THE INFLUENCE OF DIET ON THE pH OF THE DIGESTIVE TRACT OF THE ALBINO RAT

Thesis for the Degree of M. S.

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1935

THE INFLUENCE OF DIET ON THE pH OF THE DIGESTIVE TRACT OF THE ALBINO RAT

A THESIS

Submitted to the Faculty of the Graduate School

of

MICHIGAN STATE COLLEGE

in Partial Fulfillment of the Requirements for the Degree

of

MASTER OF SCIENCE

by

Russel James Davis

June, 1935

ACKNOWLEDGMENT

The writer wishes to express full appreciation to Dr. C. A. Hoppert for his guidance, direction, and assistance in the conduct of the experiment herein reported.

CONTENTS

HISTORICAL REVIEW

Determinations of the pH of the contents of different parts of the gastro-intestinal tract have been carried out by many investigators on various animals.

(1) Zucker and Matzner working only on the feces of rats on a rickets producing diet, when made into a suspension with water, gave a pH of 7.4 to 8.0. When such rats were given the active principle of cod-liver oil, the reaction of the feces changed in a few days to the acid side, usually about pH 6.2 or even as far as pH 5.7. Controls with cotton seed oil gave an unchanged alkaline reaction of the feces. (2) (3) These results obtained by Zucker and Matzner were confirmed by Jephcott and Bacharach who found that in using Sherman and Pappenheimer diet that the fecal pH always reached a value well above 7.0, The time taken was from 10 to 15 days, and the alkalinity had been maintained either to the end of the period of feeding the ricketogenic diet or until the animals had received cod-liver oil, irradiated cholesterol or had been exposed 15 to 20 minutes a day to ultra-violet radiation

from an open tungston arc. (4) Oser observed that the fecal pH curves were usually irregular. Even when a definite tendency in one direction or another obtained, there were occasional marked divergencies from the general trend. (5) Schohl and Bing observed a change in the feces from alkaline to acid when rats fed Zucker's diet were cured of rickets by cod-liver oil. This change did not occur when rats made rickets on Steenback's diet were cured by irradiation of the food or by the addition of alkaline phosphates. Hence, the cure of rickets is not necessarily associated with alteration of the pH of the feces from alkaline to acid. (6) Redman did not find it possible to obtain a definite correlation between the different states of the disease of rickets and the pH values of the feces of children.

Certain observers maintain that rickets is primarily a disturbance of calicum and phosphorus absorption inasmuch as the percentage of these elements excreted by way of the feces is abnormally high in rickets. (7) Zucker and his co-workers using a diet, which, from the point of view of balance between calicum and phos-

phorus should not be ricketogenic, were able to produce rickets by increasing the alkalinity of the intestinal tract. (8) Bergeim added common carbohydrates such as starch glucose, fructose, maltose, dextrin and lactose to the diet of the ricketic animals to make up 25 to 50 per cent of such diets, and studied the calicum and phosphorus absorption. The first five carbohydrates had no effect on absorption when composing 25 per cent of the diet and little effect when composing 50 per cent. Lactose, on the other hand, gave pronounced increases in calicum and phosphorus absorbed at the 25 per cent level. The influence of lactose and to a lesser extent dextrin on absorption was believed to be due to increased lactic acid fermentation in the intestine with resulting increased acidity of the intestinal contents.

(9) Yoder found correlations between lowered pH and calcium and phosphorus utilization and agreed with other workers that cod-liver oil lowers the pH throughout the intestinal tract of rats on ricketic ration.

(4) Oser found that on the average there was

practically no difference between normal and rickетic rats in the reaction of the contents of the upper ileum. The alkalinity tended to increase along the intestinal tract, the average being somewhat higher in ricketic rats, until a maximum was reached in the cecum. In the normal rat the pH of the contents decreased slightly but definitely below the value of the cecum. This tendency was not observed in the ricketic animals. (10) Wokes and Willimott reported averages showing an increase in the pH beyond the cecum in ricketic rats, but not in normal animals.

(11) Redman and co-workers, studying the effect of dilution on the pH of the contents of the digestive tract of normal guinea-pigs, found in general that the acidity of the contents of the stomach and duodenum tended gradually to increase during the first four hours after the ingestion of a meal. In the remainder of the tract, no appreciable differences could be observed when the time between feeding and death was from 1½ to 4½ hours. (12) The problem of whether intestinal bacterial flora had any in-

fluence on the pH of the gastro-intestinal tract was carefully studied by Rettger and Cheplin working with albino rats and human beings, and in both cases the conclusion was reached that the diet was the controlling factor.

In view of the fact that much of the work reported was of a fragmentary and often contradictory nature, it was considered advisable to carry out a fairly comprehensive study of the influence of diet on the pH of the intestinal tract of the rat and to give some attention to the influence of the age of the animals.

ELECTROMETRIC DETERMINATION

(13) The quinhydrone electrode, since its introduction by Biilmann, has been employed by a number of investigators, and found to give reliable results within the pH range of 1 to 8. (14) Robinson made comparisons between the quinhydrone electrode and the hydrogen electrode on fecal material, and the results varied only .08 pH. (11) Redman and co-workers compared the quinhydrone electrode with the colorimetric method, using different indicators, and found that duplicate results usually agreed to 0.2 pH. (15) Grazel and Miller, using the colorimetric method estimated the

average error to be o.2 pH. A comparison of the colorimetric with the electometric method "justifies reliance on comparative results as obtained colorimetrically."

A comparison of the quinhydrone electrode and the colorimetric methods brings out the advantages of the former in that it is generally more reliable and considerably more rapid and convenient because it is unnecessary to have clear solutions.

PLAN OF EXPERIMENT

The animals used for these experiments were both albinos and piebald rats. All but one group were mature animals. The rations listed below were given ad libitum to the rats from the time they were first started on the experiment, and continued for four weeks. The animals were killed by etherization and the pH determinations made as described below.

During the period the rats were on the experiment, fecal pH determinations were made. Pellets were obtained by extracting directly from the animal or by removing fresh pellets from the bottom of the cage.

Three series of experiments were carried out. The first was a comparison between a lactose free diet and

one containing twenty per cent lactose, each diet being fed without and with supplements of cod-liver oil and irradiated yeast. In the second series groups of young and mature rats received the ricketogenic diet again without and with supplements of cod-liver oil and irradiated yeast. The third series compared the effect of a high carbohydrate, high fat, and high protein diet without and with yeast supplement on the pH of the gastro-intestinal tract.

COLLECTION OF MATERIAL

The animals were killed by ether between the hours of eight and ten in the morning inasmuch as it was observed that the stomach and intestinal tract contained a more uniform content during this period. The abdomen was opened immediately, and the stomach ligatured at both ends. The esophagus was cut above the ligature, and the large intestine just above the anus. The whole of the gastro-intestinal tract was then removed and placed in physiological salt solution. The gastro-intestinal tract was divided into nine portions as follows: The stomach, into the cardiac sac and the pylorus; the small intestines into five equal portions; the cecum and the colon were taken as one portion each.

A part of each of the nine divisions was mixed with two centimeters of distilled water and triturated with quinhydrone, poured into the electrode vessel and the pH determined. The pH determinations were completed within thirty to forty minutes.

DATA AND RESULTS

The following diets were used in experiment 1, pH values were tabulated in Table 1 and plotted in Graph 1.

(1)	#1-#4	#1a	#8	#4a	#7	#2-#5	#2a	#5a
Cornmeal	35	35	35	35	35	35	35	35
Oatmeal	40	40	40	40	40	20	20	20
Lactose						20	20	20
Oil meal	10	10	10	10	10	10	10	10
Alfalfa	4	4	4	4	4	4	4	4
Corn Oil	5	5		5	5	5	5	5
Yeast	5	5	5	4		5	5	4
Nacl.	1	1	1	1	1	1	1	1
Cod-liver Oil		1	5				1	
Irrad. Yeast				1	5			

The presence of twenty per cent lactose evidently had no effect on the pH of the material in the stomach or in the first portion of the small intestines as compared with a normal lactose free diet. With the latter diet, the pH gradually rose in the lower bowel to 7.4, and then decreased to 5.6 in the cecum. The colonic

material had an average pH of 5.8. The twenty per cent lactose diet decreased the alkalinity in the lower bowel to a pH of 6.15. The pH in the cecum and colon deereased to an average of 5.45.

When the lactose free diet was supplemented with vitamin D in the form of cod-liver oil or irradiated yeast there was a slight decrease in alkalinity throughout the intestinal tract. Supplementing the twenty per cent lactose diet with cod-liver oil decreased the alkalinity on an average of .5 pH throughout the tract. Irradiated yeast increased the alkalinity slightly in the lower bowel. Both cod-liver oil and irradiated yeast increased the alkalinity between the cecum and the colon.

The lactose free diet was also supplemented with five per cent cod-liver oil and five per cent irradiated yeast, the resulting pH values were similar to the one per cent cod-liver oil and irradiated yeast supplement.

The increase in acidity of the contents of the lower bowel effected by the presence of lactose in the diet is undoubtedly due to the production of lactic acid from lactose by certain bacteria found in this portion of the digestive tract.

The following diets were used in experiment 2, pH values were tabulated in Table 2 and plotted in Graph 1.

(2)	#3-#6-#III	#3a-#IIIa	#6a-#VIa
Cornmeal	37.5	37.5	37.5
Oatmeal	37.5	37.5	37.5
Wheat gluten	20.	20.	20.
Na Cl.	1.	1.	1.
Yeast	1.		
$CaCO_3$	3.	3.	3.
Cod-liver oil		1.	
Irrad. Yeast			1.

Since the stomach was divided into two portions, some difficulty was encountered when comparing the reaction of ricketic diets on young and mature rats. From Table 2 and Graph 2 the pH results found in the stomach was very irregular. This no doubt was due to the varying amounts of material found in the stomach. However, the contents of the small intestines did not seem to be similarly affected.

In the study of the pH of the material in the small intestines, more uniform results were obtained with mature rats than younger animals on the same diet. The

pH gradually rose along the intestinal tract from an average of about 6.0 to 7.7 pH, then decreased in the cecum to an average of 6.9 and rose again slightly in the colon to 7.0. Oser (1928) observed in normal rats that the pH of the contents of the colon fell slightly but definitely below the value of the cecum, whereas in ricketic rats this did not take place. This decrease in alkalinity of the colonic contents of the normal rat was regarded as due probably to phosphorus reabsorption which normally occurs at this portion of the intestinal tract. The sustained increase in alkalinity in this region in the ricketic rat appears to be associated with inadequate phosphorus reabsorption.

The addition of vitamin D supplement in the form of cod-liver oil or irradiated yeast to the ricketogenic diet resulted in wide variations in the pH in both portions of the stomach and in the first half of the small intestines. Again, this no doubt was due to the varying amounts of material in the stomach and first portion of the small intestines. The vitamin D supplements caused a slight increase in the alkalinity of the contents of the tract of the mature rats, whereas, the alkalinity was decreased in the case of the young rat. Little difference in pH was noted in the cecum and colon

of the mature rat whether a vitamin D supplement was used or not. In the young rat fed the ricketogenic diet, an average pH of 7.2 was observed in the cecum and 7.5 in the colon, whereas, with the vitamin D supplement, the cecum gave 6.9 and the colon 7.04.

The following diets were used in experiment 3, pH tabulated in Table 2 and plotted in Graph 3.

	High carbohydrate		High Protein		High Fat	
	#9	#9a	#10	#10a	#11	#11a
Cornmeal	42	39.5	32	29.5	32	29.5
Oatmeal	42	39.5	32	29.5	32	29.5
Oilmeal	5	5	20	20	20	5
Casein	5	5	10	10	5	5
Alfalfa	5	5	5	5	5	5
Yeast		5		5		5
Na Cl	1	1.	1.	1.	1.	1.
Corn Oil					20.	20.

The third series, as shown in Graph 3 was a comparison between diets rich in carbohydrates, protein and fat, respectively. An average pH of 4.5 and 2.7 was observed in the stomach with each of these diets. The average pH 5.8 in the small intestines gradually rose to 7.4 and then dropped to 5.8 in the cecum. A slight increase in the alkalinity of the contents of the colon was observed on all of these diets.

This failure to produce any significant differences in the intestinal pH by varying the carbohydrate, protein and fat ratios of diets was essentially in agreement with

the findings of Abrahamson and Miller (1925) and also Redman, Willermott and Wokes (1927).

The addition of five per cent yeast to these different diets evidently had no effect on the pH of the digestive tract.

Discussion

This study of the intestinal tract of the rat indicated a rather striking constancy in the pH values found along the tract altho the diets varied radically in their makeup. There is apparently a well regulated control in neutralizing the acid contents that are poured into the intestinal tract, this being accomplished by the various basic secretions; namely, the pancreatic, bile and intestinal secretions. This strongly suggests that the acid poured into the stomach is compensated for accurately by the basic compounds emptied into the intestines and possibly helps to account for the fact that there is practically no change in the pH of the blood during the period of digestion.

The pH of the digestive tract can apparently be altered consistently only when considerable quantities of acid or basic compounds are included in the diet or when the diet contains a component which by bacterial activity is capable of producing an acid along the digestive tract. The first case is illustrated by the feeding of a ricketogenic diet which is characterized by its high content of calcium carbonate. This caused

an increase in alkalinity, particularly in the lower portion of the bowel. Upon supplementing the ricketogenic diet with vitamin D in the form of cod-liver oil or irradiated yeast, a fairly marked decrease in alkalinity was observed in the lower bowel of the young rat, whereas in the mature rat the effect on the alkalinity was very slight. This neutral or slightly alkaline condition of the colonic contents of the ricketic rat has been explained as being due to inadequate phosphorus reabsorption, although recent work indicates that the excretion of calcium carbonate in this portion of the lower bowel may be an important contributing factor. The difference in response between the young and mature rats may be explained as being due to the difference in calcium and phosphorus retention. The young animals obviously need considerable amounts of these elements for bone development, whereas this demand no longer exists in the mature rat.

The second case is illustrated by feeding diets containing a fairly high content of lactose. A marked increase in acidity was observed in the lower portion of the small intestines and in the cecum. At no place in the tract did the contents reach the neutral point.

This marked increase in acidity, particularly in the lower bowel was undoubtedly due to the formation of lactic acid, formed by the fermentation of lactose in this portion of the tract. When cod-liver oil was added to the lactose diet a further increase in acidity was observed, starting from about the middle portion of the small intestine and continuing through the cecum. This effect does not seem to be due to the vitamin D because irradiated yeast did not show a similar action. No explanation seems to suggest itself other than that an increased lactic acid fermentation may have prevailed. Somewhat puzzling too are the variations observed in the pH of the contents of the colon, for with both vitamin D supplements, a marked rise in pH occurred, whereas no change was noted in the case of the unsupplemented lactose diet. Whether these differences are due to variations in the absorption of the lactic acid from the colon or due to the excretion of bases is still a matter to be determined. In any event the marked influence of lactose in establishing acid conditions in the lower portion of the small intestine, in the cecum, and in the colon, perhaps accounts for decreasing the putrefactive processes normally occurring

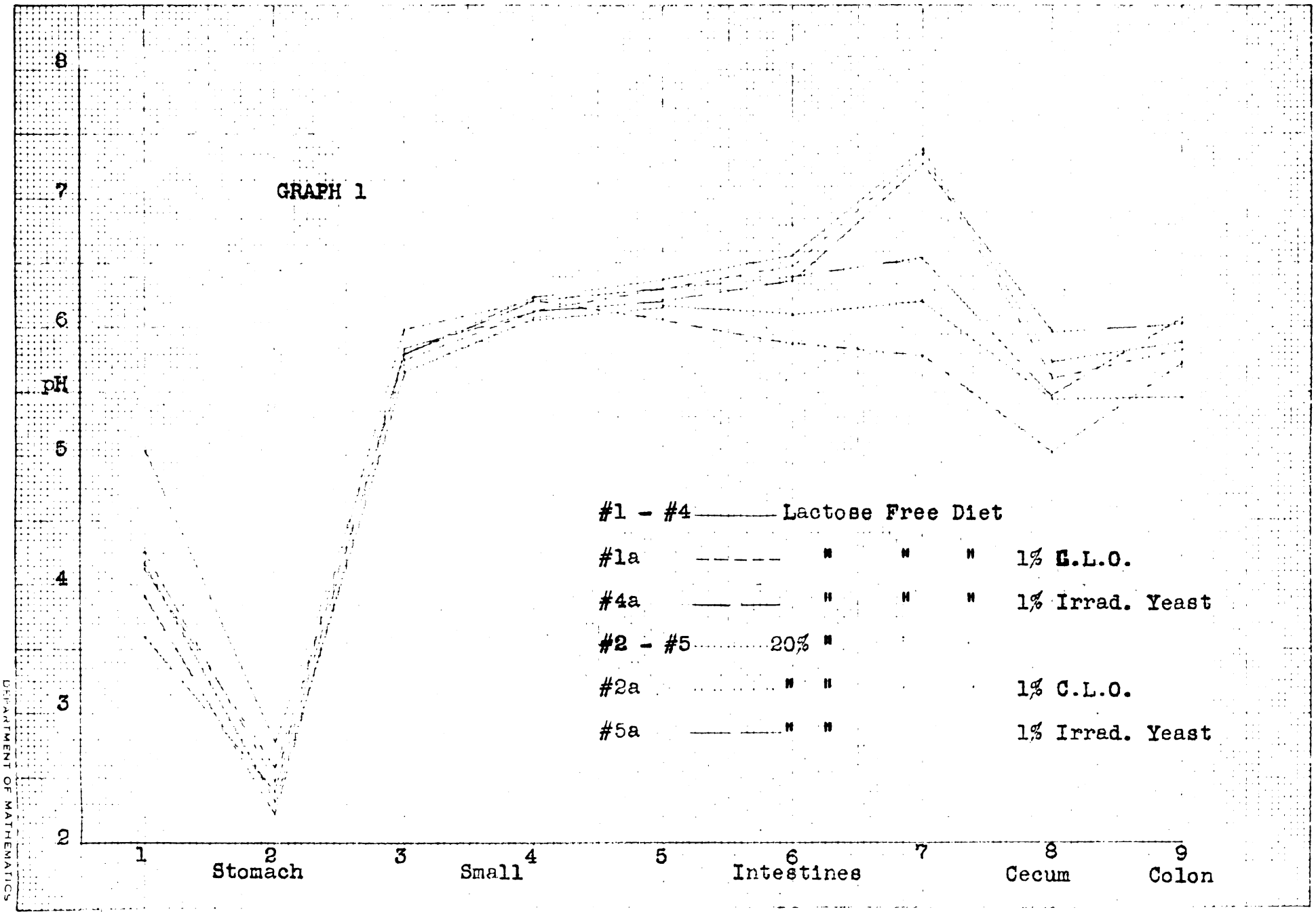
to a greater or lesser degree along the digestive tract and therefore is probably responsible for some of the benefits that are derived from the ingestion of milk.

SUMMARY

1. The pH of the digestive tract of the rat is strikingly constant, even though the diets may vary widely in composition.
2. On normal diets, containing no lactose, the average pH of the small intestines gradually increased from 5.8 to 7.3, then decreased to 5.7 in the cecum and again increased slightly in the colon.
3. Diets high in carbohydrates, proteins and fats gave pH values similar to those obtained with normal diets.
4. On ricketogenic diets, high in calcium carbonate, an increase in alkalinity throughout the tract was observed, especially in the lower bowel of both young and mature rats. When the diet was supplemented with vitamin D, a decrease in alkalinity in the lower bowel of the young rats was observed, whereas in the mature rat, the change in alkalinity was negligible.
5. Diets containing 20% of lactose showed an increase in acidity especially in the lower bowel.

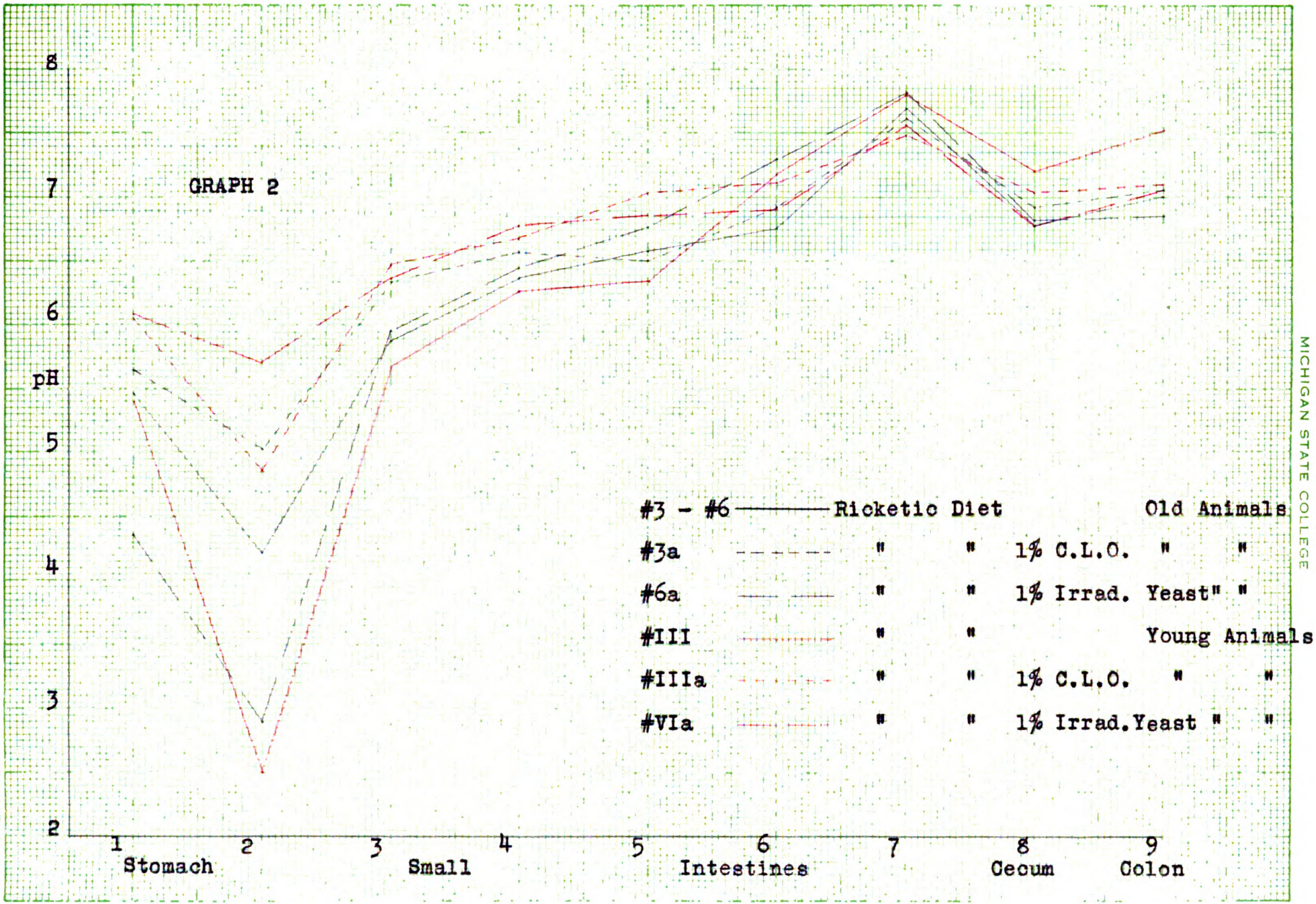
Series	No. of rats	Stomach		Small Intestines					Cecum	Colon	Feces
#1-#4	8	5.03	2.78	5.80	6.23	6.38	6.56	7.38	5.74	5.88	6.40
#1a	4	4.13	2.59	5.76	6.13	6.31	6.48	7.36	5.61	5.84	6.45
#8	4	4.55	2.65	6.03	6.26	6.32	6.51	7.21	5.73	5.73	6.52
#4	4	3.92	2.23	5.84	6.14	6.22	6.37	7.28	5.97	6.03	6.63
#7	4	5.84	3.44	6.22	6.41	6.52	6.50	7.15	5.81	6.15	6.45
#2-#5	8	4.17	2.33	5.67	6.09	6.17	6.10	6.21	5.44	5.45	5.66
#2a	4	4.25	2.48	5.98	6.22	6.07	5.88	5.78	5.02	5.73	5.62
#5a	4	3.60	2.40	5.79	6.22	6.32	6.39	6.54	5.46	6.09	6.06

TABLE I. The Values Given Represent Averages Obtained from all of the Rats used on Each Diet.



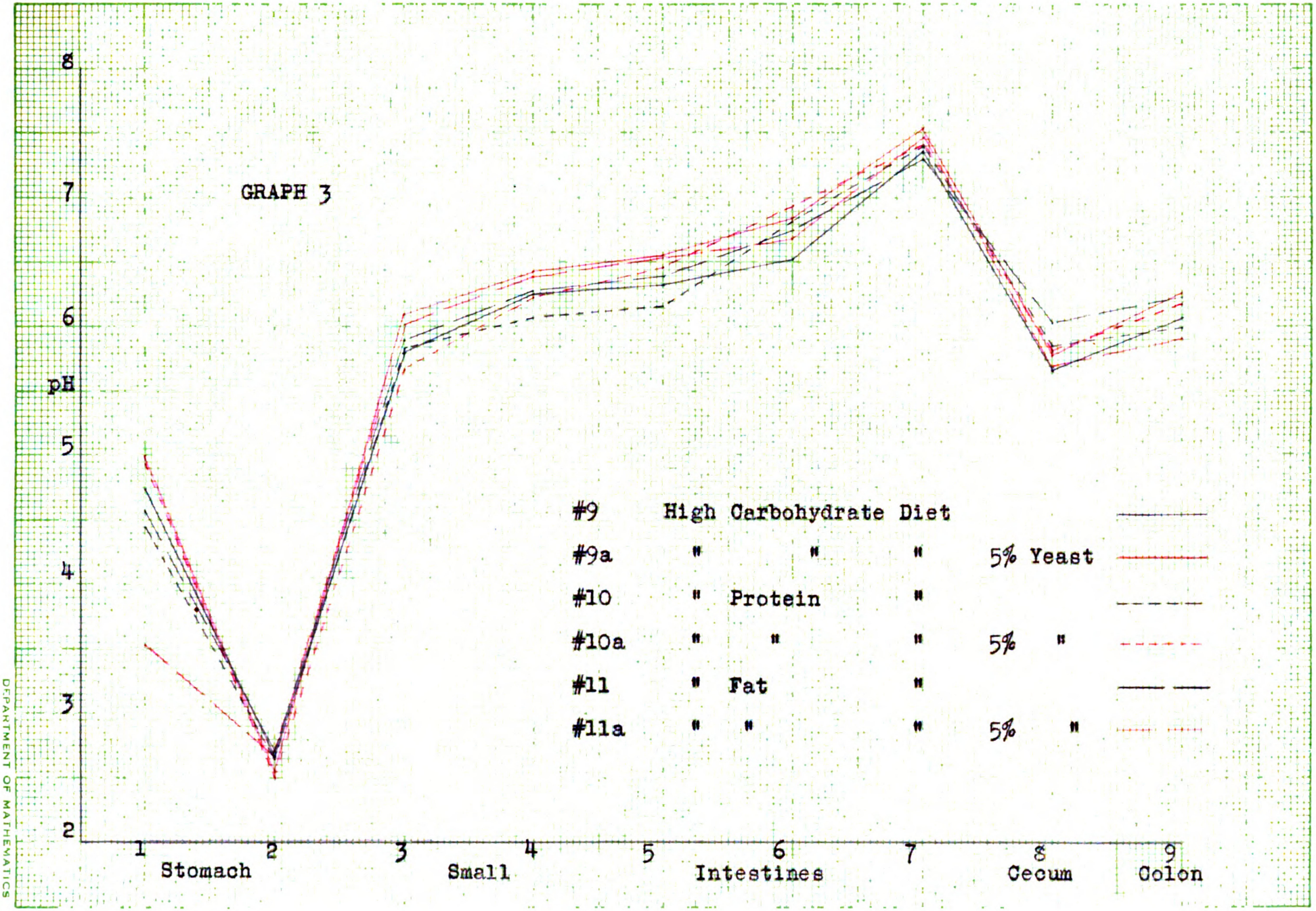
Series	No. of Rats	Stomach		Small Intestines					Cecum	Colon	Feces
#3-#6	8	5.47	4.22	5.88	6.37	6.58	6.76	7.69	6.78	7.00	6.98
#3a	4	5.65	5.02	6.35	6.57	6.50	6.93	7.62	6.92	7.05	7.20
#6a	4	4.36	2.89	5.96	6.45	6.77	7.30	7.81	6.82	6.85	6.96
III	4	5.39	2.50	5.67	6.26	6.35	7.18	7.80	7.20	7.51	7.77
IIIa	4	6.05	4.86	6.48	6.67	7.03	7.11	7.48	7.03	7.09	7.01
VIa	4	6.09	5.72	6.37	6.78	6.86	6.92	7.57	6.79	7.05	6.94

TABLE II. The Values Given Represent Averages Obtained from all of the Rats used on Each Diet.



Series	No. of Rats	Stomach		Small Intestines					Cecum	Colon	Feces
#9	4	4.74	2.67	5.81	6.26	6.33	6.52	7.36	5.67	6.07	6.43
#9a	4	4.99	2.55	6.11	6.43	6.55	6.84	7.53	5.78	6.26	6.36
#10	4	4.45	2.65	5.83	6.07	6.16	6.82	7.39	5.86	5.99	6.23
#10a	4	4.94	2.50	5.67	6.24	6.46	6.94	7.41	5.80	6.18	6.44
#11	4	4.57	2.69	5.90	6.28	6.39	6.75	7.30	6.03	6.23	6.44
#11a	4	3.52	2.67	6.01	6.39	6.54	6.69	7.47	5.69	5.90	6.26

TABLE III. The Values Given Represent Averages Obtained from all of the Rats used on each Diet.



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CHEMISTRY DEPT.
T612.015 D263 98869
Davis