BODY POOL SIZE AND SECRETION OF ACETATE IN THE RAT

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
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1969

ABSTRACT

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OF ACETATE IN THE RAT

by Shirley Chih-Hsuan Chen

Twenty-four male Sprague-Dawley rats were used for the determination of secretion rate and body pool size of acetate. Another nine rats were used for the determination of tissue *in vitro* uptake or utilization of VFA. The single-injection technique was used in the determination of secretion rate and the body acetate pool size, in which potassium-1-^{14}C acetate was the tracer. Different levels of acetate, propionate and butyrate were incubated in cecal and large intestinal plus rectal tissues to determine utilization of VFA by these tissues.

Cecum, large intestine and rectum were the major sites of the metabolism of acetate which came from the small intestine or directly from the blood. Recovery of radioactivity in GI contents and VFA concentration in blood indicated that the microflora in the cecum, large intestine and rectum were responsible for the presence of acids while the secretion of acid from the blood is too small to be significant.

Body acetate pool size was 4.196 mmoles with a turnover rate of 0.1007 mmoles/min. and a half life of 3.69 minutes.

In vitro study revealed that there was no uptake or utilization of VFA by cecal or large intestinal plus rectal tissues.

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By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Foods and Nutrition

1969

ACKNOWLEDGEMENTS

The writer expresses her hearty appreciation and gratitude to Dr. Modesto G. Yang for his encouragement, guidance and invaluable advice during the course of this work as well as the school years.

The writer also wishes to thank Drs. Robert M. Cook, Olaf Mickelsen and Steven D. Aust for their kind suggestions, guidance in the experimental procedures and generosity in providing the laboratory instruments and chemicals.

Finally, the writer dedicates this work to her dearest parents.

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INTRODUCTION

Volatile fatty acids (VFA) are the main products of cellulose and other carbohydrate digestions in the rumen. These acids are valuable energy sources for ruminants. VFA are also found in the gastrointestinal (GI) tract of monogastric animals. In rats the concentration of the acids in the cecum is as high as that found in the rumen (18). However, unlike ruminant species, the quantity of VFA derived from the digestion of carbohydrates including cellulose is not known for monogastric animals. By extrapolating studies of ruminant animals, VFA in rats are assumed to come from the digestion and/or fermentation of carbohydrates. It is also assumed that fermentation is the greatest source of the acids in the GI tract of these animals.

The acids in the GI tract of monogastric animals could also have come from secretion or conversion from other metabolites *in situ* or both. There are probably some VFA utilized by the GI tissues as a source of energy. The uptake and/or utilization of VFA locally in the tracts would lower the quantity of VFA reaching the circulatory system. The quantity of VFA in the blood and the rate of disappearance of this pool are unknown for rats. Furthermore, the literature provides very little information concerning the quantity of VFA present in the rat, the formation of these VFA and the uptake or the utilization of these VFA by the rat. Thus, this work was undertaken primarily for the following objectives:

1. To determine the body pool size of acetate in rats.
2. To determine the amount of this acid secreted into various sections of the GI tract of the rat. The sections studied were stomach, upper and lower small intestine, cecum, large intestine and rectum.
3. To determine whether VFA are utilized by cecal and large intestinal tissues *in vitro*.

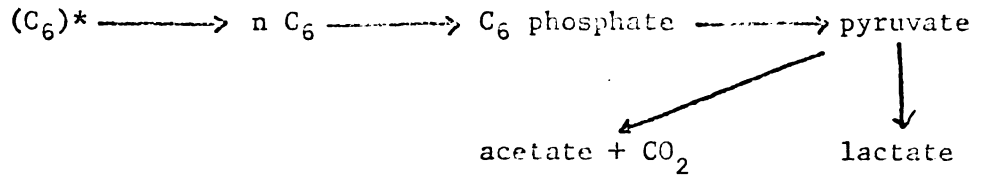
The single injection technique was used in this study and sodium-1-^{14}C acetate was the tracer. The results showed that the body pool size of acetate in rats is 4.196 mmoles. Half life was 3.69 minutes. Secretion of acetate into the GI tract was present but quantitatively insignificant. There was no indication that cecal or large intestinal tissue utilizes VFA.

REVIEW OF LITERATURE

I. General aspects of volatile fatty acid production and absorption in ruminants.

As noted by Annison and Lewis (7), Tappeiner (38) as long ago as 1882 demonstrated that the fermentation of cellulose in the rumen of the ox resulted in the formation of large amounts of volatile fatty acids (VFA), which he concluded contained at least 50% acetic acid. This observation was largely overshadowed, however, by the subsequent work of Kellner (28) in which cellulose was found to be of the same energy value as starch when fed to steers. For many decades VFA found in the rumen were considered to be of little nutritional significance and the digestion of cellulose was assumed to proceed no further than a depolymerization to glucose, which was then absorbed and metabolized as in monogastric animals. It is now established, however, that VFA found in the rumen arise largely from the fermentation of dietary carbohydrates. These acids constitute the major source of energy to the ruminants, since only a small proportion of the ingested carbohydrate escapes degradation in the rumen (7).

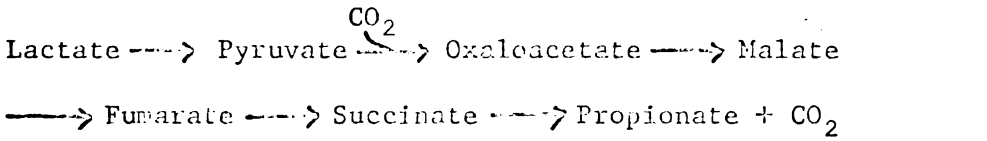
Acetate predominates in the mixtures of VFA which are found in the rumen under all dietary conditions and numerous *in vitro* studies have shown that acetate is the major endproduct of the fermentation of carbohydrates by rumen microorganisms. The following route for the production of acetic acid from carbohydrates has been proposed by Annison and Lewis (7):



*any kind of polysaccharide

Elsden (17) first conclusively demonstrated the presence of propionic acid in rumen contents and showed that it was produced during *in vitro* fermentation of cellulose, glucose and lactate. Organisms responsible for propionic acid production in the rumen and from enrichment cultures of rumen contents were identified as small Gram-positive cocci of the genus *Propionibacterium*. These organisms fermented lactic acid and glucose with the production of propionic acid and acetic acid and carbon dioxide.

Veillonella gazogenes which was identified by Johns (27) was extensively studied and the production of propionate from lactate was shown to occur by a carbon dioxide fixation mechanism:



Longer chain fatty acids in the rumen fluid are produced by secondary reactions from both acetic and propionic acid (24). There is much less direct fermentative synthesis of butyrate other than from the condensation of acetate (30). The incorporation of the major VFA into the branched-chain and longer chain acids is low (24).

The recognition of the importance of VFA as major sources of energy to the ruminant has focused attention on the problem of measuring the amounts of VFA produced in the rumen. Attempts have been made to assess the rates of production of the individual VFA after feeding by following changes in the relative proportions of acids in the rumen (23). The

system is complex, however, since the concentration of a particular acid (or any rumen metabolite) at any one time is dependent on the rates of its a) production in the rumen, b) absorption from the rumen, c) passage from the rumen to the omasum, d) dilution with saliva and food, 3) utilization by rumen microorganisms and f) conversion to other rumen metabolites.

In 1966 Gray *et al.* (25) determined the rates of production of VFA in the rumen. One part of the work measured the *in vivo* production of VFA by two isotope dilution procedures. The production of VFA by two sheep was measured by two different methods involving continuous infusion of C^{14}-labeled fatty acids into the rumen through a rumen fistula and determining the concentration of ^{14}C in the rumen acids throughout the feeding cycle. The infusion solution contained sodium acetate, propionate and butyrate in proportions (75-80: 14-15: 6-10%) which are normally found in rumen fluid. The acids were labeled with ^{14}C in the C-1, C-2 and C-1 positions, respectively, and the total specific activity was 0.03-0.04 μC_i/ml. In one procedure, the varying rate of production of volatile acids was matched by a varying rate of infusion so that a constant concentration of label was maintained in the rumen acid. In the other procedure, a constant rate of infusion was maintained and a mean value was determined for the concentration of label during the feeding cycle. Both procedures gave similar values, approximately 5 moles of VFA were produced per Kg of dry fodder. The next part of their work (41) measured the production of the individual and total volatile fatty acids. In sheep fed at 12 hour intervals a diet of lucerne and wheaten hay, production of volatile fatty acids was measured by infusion of a mixture of ^{14}C-labeled acids. They assumed that transfer of ^{14}C from one acid to another was equal, thus total ^{14}C in each acid is constant. The productions of individual acids were determined by infusion

of a single ^{14}C-labeled acid and measurement of the concentration of ^{14}C in a composite sample of the acid in the rumen fluid collected throughout the feeding cycle. With automatic sampling of rumen fluid, they showed that this procedure was suitable for routine use. They also showed that the composition of the acid mixture initially formed in the rumen was acetic 77-83, propionic 15-18 and butyric acid 1-7%. About 50-80% of the butyric acid was formed from acetic acid. Degradation of ^{14}C-labeled butyrate formed from acetate-1-^{14}C in the rumen showed 93% of the ^{14}C to be nearly equally divided between atoms C-1 and C-3.

Leng (31) used a constant intraruminal infusion of acetate-u-^{14}C, propionate-u-^{14}C and butyrate 2,3-^{3}H technique to measure simultaneously the production rates of acetic, propionic and butyric acid in the rumen of sheep. There was a close correlation between production rates and concentration of the individual acids in the rumen.

Barcroft, McAnally and Phillipson (10) first demonstrated the absorption of VFA from the rumen in anesthetized sheep, when they showed that the concentration of these acids in blood draining the rumen was higher than that of peripheral blood. Reid (36) using a chromatographic method found that about 90% of the VFA of sheep blood was acetic acid and similar findings with respect to cattle blood were reported by McClymont (34). Subsequent work using methods similar to Barcroft *et al.* suggested that the rate of disappearance of undissociated VFA from the rumen increased with chain length (16) and that pH markedly affects absorption rates (22). This dependence on hydrogen ion concentration is related to the proportion of acid present in the undissociated form. An increase of pH from the range normally associated with rumen contents (5.5-6.5) to pH 7.0-7.5 leads to decreased VFA absorption rates. Studies on the absorption of VFA from the washed out and tied

off rumen of anesthetized sheep revealed a linear relationship between acetic acid loss and movement of bicarbonate into the rumen, the absorption of two molecules of acetic acids being accompanied by the entry of one molecule of bicarbonate (32). Ash and Dobson (9) confirmed this result, and showed that the absorption of fatty acid from the rumen is accompanied by a consumption of CO_2 and by the production of bicarbonate within the rumen solution, because of the incoming of unionized acid. Near neutrality, about half of the fatty acid was left in unionized form. The amount of VFA neutralized directly in this manner was about equal to the amount neutralized by saliva. There is no evidence of active transport of VFA across rumen epithelium and the concentration gradient is the most important factor in determining rates of transfer. When solutions free of fatty acids were placed in the isolated rumen, blood VFA entered the rumen and the final concentration was close to that of plasma.

II. The metabolism of acetate, propionate and butyrate in the ruminant.

Mature ruminants absorb relatively smaller quantities of glucose from their digestive tract and depend to a large extent on gluconeogenesis for their glucose supply. Gluconeogenesis from propionate is of major importance (8). The data of Bensadoun *et al.* (11), however, suggested that gluconeogenesis from precursors other than propionic acid, e.g., lactate, could also be important. The mean value derived from the data of Warner (40) based on estimates in the literature of daily amounts of propionate absorbed by cattle, sheep and goats is 13 Kcal/$Kg^{0.75}$/day. This supports the view that propionate is a major source of glucose; however, that gluconeogenesis from other sources is likely to be important at least in the pregnant and lactating ruminant (8).

Annison *et al.* (6) studied intensively the metabolism of acetic acid, propionic acid and butyric acid in sheep. Acetate-1-^{14}C, propionate-1-^{14}C, propionate-2-^{14}C, butyrate-1-^{14}C, butyrate-2-^{14}C and butyrate-3-^{14}C were insufed into the portal vein of anesthetized sheep. The result showed these labeled fatty acids were incorporated into blood glucose, lactate and ketone bodies. The measured specific radioactivity (S.A.) are summarized as follows:

1. The S.A. of plasma glucose was 2-5% of the infused acetate or butyrate, but 30% of the infused propionic-2-^{14}C acid.

2. The S.A. of blood lactate was less than that of blood glucose except after the infusion of butyrate-2-^{14}C, when it was slightly higher.

3. The S.A. of blood β-hydroxybutyrate was about 50% of that of infused butyrate-^{14}C, about 7-15% of infused acetate and about 2% of the infused propionate-^{14}C.

4. The S.A. of blood formate was about 5% of that of infused acetate-^{14}C, propionate-^{14}C or butyrate-^{14}C.

5. The S.A. of blood acetate was less than 1% of that of infused propionate-^{14}C but about 6-10% of that of infused butyrate-^{14}C.

The infusion of acetate-1-^{14}C, propionate-1-^{14}C, butyrate-1-^{14}C or butyrate-3-^{14}C gave rise to labelling mainly C-3 and C-4 of glucose and C-1 of lactate. The infusion of acetate-2-^{14}C, propionate-2-^{14}C or butyrate-2-^{14}C labeled mainly C-1, C-2, C-5 and C-6 of glucose, and C-2 and C-3 of lactate. Thus, the metabolic pathways were concluded to be similar to those known to occur in other mammals.

The changes in blood volatile fatty acids after their infusion into the rumen of a cow were studied by Asfjes (1). A distinct and rapid rise of a volatile fatty acid in the blood followed the introduction of the acid. However, butyrate gave not only a rise of the

butyrate content of the blood but also a marked rise in the blood propionate and acetate levels.

Acetate metabolism in ruminant tissues was studied by Mayfield *et al.* (33). Acetate-1-^{14}C was incubated with various tissue homogenates prepared from tissues of fed and 7-day fasted sheep to study the site and routes of acetate metabolism in tissues of ruminants. Transfer of ^{14}C from acetate to CO_2, neutral lipids, free fatty acids, nondistillable organic acids and proteins was followed. They found that acetate utilization by ruminant tissues was most for adipose tissue, and decreased in order for kidney, muscle, heart, lung, liver and brain when expressed on the basis of per unit of protein. The 7-day fasted sheep tissues were slower in the overall acetate metabolism but CO_2 production from acetate was lowered only in liver and brain tissues.

III. The metabolism of acetate, propionate and butyrate in the rat.

Most herbivorous animals have an expanded part in their digestive system where fibrous materials can be delayed in their passage through the digestive tract; this allows fermentation to take place. In ruminants the expanded part is the rumen, whereas in the rat the cecum is the segment that is expanded. Much work has been done to determine the utilization of acetate, propionate and butyrate as energy sources for the ruminant. However, similar investigations carried out on rats are relatively few in number.

Elsden *et al.* (18) published a paper on the concentration and quantity of volatile fatty acids in the digesta of ruminants and other animals in 1946. This is the only paper that could be found which dealt with VFA in the rat. The quantity of volatile acid in proportion to the body weight is greatest in the ox and least in the rat. The

amount of volatile acids present in the digesta depends on the rate at which it is produced and the rate at which it disappears from any organ. The regions of the alimentary tract, in which fermentation occurs, were sharply defined and consist of rumen and reticulum in the ruminant and the large intestine in all other species. The most important part of this report was that on the basis of concentration the volatile acids in cecal digesta of oxen, sheep and rats were almost the same (18). In sheep the concentration was 4.3, in oxen 3.2 and in the rat 4.6% of the dry ingesta.

Early in 1943, Buchanan *et al.* (12) synthesized acetate, propionate and butyrate containing radioactive carboxyl-C and fed them with glucose to fasted white rats. Evidence was presented which indicated that propionic acid and butyric acid were converted to liver glycogen, but that acetate was not. Approximately 50% of the radioactivity of the ingested fatty acids was excreted in the respiratory gases as CO_2 in a 2-hour period.

Wood *et al.* (42) isolated the glycogen of rat liver following intraperitoneal administration of heavy carbon bicarbonate and feeding glucose by stomach tube. The position of the labeled carbon in glucose from the glycogen was determined by bacterial and chemical degradation. They found that CO_2 carbon was fixed in 3 and 4 position of glucose, thus proving CO_2 fixation is involved in the synthesis of glucose.

The incorporation of acetate and butyrate carbon into rat liver glycogen was studied by Wood *et al.* (43). Evidence showed that these acids could be converted to glycogen via pathways other than carbon dioxide fixation. The result showed that after feeding labeled ^{13}C-acetate, doubly labeled acetate or labeled ^{13}C-butyrate not only carbon atoms 3 and 4 but all degradation fractions of glycogen contained

significant amounts of ^{13}C. This furnished direct evidence that at least the carbon atom of the VFA studied was incorporated into rat liver glycogen by some means in addition to CO_2 fixation.

IV. Methods for analysis of volatile acids.

A major difficulty in the early studies of ruminal VFA and their absorption and subsequent metabolism was the lack of reliable methods for the analysis of mixtures of VFA. This difficulty was overcome by Elsden (17, 19), who devised a liquid-gel partition chromatography technique which allowed the separation and estimation of a mixture of acetic, propionic and butyric acids. The first accurate analyses of volatile fatty acids of rumen contents thus became available, and acetic, propionic and butyric acids were invariably found to be present.

A second major advance in the analysis of VFA was the gas-liquid partition chromatographic methods of James and Martin (26). This technique has revealed that in addition to acetic, propionic and butyric acid, small quantities of the naturally occuring branched-chain isomers of butyric and valeric acid (isobutyric, 2-methylbutyric and isovaleric acids) and n-valeric acid are usually present in the rumen (2).

Recently, Ramsey (35) used benzene, chloroform and the following mixtures of tert-butanol in chloroform: 1, 2, 5, 8, 12, 16, 24 and 30% (v/v) as the eluting solvents for a mixture of 17 pure organic acids as well as protein free filtrates of ruminal blood samples through the silicic acid column. The result showed that the quality of resolution is satisfactory for most of the acids from capric acid to isocitric acid. Ramsey's method is the main procedure which was used in the present experiment to analyze for the different VFA.

EXPERIMENTAL

I. Experimental animals.

Thirty-three male Sprague-Dawley rats having average body weight of 350 grams were purchased from a local supplier.* The rats were housed individually in wire bottomed cages and were fed *ad libitum* M-1 diet (Tables 1 and 2) and water. The rats were maintained on the diet until their body weights reached 450 to 500 grams, which took approximately 3 weeks. At this time, 24 of the rats were used for the determination of secretion rate and body acetate pool. The other 9 rats were used for the determination of *in vitro* tissue uptake or utilization of VFA.

II. Experimental procedures.

A. Chemical purification of sodium-1-^{14}C acetate.**

Sodium-1-^{14}C acetate was chromatographed and the profile on purity was checked by titration and by counting the radioactivity of the fractions. There were traces of ^{14}C-propionate and formate besides acetate (Fig. 1). Each batch of 20 μC_i of the purchased sodium-1-^{14}C acetate was run through silicic acid column and only tube numbers 19 to 26 were pooled and made alkaline with 20% KOH solution. This solution was then evaporated to dryness and redissolved in 10 ml saline which gave a S.A. of 2.9 x 10^5 CPM/0.1 ml. The purified K-1-^{14}C acetate was used for

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*Spartan Research Animals, Inc., Williamston, Michigan.

**Sodium-1-^{14}C acetate was purchased from Nuclear Chicago; its specific activity was 10.00 millicurie per millimole.

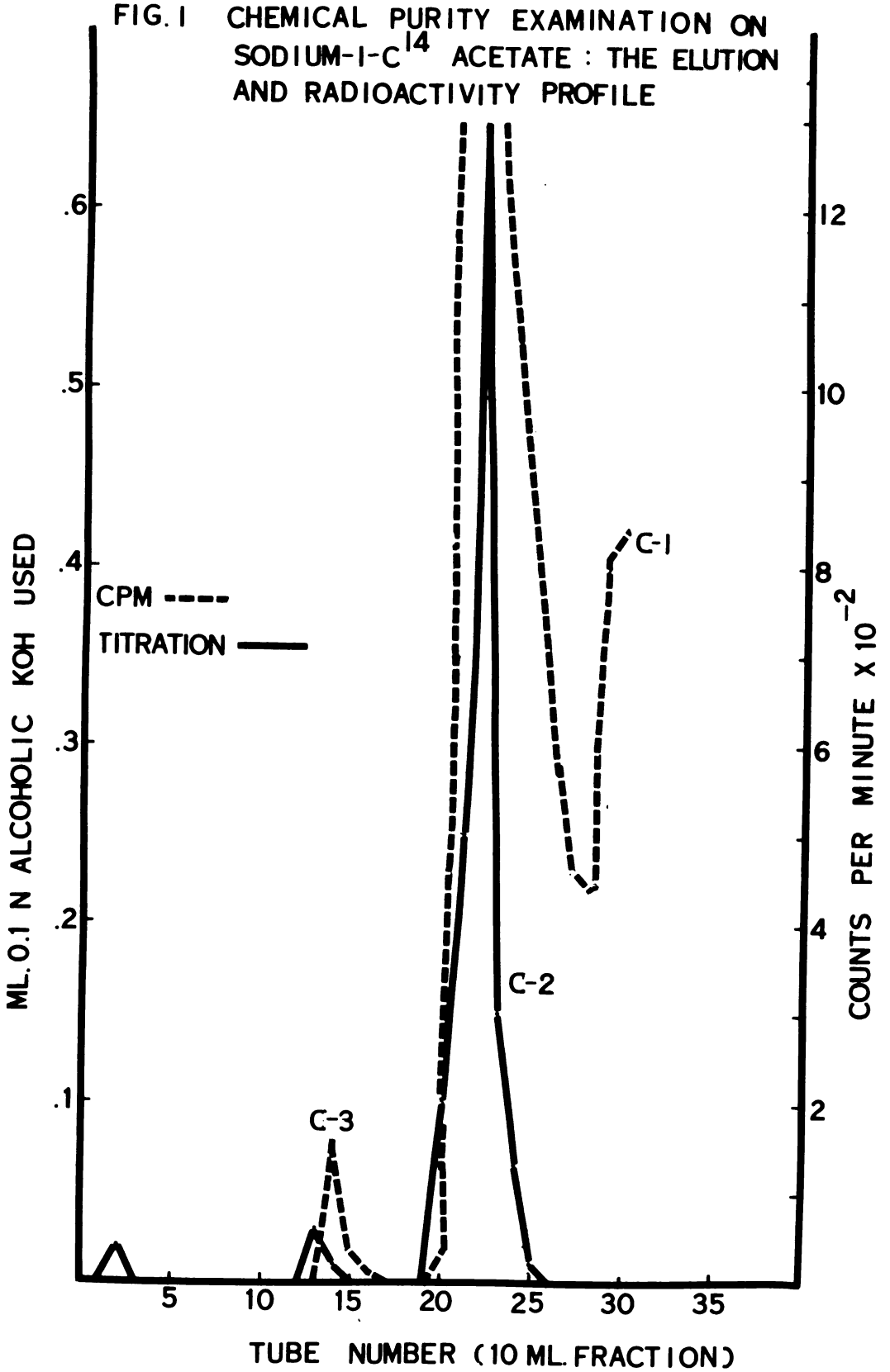
Table 1. Composition of basal diet

Ingredients	%
Ground corn	60.8
Soybean meal (50% protein)	28.0
Alfalfa meal (17% protein)	2.0
Fish meal (16% protein)	2.5
Dried whey (67% lactose)	1.6
Limestone (38% Ca)	1.6
Dicalcium phosphate (18.5% P, 23.5% Ca)	1.7
Iodized salt	0.5

Table 2. Supplemental mineral and vitamin per kilogram basal diet

	Grams
Vit. A (Pfizer 10P, 100,000 USP/gm.	0.8088
Vit. D (9F Fleishmann in yeast, 9000 USP/gm.	0.0836
Choline chloride	0.6996
Ca pantothenate	0.0055
Niacin	0.0330
Riboflavin	0.0033
Vit. B_{12} (0.1% mannitol trituration)	0.0066
α-tocopherol acetate	0.0044
Menadione	0.0022
DL-methionine	0.4994
Trace metal premix	0.9900
In %: Mn 12.2, Fe 9.6, Ca 7.5, Co 0.26, Cu 0.73, Zn 5.0, I 0.38	

FIG. 1 CHEMICAL PURITY EXAMINATION ON SODIUM-1-C^{14} ACETATE : THE ELUTION AND RADIOACTIVITY PROFILE



injection, incubation and for standard curves used for identification of unknown.

B. Sample collection.

The rats were fasted for 2 hours from 11 a.m. to 1 p.m. and then anesthetized with ether. Heart puncture technique (13) was then used to inject 0.125 μC_i of potassium-1-^{14}C acetate in 0.5 ml saline. After the time intervals indicated on Table 3, ether anesthesia was given to the rat again. A midventral incision was made to facilitate removal of the whole GI tract. After removing the GI tract, it was immersed in crushed ice immediately. Just prior to removing the GI tract, a heparinized syringe was used to draw blood from the heart and portal vein. The blood was then deproteinized by a modified method of Folin and Wu (21), centrifuged and the supernatant frozen until analyzed.

The GI tract was sectioned (Table 3) and digesta in the GI tract were removed into separate chilled and tared containers and stored frozen (-20°) until VFA were separated by column chromatography (35).

C. *In vitro* study of tissue uptake of VFA.

Nine rats which were fasted for 24 hours with water available at all times were sacrificed by over etherization and bleeding. GI tissues: a) cecum, b) large intestine and rectum were then cut into pieces of 15 to 20 mm^2 squares and were then weighed on a Smith-Roller tissue balance. All procedures mentioned above were performed in the cold. From 85 to 125 mgs of the tissues were incubated in Krebs-Ringer bicarbonate solution (39) together with 100 units Penicillin G and 0.1 mg streptomycin per ml and a known concentration of various volatile fatty acids. They were a) 25 µmoles acetic acid per ml, b) 5 µmoles propionic acid per ml, c) 15 µmoles butyric acid per ml and incubated for 10, 20,

Table 3. Blood sampling schedule and parts of GI tract used for VFA determination

GI section	Time after sodium-1-^{14}C acetate injection (min.)					
	10	15	20	30	45	60
	number of samples					
Stomach	4	4	4	4	4	4
Upper small intestine[1]	4	4	4	4	4	4
Lower small intestine[1]	4	4	4	4	4	4
Cecum	4	4	4	4	4	4
Large intestine[2]	4	4	4	4	4	4
Rectum[2]	4	4	4	4	4	4
Feces[3]	4	4	4	4	4	4
Blood	4	4	4	4	4	4

[1]The small intestine was divided into two equal portions and the anterior segment is referred to as U.S.I. and the posterior segment is referred to as L.S.I.

[2]From the cecum to the first hard pellet in the large intestine. The remaining large intestine, containing hard and well formed fecal pellets, was considered to be the rectum.

[3]Feces were collected when dropped between the time of injection and during anesthesia.

40, 60 or 90 minutes. The tissue media and VFA were placed in 10 ml. capped vials to prevent excessive evaporation. The vials were placed in a shaking water bath at 37° and incubated for the designated time. Two controls were included during each incubation: a) Krebs-Ringer bicarbonate solution plus tissue but without acid, which served as blank, b) Krebs-Ringer bicarbonate solution plus acid but no tissue, which checked the possibility of loss of acid during incubation.

At the end of incubation, the tissues were taken out, and the media were diluted ten to one with 0.7 N H_2SO_4. Ten µl of this diluted sample were injected with a Hamilton syringe into a F-M gas chromatograph. The acid concentration was detected by flame ionization with helium as the carrier gas. The concentration was determined by comparing with controls.

D. Determination of VFA concentration of GI contents.

Two grams of silicic acid which were acidified with four drops of 50% sulfuric acid were mixed thoroughly with 1.5 to 2 gms GI content. The whole mixture was chromatographed on a silicic acid column with tertiary butanol in chloroform described by Ramsey (35). The flow rate was adjusted to approximately 2 ml/min. with CO_2-free-N_2 pressure. Twenty-six to twenty-eight tubes of 10 ml. fractions were collected using a Gilson fraction collector. The fractions were titrated with ethanolic KOH while being aerated and mixed with CO_2-free-N_2 gas. Two drops of 0.004% thymolphthalein in ethanol was indicator; the endpoint of this mixture was from colorless to violet at pH 9--10. Blank titration was determined for and subtracted from each solvent. The identification of VFA : valeric acid + isovaleric acid, butyric acid + isobutyric acid, propionic acid and acetic acid were achieved by comparing with elution patterns from standard acids.

There was a yellow color in the fractions from the column, usually tubes 2, 3 and 4 but sometimes all tubes, which interfered with the titration (endpoint was less sharp). The color of the fraction probably partially quenched the radioactivity counting. This problem was taken care of by dividing those fractions with yellow into two portions and to one, a known amount of K-1-^{14}C acetate (0.005 μC_i) was added. Both were then treated and counted as described in Section C, Radioactivity counting. The difference in CPM from these 2 divided by CPM corresponding to 0.005 μC_i gave the percentage of radioactivity recovered.

E. Determination of VFA concentration in blood.

The blood sample was deproteinized by following the method of Folin and Wu (21) with little modification. One volume of whole blood was laked into 2 volumes of distilled water, one volume of sodium tungstate (10%) was added followed by adding dropwise 1.2 volume of 0.78 N sulfuric acid. The mixture was shaken vigorously and allowed to stand for 15 minutes at room temperature. The mixture was then centrifuged and the supernatant filtered through a Whatman no. 41 filter paper. The clear filtrate was made alkaline (pH 10) with a few drops of 20% KOH and stored in a freezer until analyzed. The frozen, deproteinized blood samples were thawed and the samples from 4 rats of each time interval were pooled; this amounted to about 45 ml which were then evaporated to about 1-2 ml in a flash rotating vacuum evaporator at 55°. The concentrated sample was acidified by adding a few drops of 50% H_2SO_4 to about pH 2. It was triturated with 2 gms of silicic acid and chromatographed using the same method as described for digesta.

F. Efficiency of liquid scintillation counting.

Efficiency of the liquid scintillation counting which took into consideration the instrument capabilities and quenching by ethanol which is part of the solvent system was determined by the following procedures:

a) 0.05 mC_i methyl-^{14}C-toluene (Nuclear Chicago) were dissolved in 100 ml redistilled reagent toluene (Mallinckrodt) to form a stock solution.

b) The stock solution was diluted with redistilled reagent toluene to form a working solution of 5×10^{-3} μC_i/ml.

c) One ml of working solution, 1 ml ethyl alcohol and 14 ml liquor (counting solution) were then used for determining the counting efficiency of the instrument.

G. Radioactivity counting.

The fractions eluted from column chromatographs were transferred quantitatively to glass spectrovials after being titrated with 0.01 N ethanolic KOH to determine total VFA. The mixture was then evaporated on a 60° hotplate to dryness. The radioactivity was determined by adding to the vial 1 ml absolute ethanol and 14 ml scintillation liquor of 0.5% PPO* and 0.05% POPOP** (Nuclear Chicago) in toluene and counting in a scintillation counter (Nuclear Chicago). Blank counting was made each time with 1 ml absolute ethanol and 14 ml scintillation liquor. Quenching by ethanol was determined with toluene-$^{14}C_i$ (methyl) as described in the preceding section. Channel settings were at upper F 62 and lower F 48.

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*POP: 2,5-diphenyloxazole

**POPOP: p-bis(2-(5-phenyloxazolyl))-benzene

RESULTS AND DISCUSSIONS

I. Efficiency of liquid scintillation counting.

Efficiency of liquid scintillation counting, which took into consideration the instrument capabilities and quenching by ethanol, was calculated as follows:

$$\frac{\text{CPM of } (5 \times 10^{-3}\ \mu C_i\ ^{14}C \text{ toluene} + 1 \text{ ml absolute ethanol} + 14 \text{ ml counting solution})}{\text{DPM of } 5 \times 10^{-3}\ \mu C_i\ ^{14}C \text{ toluene}} \times 100\%$$

$$= \frac{8000 \text{ CPM}}{5 \times 10^{-3} \times 2.22 \times 10^{6} \text{ DPM}} = 72.72\%$$

This value was used in correcting all the values from radioactivity counting.

II. Elution profile of standard acids: acetic, propionic, butyric, isobutyric, valeric and isovaleric acid.

Sixty ml benzene, 100 ml chloroform, 100 ml 1% t-butanol in chloroform were used in this order to elute the standard acetic, propionic, butyric, isobutyric, valeric and isovaleric acids. One tenth ml of each standard acid of 0.1 N was chromatographed to obtain the profile. Recovery of the standard acids was 100 $\pm$ 5%. Valeric plus isovaleric, butyric, isobutyric, propionic and acetic acid are distinguishable from one another (Fig. 2). This profile was used as the standard elution curve for identification of unknowns.

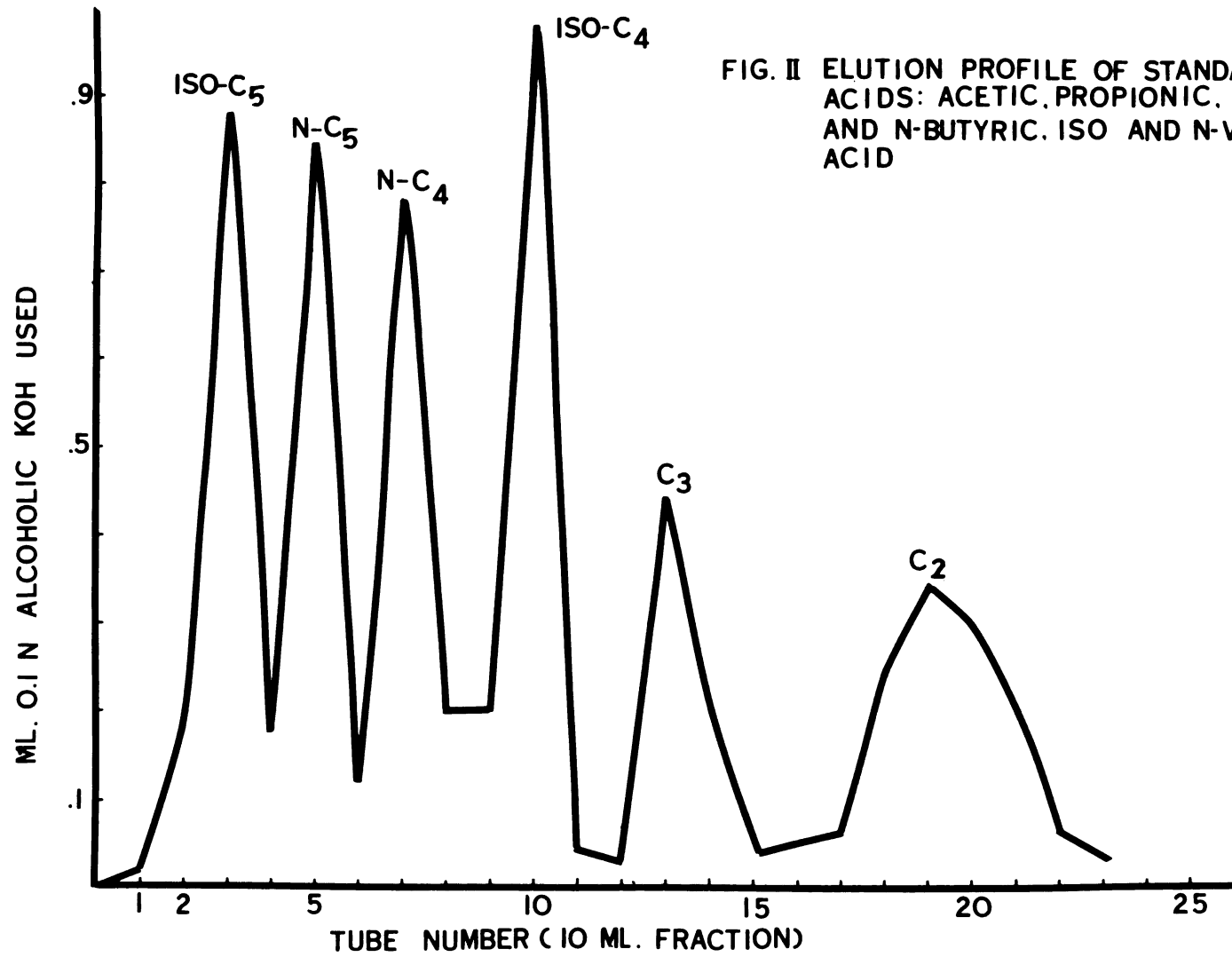

FIG. II ELUTION PROFILE OF STANDARD ACIDS: ACETIC, PROPIONIC, ISO- AND N-BUTYRIC, ISO AND N-VALERIC ACID

III. Distribution of gastrointestinal contents.

The wet digesta from the same GI portion from different rats varied considerably in weight. In order to minimize this variation, the wet digesta per 100 gm body weight was calculated (Table 11). Per unit of body weight, cecal content was the highest followed in order by stomach, lower small intestine, rectum, large intestine and upper small intestine (Table 4).

In the whole tract, cecal digesta accounted for 50.0% of the total wet digesta weight (1.3/2.6 = 0.50) (Table 4B). The cecum is thus the "expanded" part of the rat GI tract. As will be shown in the following section, it also contained the highest quantity of acetic acid expressed as micromole acid per gram wet digesta. No doubt this is the most active section as far as fermentation is concerned in the whole tract.

IV. VFA concentration of GI contents

Table 5 gives total concentration and distribution of organic acids in the GI tract contents. Stomach, small intestine and feces have more valerate than other acids, whereas cecum, large intestine and rectum have acetate as the major acid.

Several attempts were made to remove the yellow color present in the fractions from the silicic acid column. Activated-carbon adsorption, steam distillation of the eluent, and changing the polarity and amount of the solvent system proved unsatisfactory, for they also removed a large quantity of the radioactivity. The adding of external standard was adopted finally. This took care of the color quenching. However, as the endpoint of thymolphthalein was from colorless to violet, the yellow color interfered with the location of the exact endpoint; this introduced an error in the concentration determination, especially on

Table 4. Distribution of gastrointestinal and fecal contents

Sections	Range	Average	Number of animals
	A		
	Wet weight of digesta or feces (gm.)		
Stomach	0.60-7.44	3.57	24
Upper small intestine	0.13-2.21	0.71	24
Lower small intestine	1.00-4.96	3.26	24
Cecum	3.66-8.72	6.28	24
Large intestine	0.18-1.93	1.08	24
Rectum	0.40-3.63	1.59	24
Feces	0.27-1.65	0.63	24
	B		
	Wet weight of digesta or feces per 100 grams of body weight (gm.)		
Stomach	0.12-1.59	0.75	24
Upper small intestine	0.03-0.44	0.18	24
Lower small intestine	0.24-1.02	0.68	24
Cecum	0.85-1.76	1.30	24
Large intestine	0.04-0.45	0.22	24
Rectum	0.09-0.83	0.33	24
Feces	0.06-0.32	0.13	14
	C		
	Weight (gm.) of total tract content		
Range: 12.67-20.00		Average: 16.80	
	D		
	Weight of total tract content per 100 grams body weight (gm.)		
Range: 2.39-4.16		Average: 2.60	

Table 5. Total and distribution of organic acids in the GI tract and feces (micromole acid/gram digesta [wet])*

GI section or feces	Carbon chain length C_2	C_3	C_4	C_5
Stomach	16.01	3.42	5.80	45.08
Upper small intestine	7.54	3.04	1.48	10.39
Lower small intestine	5.58	2.87	2.31	8.08
Cecum	44.26	13.98	23.62	13.19
Large intestine	29.13	15.91	14.12	14.13
Rectum	24.81	13.72	15.18	25.16
Feces	17.32	7.86	15.51	30.05
Sum	144.64	60.80	78.02	120.83

*Each value is the average of four samples.

valerate, since the color usually appeared at tubes 2, 3 and 4. This yellow color probably came from bile or feed color.

The molar distribution of the acids in the cecum was 46.6% acetate, 14.7% propionate, 24.9% butyrate and 13.8% valerate and acids of higher chain lengths. Weller *et al.* (41) reported that the distribution was 77-83% of acetate, 15-18% propionate and 1-7% butyrate and traces of higher acids were in the rumen of sheep. The difference in the acid distribution pattern could be an indication of different microorganisms present in the two species.

V. Radioactivity of GI contents

Cecal digesta had the highest radioactivity among the GI sections, followed in order by rectal and large intestinal digesta (Tables 6 and 7) throughout the whole experiment. The radioactivity in the upper small intestine, stomach and feces was negligible. In general, the recovery of the injected radioactivity in the GI content was rather low (Table 6). There are several possibilities for the low recovery:

a) Low secretion rate and most of the radioactive sodium acetate still remained in the circulatory blood or was metabolized before secretion could take place. This is likely to happen, since the metabolism of acetate by rats is rather high.

b) The radioactive sodium acetate remained in the GI tissue. Specific activity of GI contents is expressed as CPM/ moles of acid (Table 8). Since only radioactive acetate was injected, radioactivity of propionate, butyrate and valerate in the contents must have come from this acid. The formation of propionate, butyrate and valerate probably involves condensation reactions. These could be nonenzymatic, as well as through intestinal microfloral fermentation. It is possible that both took place at the same time.

Table 6. Total counts in contents of each section of the GI tract and feces after injecting ^{14}C acetate into the heart chamber (average of 4 samples)*

GI section	Time after injection (min.) 10	15	20	30	45	60
	Counts per minute					
Stomach	58.1	41.3	24.1	46.4	24.4	25.8
Upper small intestine	47.9	6.3	13.7	9.6	20.5	26.0
Lower small intestine	248.8	313.5	412.3	132.7	280.0	275.0
Cecum	2394.6	3930.6	3343.8	3061.8	3306.2	2787.9
Large intestine	296.6	154.0	413.1	290.3	625.5	381.8
Rectum	305.9	849.8	238.5	459.3	369.2	484.8
Feces	70.2	41.3	4.3	110.0	22.1	19.8
Sum	3422.0	5336.9	4449.8	4111.0	4085.8	3657.5

*Average of the sum = 4177.0 CPM

Injection dose = 1.4×10^6 CPM

% recovery of radioactivity in GI content

$= (4177.0/1.4 \times 10^6) \times 100\%$

$= 0.3\%$

Table 7. Radioactivity in each section expressed as a percentage of the total GI radioactivity found in each time interval (average of 4 samples)

GI section	Time after injection (min.) 10	15	20	30	45	60
Stomach	1.69	0.79	0.54	1.10	0.50	0.64
Upper small intestine	1.40	0.01	0.31	0.20	0.40	0.66
Lower small intestine	7.27	6.03	9.27	3.32	6.05	6.87
Cecum	69.95	75.61	75.14	74.40	71.20	69.68
Large intestine	8.67	2.96	9.28	7.05	13.36	9.54
Rectum	8.95	16.35	5.36	11.16	7.95	12.12
Feces	2.05	0.79	0.10	2.70	0.47	0.49

Data on the specific activity (S.A.) of the contents in different parts of the GI tract of each rat are tabulated in Table 12 in the Appendix. Specific radioactivities in all sections of the GI tract were low. However, the following observations are apparent:

a) Stomach: The S.A. of acetate increased with time and reached a plateau at 15 to 30 minutes and then decreased. The decreasing S.A. of acetate suggested that 1) acetate passed to the lower section, 2) there was recycling of acetate of lower S.A. to the stomach. The presence of radio-labelling in propionate and valerate at 60 minutes indicated the formation of propionate and valerate from acetate.

b) Upper small intestine: Highest S.A. of acetate was found at 10 minutes after injection. It then decreased with time, suggesting that acetate was the major acid secreted into this section and that the upper small intestine is a major metabolizing site. As S.A. of acetate decreased, S.A. of propionate, butyrate and valerate increased. A maximum S.A. of butyrate was found at 20 minutes, which then decreased immediately. At the same time, S.A. of propionate increased and peaked in 30 minutes, suggesting the following reactions took place:

$$2\ C_2 \longrightarrow C_4 \qquad \text{fast}$$

$$CO_2 + C_2 \longrightarrow C_3 \qquad \text{slow}$$

and propionate and butyrate were absorbed or passed down to the following section.

c) Lower small intestine: Comparable to the upper small intestinal S.A., the S.A. of acetate in this section peaked at 10 minutes. The S.A. of acetate and propionate fell with time, suggesting that they were passed down to the cecum. There is an extremely high S.A. of butyrate at 45 and 60 minutes; this could be an experimental artifact,

Table 8. Specific activity of GI contents (CPM/μmoles)

Time after injection		Stomach	Upper small intestine	Lower small intestine	Cecum	Large intestine	Rectum
10	C_5:	0.18	0.50	1.91	4.39	3.23	1.94
	C_4:	0.00	0.00	7.87	2.72	2.51	2.88
	C_3:	0.00	0.00	6.31	1.84	1.72	1.21
	C_2:	0.28	12.31	11.17	5.03	4.13	3.96
15	C_5:	0.00	2.76	4.98	7.49	1.01	8.38
	C_4:	0.14	0.00	11.09	6.27	0.96	7.67
	C_3:	0.00	0.93	4.81	3.01	4.05	2.71
	C_2:	1.38	2.27	8.13	7.01	3.25	3.69
20	C_5:	0.00	3.27	3.19	6.14	3.71	1.90
	C_4:	0.00	7.49	14.53	6.26	6.99	2.32
	C_3:	0.00	7.17	10.61	3.58	4.28	2.16
	C_2:	1.49	3.51	1.74	5.70	6.11	3.81
30	C_5:	0.00	0.47	3.24	1.42	6.34	1.48
	C_4:	0.00	0.00	10.59	4.42	4.65	8.49
	C_3:	0.00	9.45	5.44	4.29	9.12	6.45
	C_2:	1.64	0.75	7.15	4.79	5.07	5.65
45	C_5:	0.00	2.49	5.73	3.69	1.76	3.54
	C_4:	0.00	0.00	56.00	8.75	11.20	10.00
	C_3:	0.00	6.43	5.92	7.34	9.27	13.40
	C_2:	0.24	3.99	2.10	6.03	9.07	16.60
60	C_5:	0.13	5.44	3.90	5.19	2.86	0.43
	C_4:	0.00	1.40	29.17	8.10	5.96	9.15
	C_3:	0.35	0.00	2.07	5.47	5.37	3.92
	C_2:	0.39	0.00	1.29	5.30	4.37	4.20

Each value is the average of four samples.

for at 45 minutes 4 determinations for S.A. of butyrate were 0, 0, 0 and 229.5 CPM/μmole acid. This latter value is most likely due to contamination (Table 13). If this is true, then there should be a sharp decrease instead of a sharp increase of butyrate S.A. The S.A. of valerate stayed constant throughout the whole time.

d) Cecum: S.A. of acetate was constant almost throughout the whole time. The S.A. of propionate increased steadily with time. Correlation was 0.72 for the regression line of the ln S.A. versus time. There was no significant change in the S.A. of butyrate. The S.A. of valerate decreased at 30 minutes with a concomitant increase of valerate in the large intestine. This is a good indication of the passage of valerate from the cecum to the large intestine. All the above data suggest that the rate of microfloral fermentation of VFA is in equilibrium with secretion, absorption, chemical degradation and chemical recombination. It also suggests that the cecum is a very active section of the GI tract.

e) Large intestine: The S.A. of acetate and propionate increased steadily and appeared similar to that of the cecum. A decreased butyrate and valerate S.A. in this section and a concurrent increase in the S.A. of butyrate and valerate in the rectum indicated that the acids passed from the cecum to the rectum.

f) Rectum: The S.A. of VFA in the rectum matched the trend of the S.A. of VFA in the large intestine (Table 8).

The above results showed that among these sections, the stomach contents were the most inactive in converting acetate to other VFA, while the contents in the cecum, large intestine and rectum functioned as if they were a homogeneous mass and were the most active in this aspect.

VI. VFA concentration in blood, body acetate pool size determination.

Since most of the free VFA of the body are in the extracellular fluids, injecting rapidly a tracer dose (negligible weight, high specific activity) of sodium acetate assumed rapid dilution of the radioactive material, which can be measured using the following equation: $\ln x = \ln x_o - at = b - at$, where x = specific activity of blood acid at time "t", a= constant = the reciprocal of the turnover time, and $b = \ln x_o$ = zero time specific activity.

The zero time specific activity is obtained by extrapolation. Plotting ln x against time will give a straight line with slope of -a and ordinate intercept of b. The body pool size is then equal to the amount of radioactivity injected divided by the zero time specific activity.

In applying single-injection technique, several assumptions were made:

a) The animals were assumed to be in a steady state, that is, the concentration of blood and tissue components is held at nearly constant concentrations while net synthesis and breakdown of chemical constituents proceed (44). In a situation in which the rate of entry of a molecule "A" by synthesis or transport equals the rate of exit by breakdown or transport, the concentration of "A" remains constant and a steady state is said to exist. An increase in the concentration of "A" may result not only from an increase in the rate of "A" formation but also from a decrease in the rate of "A" loss or both.

A nonsteady state exists whenever the influx of material does not balance the outflux. Thus, a growing person or person in negative nitrogen balance is not in a steady state.

b) An instantaneous mixing of injected labeled substance with the pool.

c) The extent of labeled substance recycled is negligible. That is, the degradation product of this substance will not be utilized to resynthesize this substance in the pool.

The rat was fasted for 2 hours before injection in order to obtain a constant level of blood acetate. Since the interval between injection and blood sampling was rather short, a relatively steady state should still be in existence.

An inherent error in the single-injection technique, as pointed out by Cook (15), is the rapid metabolism of the tracer dose of acetate-^{14}C before it mixes with the body pool of acetate. This can result in low values for the specific activity of blood acetate and a smaller value for b in the equation $\ln x = -at + b$ and consequently, a higher value for the body pool size than what it really should be. Another important point has to be made in applying single-injection technique for pool size determination, that is, the slope of the curve should be obtained from the early part of the specific activity versus time curve (29), since recycling of the label might occur. And if the latter portion of the curve is used for slope determination, the pool size of the acetate will appear higher than it actually is. In this study, a regression line based on all the six time-S.A. points (Fig. 3) was used for calculation of body pool size and half-life of acetate. It is obvious, then, that the values obtained here are higher than when the slope was obtained from the first three points of the graph.

Blood specific activity in CPM/μmoles acetate for each time was determined by pooling blood from 4 rats (Table 9). On an average, there was 37.35 μmoles acetate/100 ml blood.

$\hat{Y} = \log Y = 1.57292 - 0.02405\ X$ was obtained by regressing specific activity Y (CPM/μmoles) against time X (min.) after injection. The correlation is -0.88, which is statistically significant ($P < 0.01$). From the above equation, body acetate pool, turnover rate, turnover time and half life can be calculated:

Body acetate pool = $(1.4 \times 10^6$ CPM)/(anti log 1.5292 CPM/μmole)

= 37.43 μmoles

= 2.245 grams

Turnover rate = (body pool size) (slope of the curve)

= 37.43 x 0.02405

= 0.8983 mmoles/min.

Turnover time = 1/slope of the curve

= 1/0.02405

= 41.58 min.

Half life = turnover time x log 2

= 14.44 min.

If only the first three values of Fig. 3 were used, then:

Body acetate pool = 4.196 mmoles

Turnover rate = 0.1007 mmoles/min.

Turnover time = 18.47 min.

Half life = 3.69 min.

These two sets of data vary greatly from each other. The regression line puts equal weight on the first three points, which fall on a line (10, 15, and 20 minutes after injection) and the last three points (30, 45, and 60 minutes after injection). Recycling of the labeled acetate inevitably occurred at the latter stage. The values obtained by applying the regression line are obviously too high. Extrapolating the line linking the first three points to zero time gives a specific

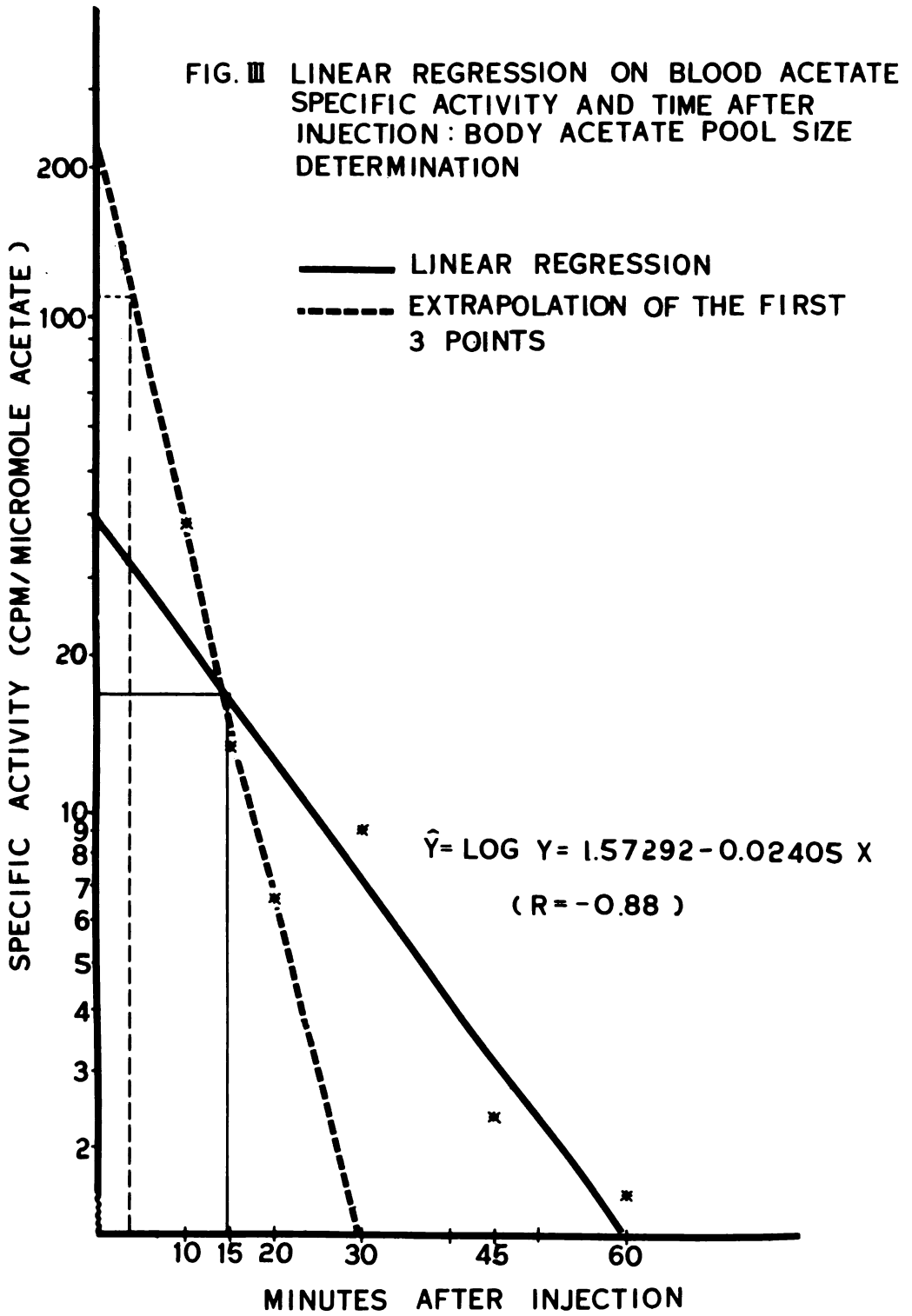
FIG. III LINEAR REGRESSION ON BLOOD ACETATE SPECIFIC ACTIVITY AND TIME AFTER INJECTION: BODY ACETATE POOL SIZE DETERMINATION
LINEAR REGRESSION
EXTRAPOLATION OF THE FIRST 3 POINTS
SPECIFIC ACTIVITY (CPM/MICROMOLE ACETATE)
200
100
20
10
9
8
7
6
5
4
3
2
$\hat{Y}$= LOG Y= 1.57292-0.02405 X
(R=-0.88)
10 15 20 30 45 60
MINUTES AFTER INJECTION

Table 9. Blood specific activity of acetate at various times

Time (min.) after injection	Pooled blood volume from 4 rats (ml)	Micromoles of acetate	CPM of the pooled blood	Specific activity (CPM/μmoles)
10	36.4	15.00	576.5	38.45
15	43.8	16.00	218.6	13.68
20	48.9	11.17	75.1	6.72
30	45.5	15.72	141.1	8.98
45	44.0	21.26	51.2	2.41
60	39.8	17.30	29.1	1.68

Average of pooled blood volume = 43.0 ml

Average micromole of acetate = 16.07

Acetate concentration in blood = (100 x 16.074)/43.02

= 37.35 micromoles/100 ml blood

activity of 233.33 CPM/μmole; this gives the acetate pool size of 4.196 mmoles (251.76 mg) and half life of 3.69 minutes. Assuming there is an equal distribution of acetate between blood and extracellular fluid, then the average concentration of acetate in the blood and extracellular fluid is 2.241 mg/100 g^m body fluid (Table 9, blood acetate concentration = 37.35 μmoles/100 ml blood), which, when divided by the size of acetate pool, will give the weight of the blood and extracellular fluid, in this case 111.8 gm. This is about 22% of the body weight for a 500 gm rat and is a reasonable value.

Since blood contained little if any propionate, butyrate or valerate, the presence of these acids in the GI contents suggested that a quick dissociation or cleavage and reassociation could have taken place in the blood between acetate and its derivative which gave acid of various kinds, i.e., acetate to valerate.

There is a relatively high concentration of VFA in each section of the GI tract at each time interval. Cecal content averaged 6.28 gm wet material and the acetate concentration from the average of six animals was 44.26 μmoles/gm wet weight. This amounted to 277.95 μmoles acetate with a specific activity maintained constant at 5-6 CMP/μmole. Thus, microfloral fermentation was probably responsible for the acetic acid presence in each section while secretion of this acid from the blood was too small to be quantitatively significant.

VII. *In vitro* study of tissue uptake of volatile fatty acids.

One control containing buffer and tissue served as the blank (0% acid). A second control which had buffer and one of the acids served as another control (100% acid). There was no uptake or utilization of VFA by cecal or large intestinal and rectal tissue under the present experimental conditions (Table 10).

It is possible that conditions were not conducive for tissue uptake of VFA. This could include improper temperature, amount of acid in the incubation media, the length of incubation, the kind of buffer, the amount of antibiotics and the balance of CO_2-O_2-N_2 surrounding the media. If there was any acid utilization or uptake by the GI tissues, it was too little to be detected by the present methods.

Table 10. Recovery of acids from the tissue incubation study

Tissue	Incubation time (min.)	Acid incubated with	% of recovery			Average
Cecum	10	acetic acid	95.6	102.0	97.3	98.3
	20		97.8	99.5	96.8	97.7
	40		96.6	99.2	97.2	97.6
	60		97.1	98.7	100.3	98.7
	90		99.3	101.4	97.4	99.4
	10	propionic acid	100.4	97.3	98.2	98.6
	20		100.8	98.5	96.7	98.7
	40		102.2	97.4	98.4	99.3
	60		97.5	96.7	95.2	96.5
	90		98.7	98.1	97.6	98.1
	10	butyric acid	99.3	96.7	95.6	97.2
	20		102.4	97.8	98.7	99.6
	40		100.5	99.7	98.3	99.5
	60		99.4	98.8	103.6	100.6
	90		98.2	94.9	97.5	96.9
Large intestine and rectum	10	acetic acid	94.0	99.6	95.7	96.4
	20		97.0	99.4	97.4	97.9
	40		94.9	100.1	95.3	96.8
	60		100.2	99.2	100.9	100.1
	90		100.4	97.2	98.4	98.7

Table 10 (cont'd.)

Tissue	Incubation time (min.)	Acid incubated with	% of recovery			Average
	10	propionic acid	100.3	98.9	96.7	98.6
	20		100.5	97.2	99.2	99.0
	40		99.3	96.9	95.3	97.2
	60		96.8	99.8	98.2	98.1
	90		97.4	98.5	98.1	98.0
	10	butyric acid	94.8	99.8	98.3	97.6
	20		100.2	99.2	97.4	98.9
	40		99.6	96.5	102.4	99.5
	60		98.7	98.2	95.3	97.4
	90		99.1	98.5	98.8	98.8

SUMMARY AND CONCLUSIONS

Twenty-four male Sprague-Dawley rats were used for the determination of secretion rate and body pool size of acetate. Another nine rats were used for the determination of tissue *in vitro* uptake or utilization of VFA. The single-injection technique was used in the determination of secretion rate and the body acetate pool size, in which potassium-1-^{14}C acetate was the tracer. Different levels of acetate, propionate and butyrate were incubated in cecal and large intestinal plus rectal tissues to determine utilization of VFA by these tissues.

Cecum, large intestine and rectum were the major sites of the metabolism of acetate which came from the small intestine or directly from the blood. Recovery of radioactivity in GI contents and VFA concentration in blood indicated that the microflora in the cecum, large intestine and rectum were responsible for the presence of acids while the secretion of acid from the blood is too small to be significant.

Body acetate pool size was 4.196 mmoles with a turnover rate of 0.1007 mmoles/min. and a half life of 3.69 minutes.

In vitro study revealed that there was no uptake or utilization of VFA by cecal or large intestinal plus rectal tissues.

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APPENDIX

Table 11. Distribution of gastrointestinal contents (I)

Rat No.	Body wt. (gm.)	Stomach 1*	Stomach 2*	Upper small intestine 1*	Upper small intestine 2*	Lower small intestine 1*	Lower small intestine 2*	Cecum 1*	Cecum 2*
1	496	7.02	1.42	0.45	.090	3.50	.706	5.80	1.17
2	492	3.47	.704	.961	.195	4.33	.880	7.40	1.50
3	470	3.43	.730	.630	.134	4.14	.880	4.81	1.02
4	486	3.44	.708	.420	.086	4.43	.912	6.40	1.62
5	469	2.75	.586	.700	.149	4.78	1.02	4.33	.923
6	468	7.44	1.59	.510	.109	3.35	.716	5.74	1.23
7	412	2.58	.623	.840	.204	1.22	.296	5.02	1.22
8	433	4.17	.963	.490	.113	3.63	.838	3.66	.845
9	422	3.17	.751	.240	.057	1.00	.237	3.67	.870
10	482	2.93	.608	.270	.056	3.39	.703	4.82	1.00
11	493	1.99	.404	.680	.138	3.02	.613	8.21	1.67
12	490	1.21	.247	.380	.078	1.82	.371	6.47	1.32
13	508	4.77	.939	2.21	.435	2.05	.404	7.17	1.41
14	531	2.80	.527	.710	.134	3.23	.608	8.37	1.58
15	499	3.70	.741	1.47	.295	2.63	.527	7.15	1.43
16	518	3.58	.691	.780	.150	3.54	.683	8.72	1.68
17	516	2.38	.461	1.02	.198	4.96	.961	6.46	1.25
18	510	.600	.118	.610	.120	2.84	.557	5.81	1.14
19	445	4.89	1.09	.300	.067	4.21	.946	5.87	1.32
20	452	3.93	.869	.130	.029	3.33	.737	5.94	1.31
21	544	4.37	.803	.730	.134	4.04	.743	6.28	1.15

Table 11 (cont'd.)

Rat No.	Body wt. (gm.)	Stomach 1*	Stomach 2*	Upper small intestine 1*	Upper small intestine 2*	Lower small intestine 1*	Lower small intestine 2*	Cecum 1*	Cecum 2*
22	453	1.60	.353	.260	.057	4.00	.883	7.99	1.76
23	437	2.01	.460	.860	.197	2.67	.611	7.57	1.73
24	469	7.40	1.58	1.32	.281	2.10	.448	7.10	1.51
Ave.		3.57	.749	.707	.175	3.26	.678	6.28	1.31

*1 Weight (gm.)
2 Weight (gm.)/100 gm. body weight

Table 11. Distribution of gastrointestinal contents (II)

Rat No.	Large intestine 1*	Large intestine 2*	Rectum 1*	Rectum 2*	Feces 1*	Feces 2*	Total GI tract 1*	Total GI tract 2*
1	.625	.126	1.74	.350	.600	.121	19.74	3.98
2	.764	.155	1.61	.327	.460	.093	18.99	3.86
3	1.60	.340	.720	.153	--**	--**	15.33	3.26
4	1.17	.241	2.21	.455	.400	.082	18.47	3.80
5	1.56	.333	1.44	.307	.270	.058	15.83	3.37
6	.940	.200	.400	.085	--**	--**	18.38	3.93
7	1.08	.262	.930	.226	1.34	.325	13.01	3.18
8	1.43	.330	.620	.143	.530	.122	14.53	3.36
9	.310	.073	1.19	.281	.510	.121	10.09	2.39
10	1.10	.228	.160	.034	--**	--**	12.67	2.63
11	1.32	.268	1.53	.310	.640	.130	17.39	3.53
12	.530	.108	1.89	.386	.460	.094	12.76	2.60
13	1.43	.281	1.76	.246	.300	.059	19.69	3.88
14	1.37	.258	2.03	.382	--**	--**	18.51	3.49
15	.530	.106	1.77	.355	.590	.118	17.84	3.58
16	.500	.096	2.88	.556	--**	--**	20.00	3.86
17	.950	.184	.930	.180	--**	--**	16.70	3.24
18	1.37	.269	1.92	.376	1.17	.324	14.80	2.90
19	1.98	.445	.550	.123	--**	--**	17.80	4.00
20	.180	.040	.900	.200	.580	.128	14.89	3.32
21	1.02	.187	3.22	.592	--**	--**	19.66	3.61

Table 11 (cont'd.)

Rat No.	Large intestine 1*	Large intestine 2*	Rectum 1*	Rectum 2*	Feces 1*	Feces 2*	Total GI tract 1*	Total GI tract 2*
22	1.62	.358	3.34	.737	--**	--**	18.81	4.15
23	.410	.094	3.63	.831	.460	.105	17.61	4.03
24	.880	.188	.69	.147	--**	--**	19.49	4.16
Ave.	1.03	.215	1.59	.328	.627	.134	16.79	3.64

*1 Weight (gm.)
2 Weight (gm.)/100 gm. body weight

**No fecal sample was obtained during the experimental interval.

Table 12. Radioactivity in GI sections (I)

	Time (min.)	Total count CPM	C_2 CPM	C_2 % of total	C_3 CPM	C_3 % of total	C_4 CPM	C_4 % of total	C_5 CPM	C_5 % of total
Upper small intestine	10	47.9	27.1	56.6	1.3	2.7	8.8	18.5	10.6	22.2
	15	6.3	0	0	0	0	0	0	6.3	100.0
	20	13.7	2.8	20.5	0	0	0.89	6.5	10.0	73.0
	30	9.6	2.1	22.3	4.7	48.8	0	0	2.7	28.5
	45	20.5	8.4	40.9	3.3	16.2	0	0	8.8	42.9
	60	26.0	0	0	0	0	1.8	6.8	24.2	93.2
Lower small intestine	10	248.8	198.3	79.9	19.5	7.8	17.1	6.9	14.0	5.6
	15	313.5	154.2	49.2	37.1	11.9	51.9	16.6	70.1	22.4
	20	412.3	70.9	17.2	127.0	30.8	126.5	30.7	88.0	21.3
	30	136.7	85.5	62.6	9.2	6.7	16.6	12.1	25.4	18.6
	45	280.8	44.3	15.8	25.1	8.9	129.2	46.0	82.1	29.3
	60	275.0	24.0	8.7	52.6	19.2	97.9	35.6	100.3	36.5
Stomach	10	58.1	42.6	70.4	0	0	0	0	15.4	26.6
	15	41.3	36.2	86.8	0	0	5.5	13.2	0	0
	20	24.1	24.1	100.0	0	0	0	0	0	0
	30	46.4	46.4	100.0	0	0	0	0	0	0
	45	24.4	24.4	100.0	0	0	0	0	0	0
	60	25.8	14.6	56.8	2.7	10.5	0	0	8.7	32.7
Cecum	10	2394.6	1648.8	68.9	254.1	10.6	360.5	15.0	131.1	5.5
	15	3930.6	2093.7	53.3	240.9	6.1	1008.0	25.7	587.2	14.9
	20	3343.8	1706.0	51.0	294.9	8.8	1231.9	36.8	110.0	3.3
	30	3061.8	1792.3	58.5	518.0	16.9	672.0	21.9	79.5	2.6
	45	3306.2	1487.0	44.9	448.0	13.6	1217.0	36.8	154.1	4.7
	60	2787.9	1103.6	39.6	416.6	14.9	1013.8	36.4	254.0	9.1
Large intestine	10	296.8	180.9	61.0	26.0	8.8	36.6	12.3	53.0	17.9
	15	154.0	89.6	58.2	28.4	18.5	14.0	10.7	19.5	12.7
	20	413.0	220.5	53.4	52.7	12.8	116.0	28.1	23.8	5.8
	30	290.3	100.0	34.5	63.3	21.8	60.9	21.0	65.9	22.7
	45	620.5	213.4	34.1	114.7	18.3	272.0	43.5	25.9	4.1
	60	381.8	163.2	49.3	61.2	16.0	113.2	29.7	19.2	5.0

Table 12. Radioactivity in GI sections (II)

	Time (min.)	Total count CPM	C_2 CPM	C_2 % of total	C_3 CPM	C_3 % of total	C_4 CPM	C_4 % of total	C_5 CPM	C_5 % of total
Rectum	10	305.9	207.8	67.9	33.9	11.1	34.2	11.2	29.1	9.8
	15	849.8	849.6	22.3	70.9	8.3	137.0	19.1	426.6	50.2
	20	238.5	116.0	47.9	47.6	19.9	52.4	21.9	24.1	10.1
	30	459.3	216.0	47.0	106.0	23.1	110.0	24.1	26.5	5.8
	45	369.2	163.3	44.2	76.2	20.7	91.7	24.9	37.0	10.2
	60	484.8	168.0	34.7	77.7	16.0	220.2	45.4	18.7	3.9
Feces	10	70.2	52.4	74.6	2.2	3.2	15.6	22.2	0	0
	15	41.3	30.4	73.3	0	0	5.8	14.1	5.1	12.5
	20	4.3	0	0	0	0	0	0	4.3	100.0
	30	111.0	68.4	61.7	13.8	12.4	27.0	24.4	1.6	1.5
	45	22.1	14.5	65.8	2.6	11.7	4.9	22.6	0	0
	60	19.8	8.3	42.8	0	0	2.6	13.6	8.5	43.7

All values are the average of four samples after correcting for background.

$$\text{percentage of total \%} = \frac{\text{CPM of one acid at one time at one section}}{\text{CPM of acid total at one time at one section}} \times 100$$

Table 13. Specific activity of GI sections (I) (CPM/μM)

Time (min.)	Animal No.	Stomach				Upper small intestine				Lower small intestine			
		C_5	C_4	C_3	C_2	C_5	C_4	C_3	C_2	C_5	C_4	C_3	C_2
10	14									0	0	0	18.75
	*	0	0	0	0	1.0	0	0	3.8				
	18									1.5	9.9	22.0	13.4
	13									1.5	19.0	1.9	9.4
		0.4	0	0	0.6	0	0	0	20.0				
	15									4.7	2.1	1.2	3.0
15	23									14.5	20.6	5.3	10.3
		0	0	0	.33	.8	0	1.9	4.5				
	24									0	0	0	8.97
	11									.45	23.7	13.1	8.20
		0	.3	0	2.4	4.7	0	0	0				
	1									4.98	0	.82	5.10
20	17									1.5	0	0	6.98
		0	0	0	2.3	42.2	0	14.4	4.3				
	3									2.9	0	0	0
	22									7.9	58.1	42.4	1.87
		0	0	0	.71	4.3	14.9	0	2.8				
	2									.46	0	0	2.62
30	12									2.3	33.3	0	4.21
		0	0	0	2.7	.47	0	9.5	.75				
	7									2.4	9.1	14.4	6.98
	20									0	0	0	13.50
		0	0	0	.55	—	—	—	—				
	9									8.3	0	7.4	3.92
45	19									1.1	0	9.7	3.90
		0	0	0	.18	2.2	0	7.4	3.4				
	8									—	—	—	—
	6									9.8	0	0	1.14
		0	0	0	.3	2.8	0	5.5	4.5				
	21									6.3	229	8.1	1.27
60	16									2.9	0	0	0
		0	0	.7	.5	4.6	2.8	0	0				
	10									2.9	12.5	.76	1.33
	5									0	40.6	3.69	2.6
		.3	0	0	.3	6.3	0	0	0				
	4									9.9	63.5	3.86	1.23

— = sample was missing

*When data appear between 2 samples, they are pool values from both.

Table 13. Specific activity of GI sections (II) (CPM/μM)

Time (min.)	Animal Number	Cecum				Large intestine				Rectum			
		C_5	C_4	C_3	C_2	C_5	C_4	C_3	C_2	C_5	C_4	C_3	C_2
10	14	16.73	1.33	.70	1.95	10.7	2.29	2.10	6.0	7.43	5.8	0	3.5
	18	0	.76	.28	3.08	1.05	.87	1.24	.83	.33	1.4	.61	3.3
	13	0	1.6	.85	3.51	1.13	2.29	2.2	7.1	0	0	2.1	1.98
	15	.84	7.19	5.53	11.6	0	4.6	1.35	3.62	0	4.4	2.2	7.1
15	23	13.11	3.5	2.08	3.81	2.51	1.3	6.75	1.52	35.0	25.4	6.51	5.3
	24	15.7	13.8	4.7	13.2	0	0	.99	2.6	0	0	0	1.1
	11	0	.95	1.89	2.26	1.54	1.08	3.07	2.47	.53	1.72	2.2	2.8
	1	1.16	6.85	3.42	8.84	0	1.44	5.4	6.44	0	3.7	2.1	5.7
20	17	22.5	1.77	2.2	3.4	13.8	1.3	2.95	1.94	6.6	5.1	3.7	5.6
	3	1.36	9.89	6.3	8.0	0	19.7	6.5	11.0	1.04	1.7	1.99	4.6
	22	0	4.9	3.3	5.3	1.1	2.7	4.1	5.2	.23	1.6	2.0	3.0
	2	.76	8.5	2.6	6.2	0	4.4	3.6	6.3	0	1.0	.97	2.2
30	12	4.21	2.4	4.1	3.7	15.4	10.9	6.2	8.9	3.0	7.7	7.0	3.5
	7	0	4.8	3.9	4.5	0	7.7	9.3	6.7	2.9	16.2	4.0	5.2
	20	0	2.8	4.2	3.4	9.6	0	4.5	2.2	0	3.0	5.3	6.0
	9	1.48	7.7	5.0	7.5	0	0	16.5	2.6	0	7.1	9.5	7.9
45	19	4.39	6.2	7.7	2.8	2.8	6.9	8.2	8.2	6.9	12.3	19.8	30.7
	8	6.64	11.2	8.7	8.8	2.7	25.4	18.7	15.9	3.3	14.5	15.7	15.3
	6	3.74	10.9	7.2	6.8	.68	9.5	6.3	6.9	--	--	--	--
	21	0	6.8	5.8	5.8	.85	3.0	3.9	5.4	.82	3.3	4.0	3.6
60	16	14.78	10.7	7.7	6.8	6.0	4.2	6.9	5.7	.82	6.0	5.6	4.5
	10	0	6.7	4.6	5.4	4.7	5.5	5.3	4.2	0	11.5	4.0	4.7
	5	2.79	7.4	4.3	4.6	0	6.2	4.7	4.3	0	3.4	2.3	3.3
	4	3.22	7.6	5.4	4.4	.72	7.9	4.6	3.4	.93	11.8	3.8	4.4

-- = sample lost