamins. Abdel-Salam and Leong (1938) demonstrated the

synthesis of thiamin by a mixed flora taken from the rat cecum and grown *in vitro* although they failed to detect any diffusion of the synthesized vitamin B_1 from the bacterial cells into the broth medium in which the organisms were grown. Similar evidence of the synthesis of B vitamins by the intestinal flora in the rat cecum has been put forward by Mitchell and Isbell (1942), who think that the amount synthesized is markedly influenced by the type of flora and their numbers. Their investigation has also emphasized the marked effect that diet has on both the quality and quantity of the intestinal flora. Dam and associates (1941) considered *E. coli* as a probable organism responsible for the synthesis of vitamin K. The work of Taylor, Pennington and Thacker (1942) indicates, further, that the cecum contributes to the rats' supply of some of the B vitamins depending on whether the diet is of the type that will support a cecal flora of the right quantity and, or, quality. Day and associates (1943) have also shown this by experimenting on rats deprived of their ceca.

In their work on the study of the influence of diet on the distribution of bacteria in the various parts of the gastro-intestinal canal of rats fed on stock diet, Porter and Rettger (1940) have shown that whereas the normal bacterial flora of the young white rat is comparatively simple in the upper segments of the alimentary tract, it becomes increasingly more abundant in the lower sections of the small intestine. These workers found the cecum to harbor the highest number of bacteria and, with the exception of the animals fed on a high protein diet, all rats were shown to harbor large numbers of lactobacilli in their ceca. Coliform organisms were always found to be present in the cecum of these rats, regardless of the diet, as also other forms such as streptococci and anaerobes.

From the works cited above, it would naturally follow that the cecum in rats forms an important part of the alimentary tract and with

its large bacterial populations, it is not surprising that it has been shown to be an active portion of the intestinal canal where synthesis of B vitamins has been noticed to take place.

Rats fed on purified diets to which sulfonamides were added were discovered by Gant et al (1943) to bring about a marked diminution in their intestinal flora. These workers discovered that this reduction in the numbers of microflora had a definite effect on their rate of growth and resulted in the development of symptoms of vitamin deficiency.

During her study of the bacterial flora of the feces of rats, Miller (1945) observed that the number of organisms present varied from rat to rat and in the same animal from day to day. She did not find any significant change in the fecal flora following change of diet from Purina chow to a highly purified diet containing adequate amounts of all the growth factors. A drop in the number of coliform organisms as a result of addition of succinylsulfathiazole and phthalylsulfathiazole to the purified diet was, however, noted although this did not affect the number of aerobes present and it was presumed that the decrease in coliform bacteria resulted in the corresponding increase of other organisms. Rats fed on these diets containing succinylsulfathiazole and phthalylsulfathiazole were noted to exhibit symptoms usually associated with dietary deficiency and are a further indication of the role of these bacteria in the synthesis of some of the accessory growth factors.

The work of Taylor and associates (1942) on the vitamin requirements of cecectomized rats already referred to above has indicated that a diet deficient in several of the B vitamins when fed to cecectomized rats produces a severe effect on their health, whereas rats subjected to no cecectomy kept on the same diet thrive well. They have further concluded

from their experiments that where the diet supports the right intestinal flora, the cecum contributes to the rat's supply of several of the B vitamins. Which type or types of bacteria inhabiting the large intestines would be the most active in this respect has yet to be ascertained and is a matter for further research. Further work is also necessary to determine whether, in the event of cecectomy, any of these bacteria would establish themselves in other parts of the large intestine in sufficiently large numbers to be able to carry out the function of vitamin synthesis as indicated by these workers. That there is such an attempt on the part of the neighboring portions of the colon and the junction of the colon with the ileum in the cecectomized rats is evidenced by the changes of a compensatory nature observed by these workers in their rats.

Nutritional studies in progress at the Michigan State College under the auspicies of the Department of Home Economics have recently revealed the development of enlargements of the ceca (probably similar to those observed by Taylor et al, 1942, in their experimental animals) in rats fed on a diet consisting of Carnation Milk supplemented by a mixture of minerals containing iron phosphate, magnesium sulfate and copper sulfate, while no such abnormalities were observed in the control group of rats fed on a stock diet having the following composition:

Yellow corn meal	5,000 parts
Linseed oil meal	1,600 parts
Alfalfa meal	200 parts
Salt	50 parts
Calcium carbonate	50 parts
Casein	500 parts
Wheat germ	1,000 parts
Yeast	500 parts
Powdered milk	500 parts

PURPOSE

The work reported in this paper was undertaken to study the relative numbers of the various groups of microbes in the feces of

cecectomized and uncecectomized rats with a view to get an idea of the effect of cecectomy on bacterial population. Since the counts in these determinations varied considerably from rat to rat, the work was further extended to study their numbers in the feces of young rats before and after cecectomy to obtain a more comprehensive picture of the changes in the intestinal flora that might result from the removal of their ceca. A comparative idea of the relative numbers of different important species of intestinal microbes in the cecal contents and feces of normal rats was obtained by making tests on samples of relevant material from two of the rats collected at the time of cecectomy.

MATERIALS AND METHODS

A total of 32 weanling male albino rats was used in the feeding experiments referred to above. Of these, a group of 16 rats was fed on Carnation Milk supplemented by the minerals and the second group, comprising the remaining 16 animals, was fed on the stock diet as per formula given above. Half the number of animals in each group were cecectomized and the remainder of the animals in the group served as normal controls which were not cecectomized.

Two rats from each of these four groups were selected at random and counts of the principal groups of bacteria in the feces of the cecectomized and control rats fed on the two diets were determined at varying intervals after cecectomy.

Media:—The choice of media for this investigation was made comparatively simple as a result of the recent work of Evenson and others (1946) who have reported a very useful set of media for use in this kind of work. They have developed a medium for each type of important microbes that commonly occurs in the intestinal tract, particularly of white rats. Liver infusion broth made from bacto-tryptone,

glucose and liver extract, as used by these authors, has proven excellent for getting a "total count" of viable bacteria and is very simple to make. The only modification made in this medium has been the use of dehydrated bacto-liver powder in place of fresh liver, which is often difficult to obtain. Six grams of the powder in 100 ml of water steamed for 30 minutes in the Arnold Steamer gave sufficient liver extract for a litre of the medium. This liver powder also replaced the dried liver chunks introduced to provide anaerobic conditions in the medium and a quantity of the powder sufficient to cover the flat end of a small sized spatula was all that was necessary to be added to each tube before dispensing the infusion broth. The medium was finally sterilized by autoclaving at 15 pounds for 15 minutes.

E. C. Medium for growing the coliform organisms was at first made after the formula of Hajna and Perry (1943). This medium was subsequently discovered to be available in a dehydrated form.

The S. F. Medium for growing enterococci and Winblad's acetic acid medium were adopted for use without any modifications. There being no facilities available for incubation of the culture at 45 degrees C, the S. F. cultures were incubated at 37 degrees C. This allowed the growth of some other organisms besides the Streptococcus fecalis. Examination of smears from all the tubes showing a change in the color of the medium and a few following dilutions showing no color change, however, was enough to decide the presence of streptococci. It was noticed from the results of smear examination that all tubes showing a change in color revealed the presence of streptococcal chains and all the others were found to be negative. As a result of this conformity between the color change and smear examination, the practice of examining smears was later abandoned to save time.

The Winblad's acetic acid medium used for growing lactobacilli and yeast-like organisms proved equally efficient as claimed by Evenson et al (1946). It was noted, on microscopic examination, that all tubes showing gas in the inserts revealed the presence of yeast-like cells in the smears. This was not true of the tubes in which gas was not trapped in spite of shaking the culture tubes several times during incubation as recommended by the original authors, and smears from such tubes always proved negative for these micoorganisms.

The formulas of the media used in this work are listed in Appendix I.

Animals:—As mentioned previously, young albino rats, about 3 weeks of age, were fed on the two diets. Half the number of animals in each feed group were cecectomized and the other half left as normal controls. Two rats from each of the four groups (each group consisting of eight animals) were used for bacterial examination of their feces. For making a comparative count of the various groups of flora in the cecum and feces, cecal contents were collected from two rats at the time of cecectomy by squeezing into sterile petridishes, under aseptic conditions, the contents of the ceca immediately after their removal from the body. All fecal samples were collected in sterile petridishes by gently squeezing, with all possible precautions of asepsis, a little of the fecal material from the rectum of the rats held from the tail.

For the determination of counts, a small amount of the material (about 0.1 gram) was transferred to previously weighed sterile test tubes containing glass beads and the actual amount of material used determined by weighing the tubes with the samples in them. Sufficient sterile distilled water was added to give a known dilution (usually 1:100) and a homogeneous suspension was obtained by a thorough and vigorous shaking. Further decimal serial dilutions of the material to 10^{-11} were obtained

by transferring 10 ml of the suspension into stoppered bottles containing 90 cc sterile distilled water. By inoculation of 1 ml quantities of each dilution (10^{-3} to 10^{-11}) into sets of three tubes of each kind of medium and incubation at 37 degrees C for 3 days, the number of tubes showing growth in each dilution was determined. Counts of various types of organisms per gram weight of the material examined were obtained by the dilution count method. Growth was judged by the presence of turbidity, acid production and gas production. Examination of smears after staining by Gram's method from each type of medium after growth had been obtained showed the predominance of the particular type of organisms for which the medium was intended, thereby proving the suitability of each medium used. The actual counts were obtained by a reference to the Dilution Count Tables (Buchanan and Fulmer, 1928).

RESULTS

Table I shows the counts of the various types of bacteria in the cecal contents and feces collected from two rats at the time of cecectomy.

The average counts of the fecal flora obtained in cecectomized and uncecectomized rats belonging to the two groups fed on the Carnation Milk diet and stock diet are recorded in Tables II and III respectively. The graph (Fig. 1) shows the composite growth curves of the two groups of rats covering the entire period of observation of the feeding experiment. Table IV is included to show the counts as determined on a group of four white rats 10, 5 and 0 days before cecectomy and 6, 11 and 17 to 18 days after the operation. This table also shows the average counts of the whole group of four rats.

DISCUSSION

It will be observed from Table I that bacterial counts of most

TABLE I:—BACTERIAL FLORA OF CECAL CONTENTS AND FECES OF WHITE RATS

Rat No.	Material	Total and Group Counts—From Dilution Count Data				
		Total Count	Coliforms	Lactobacilli	Enterococci	Yeast-like Organisms
23	Cecal contents	450,000,000	250,000,000	250,000	2,500	<250
23	Feces	9,500,000,000	950,000,000	950,000	2,500	<250
25	Cecal contents	450,000,000	9,500,000	25,000,000	450	45,000
25	Feces	2,500,000,000	95,000,000	250,000,000	4,500	4,500

TABLE II:—FECAL FLORA OF CECECTOMIZED AND UNCECECTOMIZED RATS ON CARNATION MILK DIET—AVERAGE FROM DILUTION COUNT DATA

Days after Cecectomy or Starting the Feed	No. of Animals in the Group	Total Counts	Coliforms	Lactobacilli	Enterococci	Yeast-like Organisms
14	2 cecectomized	49,750,000,000	14,750,000,000	1,725,000	2,375,000,000	<250
14	2 uncecectomized	2,500,000,000	450,000,000	22,500,000	5,750,000	2,725
28	2 cecectomized	27,250,000,000	7,000,000,000	1,325,000	147,500,000	<250
28	2 uncecectomized	2,500,000,000	1,251,000,000	7,000,000	5,225,000	<1,375
48	2 cecectomized	17,250,000,000	49,750,000,000	497,500,000	1,375,000,000	<250
42	2 uncectomized	1,375,000,000	70,000,000	6,000,000	1,475,000	10,750
63	2 cecectomized	49,750,000,000	2,450,000,000	62,500,000	12,975,000,000	41,000
68	2 uncectomized	272,500,000	32,500,000	275,000	2,297,500	12,700

TABLE III:—FECAL FLORA OF CECECTOMIZED AND UNCECTOMIZED RATS

ON STOCK DIET—AVERAGE FROM DILUTION COUNT DATA

Days after Cecectomy or Starting the Feed	No. of Animals in the Group	Total Counts	Coliforms	Lactobacilli	Enterococci	Yeast-like Organisms
14	2 cecectomized	3,500,000,000	1,252,250,000	25,000,000	252,500	2,297,500
15	2 uncecectomized	5,225,000,000	229,750,000	95,000,000	32,500	85,000
27	2 cecectomized	7,000,000,000	48,750,000	1,012,500	2,375,000	17,250
29	2 uncecectomized	12,725,000,000	1,262,500	7,000	2,725,000	2,475
54	2 cecectomized	14,750,000,000	70,000,000	1,262,500,000	1,375,000	147,500
56	2 uncecectomized	2,297,500,000	1,375,000	35,000,000	2,725,000	15,000
67	2 cecectomized	2,475,000,000	1,252,250,000	1,500,000	<22,500	70,000
68	2 uncecectomized	1,125,000,000	1,225,000	35,000	<250	<2,250

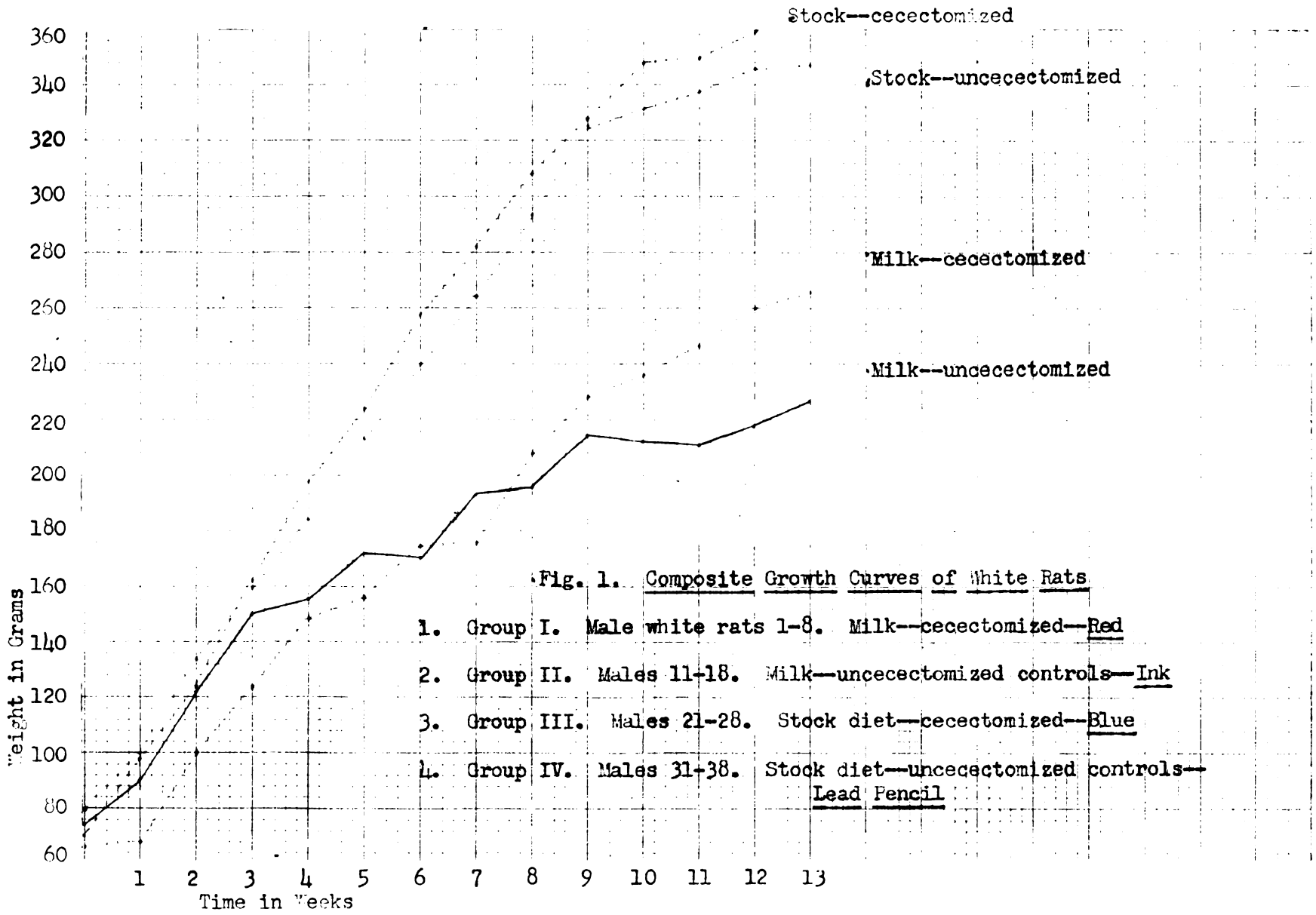

Fig. 1. Composite Growth Curves of White Rats

1. Group I. Male white rats 1-8. Milk--cecectomized--Red
2. Group II. Males 11-18. Milk--uncecectomized controls--Ink
3. Group III. Males 21-28. Stock diet--cecectomized--Blue
4. Group IV. Males 31-38. Stock diet--uncecectomized controls--Lead Pencil

TABLE IV:—FECAL FLORA OF WHITE RATS ON STOCK DIET BEFORE AND AFTER CECECTOMY—
COMPARISON OF COUNTS IN INDIVIDUAL RATS—COUNTS FROM DILUTION COUNT DATA

Rat No.	Days before or after Cecectomy	Bacterial Counts				
		Total Counts	Coliforms	Lactobacilli	Enterococci	Yeast-like Organisms
1	10 days before	4,500,000,000	1,500,000	95,000,000	95,000	950,000
2		25,000,000,000	450,000	150,000,000	2,500,000	450,000
3		15,000,000,000	2,500,000	150,000,000	95,000	25,000
4		4,500,000,000	4,500,000	20,000,000	450,000	250,000
Average of 10 day counts		12,250,000,000	2,240,000	104,000,000		
1	5 days before	15,000,000,000	2,500,000	450,000,000	4,500,000	250,000
2		9,500,000,000	1,500,000	95,000,000	2,500,000	9,500
3		25,000,000,000	450,000	950,000,000	9,500	9,500
4		2,500,000,000	400,000	45,000,000	95,000	20,000
Average of 5 day counts		13,000,000,000	1,200,000	385,000,000		
1	0 days before	20,000,000,000	950,000	75,000,000	1,500,000	150,000
2		9,500,000,000	95,000	1,500,000	950,000	150,000
3		9,500,000,000	650,000	4,500,000	150,000	95,000
4		2,500,000,000	250,000	4,500,000	45,000	95,000
Average of 0 day counts		10,400,000,000	490,000	21,400,000		
1	6 days after	4,500,000,000	25,000,000	2,500,000	200,000	95,000
2		9,500,000,000	4,500,000	75,000	150,000	25,000
3		4,500,000,000	95,000,000	95,000,000	450,000	75,000
4		4,500,000,000	25,000,000	200,000,000	200,000	150,000
Average of 6 day counts		5,750,000,000	37,000,000	74,400,000		
1	11 days after	95,000,000,000	950,000,000	20,000,000	250,000,000	9,500
2		9,500,000,000	250,000,000	4,500,000	2,500,000	7,500
3		25,000,000,000	9,500,000	450,000	400,000	2,500
4		4,500,000,000	9,500,000	9,500,000	95,000	1,500
Average of 11 day counts		33,500,000,000	300,000,000	8,600,000		
	18 days after	25,000,000,000	9,500,000	150,000,000	25,000	200,000
		4,500,000,000	4,000,000	25,000,000	<250	350,000
	17 days after	25,000,000,000	95,000,000	450,000,000	2,500	950,000
		9,500,000,000	25,000,000	200,000,000	<250	95,000
Average of 17-18 day counts		16,000,000,000	33,000,000	206,000,000		

of the species in the feces are comparatively higher than the corresponding counts in the cecal contents. It is difficult to draw any conclusions from data obtained from only two rats and attribute any significance to these higher fecal counts. These findings are, however, contrary to those of Porter and Rettger (1940), who found cecum to harbor the highest number of bacteria and indicate that bacterial proliferation does continue in the intestinal tract beyond the cecum.

The higher incidence of total numbers of bacteria, coliform organisms and enterococci in cecectomized rats fed on the Carnation Milk diet as compared with the corresponding counts in the control animals in the same feed group (see Table II) is a striking feature of this study. The number of lactobacilli which was lower in the cecectomized rats in the first two determinations also rose about 100 to 200 times in the last two determinations. It is surprising to note that in spite of being devoid of their ceca these rats were observed to maintain the same rate of growth as those with their ceca intact. In the case of the group of rats fed on stock diet, the "total counts" were not markedly different in the cecectomized rats as compared with the uncecectomized, and the enterococci counts were variable, but the coliform counts were throughout higher and so were the lactobacilli except in the first sampling. Even in this group the growth curves of the cecectomized and uncecectomized rats are exactly identical. Whether higher bacterial counts would have any bearing on the rate of growth of these animals is purely a matter of conjecture and hard to conceive. But since these two groups of rats were kept under absolutely identical conditions with the absence of cecum as the only variable factor besides the variation in bacterial population, the higher numbers of bacteria might influence the growth through their ability to synthesize some of the accessory

food factors of which they would otherwise be deprived by the removal of the cecum which has been shown to be a seat of synthesis of these factors by some of the microbes in the intestinal tract.

The effect of cecectomy on the fecal flora is further illustrated by the results of determinations carried out on a group of four rats at varying intervals before and after cecectomy (Table IV). It will be observed from this table that the total counts in these rats were fairly constant throughout the period of observation and were in close proximity with those obtained by some recent workers (Everson et al, 1946) in their control group of rats. The counts of lactobacilli, enterococci and the yeast-like organisms have been quite variable both before and after cecectomy and give no indication of the effect of the removal of ceca. The coliform organisms, however, have been found to show a definite increase in numbers in all the rats in tests made subsequent to the operation as compared with the counts obtained in the same rats before cecectomy. These higher counts appear to have been maintained in all the tests made during a period of 18 days after the removal of the cecum. The number of animals used in the experiment, however, is rather small and the results cannot be accepted as generally true unless the experiment is repeated on a larger number of rats fed on different kinds of diets to make the data statistically sound. The fact that these rats continued to maintain a good general level of health following cecectomy as indicated by their general appearance and their composite growth curve (Fig. 2) leads one to presume that this increase in the coliform counts might have a favorable influence on their health. This presumption would be supported by the observations of Miller (1945) on her rats which exhibited symptoms of dietary deficiency when a drop in the number of coliform organisms was brought about by the addition to their purified

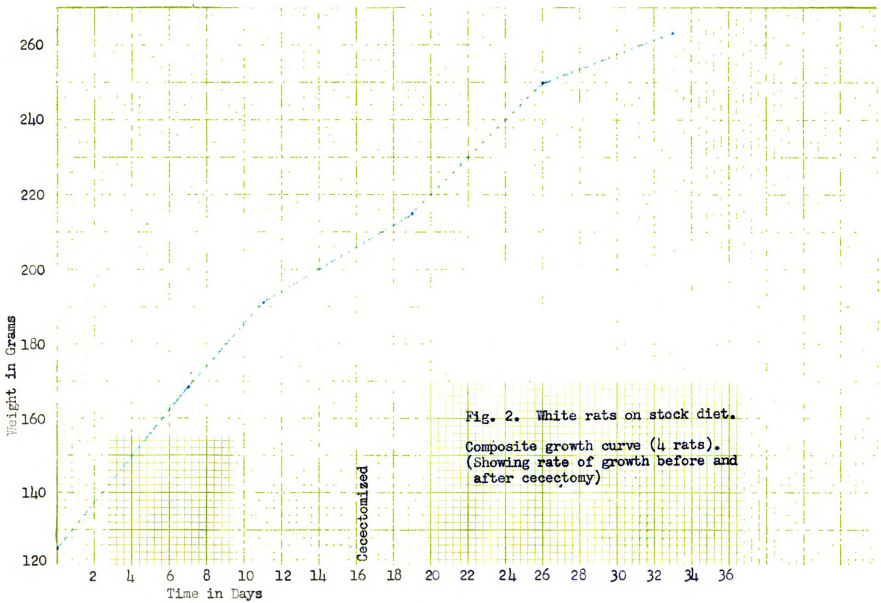

Fig. 2. White rats on stock diet.

Composite growth curve (4 rats).
(Showing rate of growth before and after cecectomy)

diet of succinylsulfathiazole and phthalylsulfathiazole.

SUMMARY

A study of the bacterial populations of the cecal contents and feces of two young albino rats revealed the numbers of various groups of intestinal bacteria to be larger in the feces than in the cecal contents.

Young cecectomized rats fed on Carnation Milk showed higher "total bacterial counts" as well as coliform and enterococci counts in their feces as compared with the corresponding counts in uncecectomized rats. The lactobacilli counts were lower in cecectomized rats in second and fourth week samplings but increased considerably in the subsequent determinations. In the group of rats fed on stock diet, the "total counts" were comparable in the cecectomized and uncecectomized rats, the coliform and lactobacilli counts were definitely higher in the cecectomized, but the numbers of enterococci and yeast cells were very inconsistent. The composite growth curves of these animals for a period of 12 to 13 weeks were much better in rats fed on stock diet than those fed on Carnation Milk but the cecectomized rats showed growth curves identical with those with their ceca intact, thereby showing that cecectomy has no untoward effect on the rate of growth.

Examination of feces of a group of four young rats for different groups of microbes before and after cecectomy has shown the "total counts" to be fairly consistent and other groups to be quite variable. The animals showed a definite increase in their coliform counts in determinations made subsequent to cecectomy.

It is suggested that the increase in the number of bacteria in the intestinal canal of rats following cecectomy might have a favorable influence on their health and might possibly be a compensatory mechanism which provides a substitute for lost physiological function.

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APPENDIX

FORMULAS OF MEDIA USED IN THIS WORK

Liver Infusion Broth

Bacto-tryptone	5 gm
Glucose	5 gm
Liver extract	100 ml
Tap water to make	1,000 ml

Tubed with added liver powder (Bacto)
Autoclave at 15 pounds steam pressure for 15 minutes

Liver Extract

Bacto-liver powder	60 g
Tap water	1,000 ml

Steam in Arnold Steamer for 30 minutes; filter through gauze

E. C. Medium (Hajna and Perry, 1943)

Distilled water	1,000 ml
Bacto tryptose	20 gm
Sodium chloride	5 gm
Lactose	5 gm
Dipotassium hydrogen phosphate (K_2HPO_4)	4 gm
Monopotassium hydrogen phosphate (KH_2PO_4)	1.5 gm
Bacto bile salts No. 3	1.5 gm

Titration of pH and filtration not necessary; final pH 6.9
Autoclave at 15 pounds steam pressure for 15 minutes

S. F. Medium (Hajna and Perry, 1943)

Distilled water	1,000 ml
Bacto tryptone	20 gm
Sodium chloride	5 gm
Glucose	5 gm
K_2HPO_4	4 gm
KH_2PO_4	1.5 gm
Sodium azide	0.5 gm
Brom cresol purple, 1.6% alc. sol.	2 ml

Autoclave at 15 pounds steam pressure for 15 minutes

Winblad's acetic Acid Medium for Lactobacilli and Yeast Cells

Distilled Water	1000 ml
Beef extract	3 gm
Bacto tryptose	10 gm
Bact dextrose	5 gm
Sodium chloride	5 gm

Acetic acid sufficient to bring the concentration to 0.12%

Dispense with enclosed fermentation tubes up to a level of the inserts

Autoclave at 15 pounds steam pressure for 15 minutes

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