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A study of several factors incident to the absorption of choline from the small intestine of the albino rat

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A STUDY OF SEVERAL FACTORS INCIDENT TO THE ABSORPTION OF CHOLINE FROM THE SMALL INTESTINE OF THE ALBINO RAT

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TABLE OF CONTENTS

CHAPTER PAGE

LIST OF TABLES

CHAPTER I

INTRODUCTION

The substance choline, one of the quaternary nitrogen bases, has been the subject of a number of reviews and investigations (1-3). It has been described as a cholinergic agent, a lipotropic agent, a vitamin, and perhaps in other ways. In spite of the interest shown in its action and uses, there is as yet very little information available as to the mechanism by which it is absorbed by the intestinal mucosa.

There are three possible routes by which choline can disappear from the lumen of the small intestine. The first two routes are by absorption, and include either passive or active transport. Passive transfer is dependent on such factors as pH, concentration, and presence of electrolytes, whereas active transport is dependent on the presence of some enzyme system. The third route is by bacterial conversion of choline to trimethylamine. It is generally accepted that loss of choline in the feces does not occur; de la Huerga and Popper (4) have eliminated this possibility. As yet, there is not sufficient evidence to ascribe the absorption of choline to either passive transfer, or active transport, or both. The degree to which bacterial conversion is a factor in the disappearance of choline

from the small intestine is still in question. This study is concerned with an investigation of several of these factors.

I. REVIEW OF THE LITERATURE

Passive and active transport. One of the first studies dealing with absorption of choline was done by Riedesel and Hines (1). Rats were used as the test animals in demonstrating that choline disappears from the lumen of the small intestine at a constant rate. They also reported that the rate of disappearance of choline was increased with experimentally induced liver damage, and after bile duct ligation. In such instances, the concentration of bile in the small intestine is decreased. Conversely, Riedesel and Hines reported that introduction of exogenous fresh rat bile into the lumen of the small intestine resulted in a decreased rate of disappearance of choline. They suggested that their observations were indicative of an active transport mechanism.

Rohse and Searle (5), in studying choline absorption from acute ileal and Thiry-Vella loops of dog intestine, also suggested the existence of an active transport system. They reported that the rate of absorption from the Thiry-Vella loops was relatively constant over a four hour period, and was unaffected by size of dose. In contrast,

the acute ileal loops exhibited a rate of absorption that was greatest in the first hour and decreased thereafter. Rate of absorption from the acute ileal loops varied with size of dose. These observations led Rohse and Searle to believe that not only active transport, but passive transfer as well are factors in choline absorption.

Bacterial conversion of choline. The major work with respect to bacterial conversion of choline, was reported by de la Huerga and Popper (4, 6-9). The method they used consisted of administering choline and then measuring concentration of choline and trimethylamine in the urine. Their studies were performed using both clinical patients and rats. They reported that two-thirds of ingested choline could be accounted for as urinary trimethylamine. Further, they observed that choline did not appear in the urine after oral administration, but did appear in the urine after parenteral administration. These observations were interpreted as indicating that intestinal bacterial conversion is for the most part responsible for the fate of choline in the intestine.

De la Huerga and Popper reported several studies favorable to the bacterial conversion theory. They demonstrated that incubation of Proteus and Shigella bacteria in a tryptose medium containing choline resulted in conversion of the choline to trimethylamine (3). They

also showed that sterilization of the intestinal tract with such antibiotics as Chlortetracycline, Oxytetracycline and Penicillin O resulted in decreased urinary trimethylamine.

Strong evidence to support the bacterial conversion theory is offered, also, by the work of Prentiss (10). His studies closely resembled those of de la Huerga and Popper except that his test animals were germ-free rats. In seven such animals no trimethylamine appeared in the urine after oral administration of choline.

Even though there is strong evidence to support the bacterial conversion theory of the fate of choline, several observations have been reported which seem to be inconsistent with the theory. If intestinal bacteria are responsible for trimethylamine formation, it would seem that parenteral administration of choline would result in no urinary trimethylamine, or at least, decreased urinary trimethylamine. De la Huerga and Popper, however, observed a slight increase (8). They offered two possible explanations for this observation. Choline could have been secreted into the lumen of the small intestine, converted to trimethylamine and absorbed as such. Also, the liver could have been responsible for the formation of a small amount of trimethylamine. This second explanation is based on the observation of Artom and Crowder (11), that liver slices can convert choline to trimethylamine.

De la Huerga and Popper (6) also reported that after oral administration of choline, patients with hepatobiliary disease excrete one-half as much trimethylamine as control patients. Having accepted the theory that intestinal bacteria are responsible for the conversion of choline to trimethylamine, they found it necessary to define a mechanism by which hepatobiliary disease had exerted its effect on trimethylamine excretion. It is conceivable that liver disease might have decreased intestinal absorption or renal excretion of trimethylamine. De la Huerga and Popper (4) demonstrated, however, that ingested trimethylamine could be recovered rapidly and completely in the urine, in both control patients and patients with hepatobiliary disease. Thus, they concluded that liver disease had, in some manner, inhibited the bacterial conversion of choline in the intestine.

II. THE PROBLEM

The studies referred to thus far leave the field open for additional investigation. No mention was found in the literature of studies on selective areas of absorption of choline in the small intestine, or whether enzyme inhibitors might produce an effect on rate of absorption. In view of the possibility that active transport, and thus enzyme activity, might be involved in choline absorption,

it is conceivable that rate of choline absorption might be altered by such enzyme inhibitors as phlorizin and 2, 4-dinitrophenol. Selective areas of choline absorption, and the effects of several enzyme inhibitors were, then, selected as the areas for investigation in the study described here.

CHAPTER II

METHODS AND PROCEDURES

I. MATERIALS

All chemical reagents and drugs used throughout this investigation are listed in the appendix.

II. EXPERIMENTAL

Animals

The animals used in this investigation were male albino rats weighing 100 to 150 grams. They were obtained from a licensed supplier of biological materials, and maintained on Purina Laboratory Chow and water, ad libitum.

In order that the entire intestinal tract be free of contents at the time of the absorption study, the rats were allowed only water for a period of forty-eight hours before the time of the study. Inasmuch as rats tend to be coprozoic when regular food is withheld, the animals were kept in wire bottom cages which allowed fecal matter to drop into a tray below.

Procedures

Administration and collection of choline. At the time of the absorption study each rat was anesthetized with ether and the abdomen shaved with electric clippers. The

peritoneal cavity was opened by an incision along the linea alba. The section of intestine to be used was isolated by ligation and a measured quantity of choline chloride, 200 milligrams per kilogram, body weight, was injected into the cephalad end of the isolated section. The abdominal incision was closed with suture in two layers, and each rat was placed in a cage to recover. All absorption studies were two hours in duration. At the end of the two-hour period each rat was sacrificed and the ligated section of intestine removed. The contents of the intestinal section were washed into an erlenmyer flask with thirty milliliters of 0.85 per cent sodium chloride. The quantity of unabsorbed choline was then determined. The procedures used for precipitation of proteins and gravimetric determination of choline are a modification of the methods used by Reidesel and Hines (1).

Precipitation of Proteins. In order to determine the quantity of choline remaining in the intestinal fluid after the absorption study, it was necessary to clear the fluid of proteinacious matter. This was accomplished in the following manner. Twenty milliliters of barium hydroxide solution were added to the contents of the erlenmyer flask along with five drops of phenolphthalein indicator. Zinc sulfate solution was added slowly until the pink color disappeared. If, at this time, the supernatent liquid was

not clear, additional small portions barium hydroxide and zinc sulfate solutions were added until clearing occurred. Finally sufficient barium hydroxide solution was added to turn the solution pink and, thus, precipitate any excess zinc sulfate. With the use of Hydrion paper, the final alkalinity was adjusted to pH 10 using sodium hydroxide solution.

To this point the procedure served to precipitate the proteinacious matter of the intestinal contents. The mixture was then filtered with choline chloride appearing in the filtrate. The filtration apparatus consisted of Buchner funnels in series with a vacuum pump and asbestos filters prepared by the classical method. The precipitate was washed repeatedly to insure recovery of all of the choline in the filtrate, keeping the final volume of the filtrate at approximately one hundred and fifty milliliters.

Gravimetric determination of choline. The choline was precipitated as the reineckate salt by slowly adding an excess of a saturated solution of ammonium reineckate in methanol. Agitation was kept to a minimum in order to allow large crystals to form. The best crystal formation occured when the choline reineckate mixture was left for a period of four hours at room temperature, and then transferred to a refrigerator at 0.5° for a minimal period of four hours. The precipitate of choline reineckate was

filtered on a sintered glass crucible of fine porosity by vacuum filtration. Distilled water chilled to 0.5°, was used to wash all the choline reineckate crystals onto the filter and the entire precipitate was washed with repeated minimal quantities of the chilled water under suction. The crucible with its washed precipitate was then placed in the drying oven at 100° for one hour, cooled in a desiccator, and weighed on an Ainsworth Type Ten Balance. The choline reineckate in the crucible was removed by dissolving in acetone, after which the crucible was again dried and weighed. The weight of crucible with choline reineckate, minus the weight of empty crucible, gave the weight of choline reineckate collected. This value was multiplied by a factor of 0.3304 (1) in order to determine the equivalent weight of choline chloride. This final value represented the amount of choline chloride left in the small intestine after the absorption period and by simple arithmetic gave the quantity of choline chloride absorbed.

Preparation of the entire small intestine. In the absorption studies involving the entire length of small intestine, a ligature was placed at both the pyloric and ileocecal sphincters.

Preparation of selected areas of the small intestine. The studies on selected areas of the small intestine were

designed to compare absorption rates from six equally divided sections of the small intestine. Thus, the section of intestine isolated by ligation was located along the length of the small intestine such that it represented one of the six sections. The sections, called A, B, C, D, E, and F, were arranged such that section A began with the pyloric sphincter, section F ended with the ileocecal sphincter, and the other sections were located in their respective positions between A and F.

Use of enzyme inhibitors. The effect enzyme inhibitors exert on rate of choline absorption was measured in the entire length of the small intestine and in section E. The composition of the solutions containing the various enzyme inhibitors are listed in the appendix.

CHAPTER III

RESULTS

The data of all the absorption studies are expressed in milligrams of choline absorbed per one hundred grams body weight and in per cent of choline absorbed.

Absorption from the entire small intestine. The data of the study dealing with the rate of absorption of choline from the entire length of the small intestine are found in Table I. It shows that 8.08 milligrams of choline per 100 grams body weight, or 40 per cent of the injected choline is absorbed during the two-hour absorption study.

Absorption from selected areas of the small intestine. The data of the study dealing with selected areas of the small intestine are found in Table II. The raw data are found in Tables I through VI of the appendix. Reference to Table II shows that the following may be inferred from the data. First, section B exhibits the lowest rate of absorption. Second, the mean rates of absorption for sections B and E are consistently different. Lastly, even though the rates of absorption from sections A, C, D, E, and F are not statistically different, the data suggest that section E tends to have the greatest rate of absorption.

Effect of Enzyme inhibitors. The data dealing with the measurement of the effect of enzyme inhibitors on choline absorption is found in Table III. As this study was intended as a screening test, there were not sufficient data to be given statistical evaluation. However, in no case did inclusion of an enzyme inhibitor in the choline solution result in a decreased rate of absorption. In several cases rate of absorption was actually increased. The data suggest then, that if the individual enzyme inhibitors used exerted an effect on the rate of choline absorption, it was to cause an increase.

The implication of these studies are taken up in the section under Discussion.

TABLE I

DISAPPEARANCE OF CHOLINE FROM THE ENTIRE LENGTH OF THE SMALL INTESTINE OF THE ALBINO RAT OVER A TWO-HOUR PERIOD.

Weight Of Rat In Gm.	Per cent Choline Absorbed	Mg. Choline Absorbed/100 Gm. Body Weight
136	35.9	7.35
126	51.6	10.2
128	64.0	13.1
134	30.0	6.11
135	28.1	5.63
126	38.8	7.70
136	38.5	7.65
138	33.7	7.03
140	35.8	7.43
140	43.2	8.64

Average per cent of choline absorbed is 39.96. Average milligrams of choline absorbed per one hundred grams body weight is 8.08 with standard deviation of 2.07.

TABLE II

A COMPARISON OF DISAPPEARANCE OF CHOLINE FROM SECTIONS A THROUGH F OF THE SMALL INTESTINE OF THE ALBINO RAT OVER A TWO-HOUR PERIOD.

Section	Number of Animals	% Absorbed	Mg. Choline Absorbed/100 Gm. Body Weight
A	6	31.3	6.36
S.D.*			2.7
B	7	17.1	3.46
S.D.			0.35
C	10	25.2	5.02
S.D.			1.3
D	12	28.3	5.94
S.D.			1.4
E	17	33.8	6.86
S.D.			1.9
F	9	26.2	5.21
S.D.			1.75

*Standard deviation.

TABLE III

DISAPPEARANCE OF CHOLINE FROM THE ENTIRE LENGTH AND SECTION E OF THE SMALL INTESTINE OF THE ALBINO RAT OVER A TWO-HOUR PERIOD IN THE PRESENCE OF ENZYME INHIBITORS.

Enzyme Inhibitor	Portion Of Intestine	Molar Conc.	% Absorbed	mg. Choline Absorbed/100 Gm. Body Weight
D*	e.l.**	3×10^{-2}	46.1	9.24
D	E	3×10^{-2}	32.3	6.54
P	e.l.	10^{-5}	37.8	7.65
P	E	10^{-5}	47.1	9.43
P	e.l.	10^{-2}	41.1	8.12
P	E	10^{-2}	39.3	7.92
SF	e.l.	4.8×10^{-2}	35.3	7.07
SF	E	4.8×10^{-2}	32.7	6.54
SF	e.l.	4.8×10^{-1}	68.4	13.6
SF	E	4.8×10^{-1}	42.1	8.42
MA	e.l.	4.8×10^{-3}	56.2	11.0
MA	E	4.8×10^{-3}	51.5	10.1

*D = 2,4-Dinitrophenol; P = Phlorizin; SF = Sodium Fluoride; MA = Monoiodoacetic Acid.

**e.l. = Entire Length; E = Section E.

CHAPTER IV

DISCUSSION

A first impression of the results in this study might be that the findings lack consistency. On further examination however, several factors appear in sharp relief. The absorption studies on the entire length of the small intestine are in close agreement with the studies of Riedesel and Hines (1), and Vetper et al (12). Attention is called to this as varification of the validity of the methodology. What is of concern, however, is the variation found in absorption rates from the different sections of the small intestine. Because the standard deviation from the means of the individual sections is large, no attempt was made to attach significance to the differences from one section to the next except in sections B and E. In these two sections there is a consistent difference between the means of the absorption rates.

There are two possible explanations for this difference between the more cephalad and caudad sections. Riedesel and Hines (1) noted that when there is an absence or simple deficiency of bile, the rate of choline absorption is increased. Baker and Searle (13) reported that bile salts are selectively absorbed from the small intestine at a rate which increases four fold as the intestine

is descended from the duodenum to the terminal ileum. On this basis there is less bile in section E which also demonstrated the highest rate of choline absorption, and more bile in section B which demonstrated the lowest rate of choline absorption. This agrees with the claim of Riedesel and Hines that a deficiency of bile results in an increased rate of choline absorption.

The second explanation is based on the acceptance of the theory that bacterial conversion is the principal avenue of choline disappearance from the intestine. De la Huerga and Popper (9) noted that there are no bacteria in that portion of the intestine immediately caudad to the entrance of the common bile duct, but that there are bacteria farther down the tract. It might be assumed that since less choline disappeared from the intestine closer to the common bile duct, it is because there were no bacteria there to metabolize the material. In the lower end, however, where much more choline disappeared, there were bacteria.

The theory that such a rapid loss of choline from the intestine could be brought about by bacteria leaves something to be desired. Riedesel and Hines (1) observed that sterilization of the intestine with sulfonamides and antibiotics did not lessen the rate of choline disappearance. Further, Vetper et al. (14) reported that choline injected

into a loop of intestine appeared in the mesenteric venous blood unchanged. They found that after two hours 30 to 40 per cent of choline disappeared from the loop and that at least 90 per cent of instilled choline could be accounted for by analysis of remaining intestinal content and mesenteric venous blood content of choline.

There is another matter which cannot be so easily disposed of, namely the difference which exists between the rate of choline absorption for the intact intestine and the average of the mean rates of absorption for the individual sections of intestine. It is incongruous that this average mean of the various sections should be less than the mean rate of choline absorption for the entire intestine. Since the initial studies indicated that the methods for measuring the rate of choline disappearance are valid insofar as they agree with reports by other investigators, there is no reason to assume that the methods are suddenly unreliable. There is the possibility that the difference between the two types of study result from purely physical-biological phenomena associated with absorption of materials from the intestine. It is of rather common knowledge among physiologists that when a hypertonic solution is placed into the intestine, fluid pours into this solution from surrounding tissue. Ultimately the solution becomes isosmotic with tissue fluid at

which time absorption becomes maximum. It seems highly probable that such an incidence has happened in these studies. In any case, further work is indicated to rule out such a possibility. Whether the entire length of the intestine was being studied or an individual section, the same amount of choline chloride solution was administered on the basis of two hundred milligrams per kilogram. The entire length of the small intestine was able to receive the volume injected without trauma. The smaller section, however, would have a greater strain placed on its ability to dilute the injected solution and cause it to be isotonic with the body fluids. This, in turn, would reduce the total amount of choline chloride which could be absorbed within the two-hour absorption period. On this basis, the somewhat inconsistent values for standard deviation and means take on a different meaning. The rate of dilution of intestinal content will depend upon a number of factors such as hydration, kidney function, and hormonal control, all of which were parameters outside of this particular study.

The portion of the investigation dealing with enzyme inhibitors was intended primarily as a screening study for possible further investigation, and the results are not conclusive. They are reported, however, because of the potential value they may have in the planning of further

study. At first glance the results of the study appear to be purely negative in that there is no inhibitory effect on the rate of choline absorption. The question may be raised that perhaps administration of the enzyme inhibitors in the choline solution is not effective and that systemic administration of the inhibitors prior to the absorption study is called for. Ponz and Larraide (15), however, reported that glucose absorption was inhibited by enzyme inhibitors which were administered in the glucose solution. Thus, the route of administration selected for this study was potentially effective, and may well have been so. Further study of the data produces suggestive evidence that the rate of absorption is actually increased in the presence of enzyme inhibitors. Riedesel and Hines had observed (1) that thyroidectomy increased rather than decreased the rate of choline disappearance from the small intestine. Although there is insufficient experimental evidence to make any deductions at this time, there seems to be some possibility of relationship between the decreased enzyme activity following thyroidectomy and the decreased enzyme activity following administration of a known inhibitor. This then remains one of the factors requiring much more intensive investigation. At the present time it is more a matter of conjecture than proof that inhibition of some enzyme system actually increases rather than decreases absorption.

There is ample precedence for such an interpretation, as illustrated with the biguanides used as oral hypoglycemic agents. In this instance, the biguanide (16) appears to inhibit certain membrane enzymes which normally prevent glucose absorption by the cell membrane. In the presence of biguanides, massive quantities of glucose are allowed to be transferred into the cell.

If such an enzyme system is involved in choline absorption, it may be responsible for the suggested negative correlation between rate of choline absorption and presence of bile. Nothing was found in the literature, however, to suggest that there are actual enzymes involved in this particular type of absorption. Further investigation will be necessary.

CHAPTER V

CONCLUSION

This thesis reports on studies measuring rates of disappearance of choline from six different sections of the small intestine. Also reported are measurements of rate of disappearance of choline from the entire small intestine. Section B of the small intestine exhibited the lowest rate of disappearance of choline. Section E exhibited the most rapid rate of disappearance. Sections A, C, D, and F exhibited intermediate rates of disappearance.

A review of the literature indicated that variation in bile concentration throughout the intestine might account for the variation in rate of choline absorption observed in the various sections. The difference observed between mean rate of absorption for the entire intestine and the average of the mean rates of absorption for the various sections was explained in terms of the decreased capacity of the smaller sections to make the instilled choline solution isosmotic with surrounding tissue fluid.

The effects of enzyme inhibitors were also studied. The enzyme inhibitors did not decrease choline absorption. In some cases choline absorption was increased. This suggests the possibility that an enzyme system is present which, when inhibited, increases choline absorption.

The possible implications are discussed in detail.

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APPENDIX

APPENDIX

I. REAGENT GRADE MATERIALS

Choline Chloride, dried at one hundred degrees Centigrade for twelve hours and kept in a dessicator.

Sodium Chloride.

Barium Hydroxide, Octahydrate.

Zinc Sulfate, Heptahydrate.

Ammonium Reineckate.

Number Fourty-two Whatman Filter Paper.

Asbestos.

Methanol, Anhydrous.

Acetone.

II. U. S. P. GRADE REAGENT

Phenolphthalein Indicator.

III. SPECIAL SOLUTIONS

Choline Chloride Solution, one hundred milligrams of choline chloride and 8.5 milligrams of sodium chloride per milliliter of solution.

Barium Hydroxide Solution, a concentration of 0.3 normal in distilled water.

Zinc Sulfate Solution, a concentration of 0.3 normal in distilled water.

Choline Chloride Solutions Containing Enzyme Inhibitors.

These solutions all contain one hundred milligrams of choline chloride and 8.5 milligrams of sodium chloride per milliliter of solution.

Choline chloride and 2,4-dinitrophenol solution, $3X10^{-2}$ molar concentration.

Choline chloride and Phlorizin solution, 10^{-5} and 10^{-2} molar concentrations.

Choline chloride and sodium fluoride solution, $4.8X10^{-2}$ and $4.8X10^{-1}$ molar concentrations.

Choline chloride and monoiodoacetic acid solution, $4.8X10^{-3}$ molar concentration.

IV. GLASSWARE

All glassware was washed in trisodium phosphate, and rinsed in tap water and in distilled water. It was then soaked in a potassium dichromate-concentrated sulfuric acid bath for twelve hours. The glassware was then rinsed repeatedly in tap water and, finally, distilled water and allowed to drip dry.

V. SYRINGE AND NEEDLE

All injections were made with a one cubic centimeter tuberculin syringe and twenty-four gauge needle.

TABLE I

DISAPPEARANCE OF CHOLINE FROM SECTION A OF THE SMALL INTESTINE OF THE ALBINO RAT OVER A TWO-HOUR PERIOD.

Weight Of Rat In Gm.	Choline Injected In mg.	% Choline Absorbed	Mg. Choline Absorbed/100 Gm. Body Weight
125	25	10.4	2.08
124	25	44.0	8.87
150	30	47.7	10.5
140	28	25.7	5.14
145	29	25.4	5.10
145	29	34.4	6.90

Average per cent of choline absorbed is 31.3. Average milligrams of choline absorbed per one hundred grams body weight is 6.36 with standard deviation of 2.7.

TABLE II

DISAPPEARANCE OF CHOLINE FROM SECTION B OF THE SMALL INTESTINE OF THE ALBINO RAT OVER A TWO-HOUR PERIOD.

Weight Of Rats in Gm.	Choline Injected in mg.	% Choline Absorbed	Mg. Choline Absorbed/100 Gm. Body Weight
145	29	16.1	3.24
94	19	18.4	3.72
117	23	16.9	3.33
112	23	16.0	3.30
110	22	10.9	4.18
134	27	15.9	3.20
110	22	15.4	3.24

Average per cent of choline absorbed is 17.1. Average milligrams of choline absorbed per one hundred grams body weight is 3.46 with standard deviation of 0.35.

TABLE III

DISAPPEARANCE OF CHOLINE FROM SECTION C OF THE SMALL INTESTINE OF THE ALBINO RAT OVER A TWO-HOUR PERIOD.

Weight Of Rat In Gm.	Choline Injected In mg.	% Choline Absorbed	mg. Choline Absorbed/100 Gm. Body Weight
104	21	29.5	5.95
116	23	16.9	3.36
110	22	22.7	4.54
112	22	25.0	4.91
126	25	25.6	5.08
126	25	27.5	5.48
117	23	16.5	3.25
145	29	18.2	3.65
140	28	22.4	4.50
140	28	37.5	7.50

Average per cent of choline absorbed is 25.2. Average milligrams of choline absorbed per one hundred grams body weight is 5.02 with standard deviation of 1.3.

TABLE IV

DISAPPEARANCE OF CHOLINE FROM SECTION D OF THE SMALL INTESTINE OF THE ALBINO RAT OVER A TWO-HOUR PERIOD.

Weight Of Rat In Gm.	Choline Injected In mg.	% Choline Absorbed	mg. Choline Absorbed/100 Gm. Body Weight
130	26	24.6	4.93
116	23	31.7	6.29
116	23	25.2	5.00
112	22	18.6	3.66
136	27	34.0	6.76
140	28	17.1	4.15
132	26	21.5	4.24
119	24	28.2	5.71
135	27	31.4	6.30
127	27	40.0	8.50
116	23	28.8	5.60
126	25	38.4	7.62

Average per cent of choline absorbed is 28.3. Average milligrams of choline absorbed per one hundred grams body weight is 5.94 with standard deviation of 1.4.

TABLE V

DISAPPEARANCE OF CHOLINE FROM SECTION E OF THE SMALL INTESTINE OF THE ALBINO RAT OVER A TWO-HOUR PERIOD.

Weight Of Rat In Gm.	Choline Injected In mg.	% Choline Absorbed	mg. Choline Absorbed/100 Gm. Body Weight
110	22	20.4	4.09
100	20	29.0	5.80
100	20	21.6	4.00
110	22	24.5	4.91
114	23	28.2	5.70
110	22	25.8	5.18
92	18	33.9	6.63
116	23	41.3	8.20
114	23	43.9	8.86
94	21	45.2	10.1
108	22	21.4	4.35
78	17	33.5	7.31
86	17	39.4	7.80
90	18	41.2	8.44
84	17	45.9	9.29
86	17	37.6	7.45
110	22	42.3	8.45

Average per cent of choline absorbed is 33.8. Average milligrams of choline absorbed per one hundred grams body weight is 6.86 with standard deviation of 1.9.

TABLE VI

DISAPPEARANCE OF CHOLINE FROM SECTION F OF THE SMALL INTESTINE OF THE ALBINO RAT OVER A TWO-HOUR PERIOD.

Weight Of Rat In Gm.	Choline Injected In mg.	% Choline Absorbed	mg. Choline Absorbed/100 Gm. Body Weight
98	20	39.0	7.96
82	16	19.3	3.78
114	23	14.8	4.82
92	18	42.2	8.27
100	20	23.5	4.70
102	20	19.5	3.82
105	21	19.5	3.90
100	20	29.0	5.80
100	20	19.0	3.80

Average per cent of choline absorbed is 26.2. Average milligrams of choline absorbed per one hundred grams body weight is 5.21 with standard deviation of 1.75.