THE EFFECTS OF ANESTHESIA AND OF ROUTES OF TRACER ADMINISTRATION UPON LIPOGENESIS

by

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I. HISTORICAL

A. The Field of Lipogenesis

It is known that free acetic acid is metabolically inert and in order to be metabolized requires conversion to an "active" form, but only in the last few years has this active form been identified as acetyl-coenzyme A. Coenzyme A (CoA) is (in word form) specifically adenine-(ribose-phosphate)-pyrophosphate-pantothenic acid-beta mercapto-ethylamine. It contains a free sulfhydryl group, i.e. SH group, in the beta mercaptoethylamine portion of the molecule, and it is this SH group which combines with the carboxyl group of acetate through a thio-acyl linkage. This bond appears to have an energy of 12,000 to 13,000 calories per mol, a value near that of the terminal phosphate of adenosine triphosphate (ATP). This gives to the complex a high potential reactivity in metabolic systems.

Rittenberg and Block[1] using C^{13} and deuterium showed that fatty acids can be synthesized wholly from condensation of C_2 units now recognized as being acetyl-CoA units. Synthesis of fatty acids is probably in essence, thus, a reversal of the beta oxidation scheme, suggested in principle by Knoop. As to the degradation of fatty acids, the rates of fatty acid turnover expressed as half life in days is approximately one to three days in liver and six to nine days in the carcass of the rat (West and Todd[2]).

How are acetate and fatty acid metabolisms related to cholesterol metabolism? Rittenberg and Schoenheimer[3], using deuterium, showed that one-half of the hydrogen atoms of the cholesterol molecule come from

body water. From this they concluded that the biological synthesis of cholesterol is brought about by the condensation of a large number of small molecules. It has since been shown that labeled acetic acid is converted to cholesterol in animal tissue[4]. Degradation experiments have also shown that all the carbon atoms of cholesterol can originate from the methyl and carboxyl carbons of acetate[5,6]. It is now known that three molecules of acetate condense to form a C_5 compound and that six of these C_5 units further condense to form cholesterol. As to the turnover time of cholesterol, again expressed as half life in days, for liver cholesterol it is approximately five to seven days, and for carcass cholesterol twelve to fifteen days[2]. Recent work indicated that the previous figures for liver may be unduly high[7]. To further relate fat and cholesterol metabolism, Lipmann[8] has recently reported that coenzyme A is necessary for steroid synthesis.

The preceding short description of synthesis and turnover of lipids is pertinent to an explanation of why radioactive acetate is used to study the lipogenic ability of various tissues. To quote from the work of Hutchens, Van Bruggen, and West[7]: "The major assumption involved in calculating the lipid synthesis rate is that an acetylating agent, $-C_2$, derived from pyruvate, fatty acid, certain amino acids, and exogenous acetate, serves as the carbon source for all the carbon atoms for fatty acid and cholesterol synthesis, and for the acetylation of oxalacetate to form citrate for the tricarboxylic acid cycle." Thus we see that the injection of a tracer dose of acetate into an animal will tag the common C_2 pool but not materially affect its concentration and should give an indication of the activity of various tissues in lipogenic reactions.

Another important aspect is the rate at which the fatty acids and also cholesterol are catabolized in various tissues. The shortest half life of any lipid reported is one to two days for fatty acids in liver. Experiments to be described continued for four hours after the injection of acetate. Only a small fraction of the liver fatty acid could "turn over" in this time period. The degree of incorporation of acetate-1-C^{14} thus should be little changed in a four hour period.

In the work to be reported, the CO_2 eliminated in the expired air by the rat during the four hour metabolism period was collected. By far the greatest amount of C^{14} tracer activity is eliminated by this route. In the tricarboxylic acid oxidative cycle, acetate is an obligatory active member. In this cycle acetyl-CoA combines with oxaloacetate to form citrate which, through a succession of changes involving dehydration, hydration, oxidation, and decarboxylation, produces CO_2 and H_2O and reforms oxaloacetate. The only major source of respiratory CO_2 outside of the cycle is the decarboxylation of pyruvate. In addition, pyruvate combines with CO_2 and forms oxaloacetate which is a necessary component of the oxidative cycle. CO_2 data thus may yield useful information about metabolic reactions.

This thesis is concerned with studies on lipogenesis carried out in the Isotope Laboratory of the Biochemistry Department of the University of Oregon Medical School. Using radioactive acetate as a tool, it has been shown that many tissues in the body are capable of synthesizing fatty acids and cholesterol. It has also been demonstrated that rats show a maximum incorporation of acetate into fatty acid and cholesterol fractions of skin, liver, gut, and carcass tissue in less than twenty

minutes. Significant further changes were not observed in the following four hours[9]. In other experiments, on fasted animals, the cholesterol content of the tissues was shown to remain constant, whereas the fatty acid content was reduced 75% in 120 hours of fasting. In rats fasted 120 hours and given a tracer dose of C^{14} acetate and allowed to metabolize it for one hour, the activity of CO_2 did not change from that in normal animals, but the C^{14} appearing in fatty acids was reduced to less than 32% of the normal and cholesterol activity was reduced to 43% of the normal[10]. This fasting study was extended to 240 hours and all tissues showed significant decreases in the incorporation of label, but liver decreased most. In many of these studies, the incorporation of label into gut lipid was extensive. This high gut activity will be examined critically in our study[11].

Radioactive C^{14} is particularly valuable as a label in metabolic studies. It has the long half life of 5,000 years. With this long half life, the experimental techniques, the counting procedures used, and the period required for the experiment need not be limited as to time. The radiation emitted by C^{14} is a beta particle. Its range is only one cm. in air, and therefore there is no personal danger from being in close proximity to radioactive substances containing C^{14}. Compounds containing it should, however, not be ingested.

B. Specific Problem

Since the advent of both radioactive and stable isotopes, there has been a revolution in the field of biochemistry. In in-vitro techniques, problems of tissue permeability to the label are of serious concern, in

spite of apparent substrate label availability. In in-vivo studies, some investigators have used the intraperitoneal (I.P.) route of label administration exclusively, some the intravenous route (I.V.), and some both routes. Practically no information is available as to whether or not the route of administration affects the results obtained.

This laboratory has been active in the field of lipid metabolism for a number of years, and it seemed imperative to determine if the high incorporation of acetate label into intestine and liver tissue of Sprague Dawley rats is influenced by the route of tracer administration[11]. Tissue utilization of acetate is so rapid that the availability of the label to a given tissue might have an important effect on the incorporation of acetate by the tissue. How quickly is acetate utilized by the tissues? How immediate is the distribution of acetate to tissues? Hutchens and Van Bruggen[12] reported that half the C^{14} activity disappears from the peritoneal space in one minute, whereas the half time for blood acetate was found to be seven minutes. However, Busch and Baltrush[13] found the half time of absorption of acetate-1-C^{14} from the blood to be 18 seconds, and, ten minutes after injection, less than 10% of the total activity was found in the blood. One minute after injection of the tracer into the tail vein of rats, 74 to 100% of acetate had been transferred to other substances. Harper and his group[14] reported that tracer or non-tracer amounts of acetate injected into the fore leg vein of dogs gave the same pattern of utilization, but showed a difference in time. The non-tracer dose was utilized completely in 30 minutes and the tracer dose of acetate in five or six minutes. Also he reported a high "turnover" of liver fatty acid, one gram/hour/100 grams of liver. If this is

true in the rat, then it appears that in four hours the % incorporation into liver fatty acid would show a reduction from the initial % incorporation because of fatty acid breakdown. However, Van Bruggen, Hutchens, and Claycomb[9] reported no change in % incorporation of acetate into liver fatty acid up to 126 minutes after tracer acetate was injected.

Ciaranfi and Formesu[15] showed that normal blood values of acetate for dogs average 7 mg. %. Injected non-tracer acetate disappears very rapidly from the blood at a rate which is related to the initial blood concentration. They noted that only a negligible amount of acetate is eliminated by the kidneys. They concluded that injected acetate is rapidly metabolized by the tissues and that a non-tracer dose disappears from the blood stream in five to ten minutes. It is clear from these experiments that acetate is readily utilized by the tissues. Most experimenters have reported a utilization time of five to ten minutes for acetate. The only extreme divergence from this figure was reported by Busch[13] who found an acetate half life of 18 seconds.

The fact that acetate is so rapidly metabolized suggests that the route of administration may affect the incorporation of acetate by tissues. The study reported here compared four tissues, liver, gut, carcass, and skin, of the Sprague Dawley white male rat and seeks to determine the influence of the administration route upon acetate utilization during lipogenesis. All previous studies on these four tissues reported from this laboratory have involved the intraperitoneal route of tracer administration. In our work the acetate was given by both intraperitoneal and intravenous routes in an attempt to better explain the divergent results reported for acetate utilization time.

Since it was necessary to anesthetize the animals for intravenous injection, control experiments were done in which anesthetized animals were given the tracer dose by the I.P. route. These control animals were then compared to other rats which were injected I.P. and subjected to identical experimental conditions except that no anesthesia was used. Nembutal (Pentobarbital Sodium) was chosen since it is easily administered, has a short period of action, and has been reported to have no direct effect upon hepatic function, circulation, or basal metabolic rate. Nembutal has been shown to depress the central nervous system with resultant slight depression of the respiratory center[16].

Some in-vitro homogenate work involving barbiturates showed inhibition of inorganic phosphate uptake by liver when metabolizing pyruvate. Brody and Bain[17] found that along with a 20 to 40% decrease in oxygen uptake, caused by pentothal (a drug quite similar to nembutal), inorganic phosphate uptake also was almost completely inhibited. They concluded that the barbiturates appear to uncouple phosphorylation from oxidation and thus decrease neuronal activity.

It is known that adenosine triphosphate (ATP) is the primary source of energy for tissue activity. To be used in lipogenesis, acetate must be activated by conversion to acetyl CoA through the following reactions in which AMP represents adenosine monophosphate, PP represents pyrophosphate, and HS-CoA represents coenzyme A:

$$\text{Enzyme} + \text{ATP} \xrightarrow{Mg^{++}} \text{Enzyme} - \text{AMP} + \text{PP}$$

$$\text{Enzyme} - \text{AMP} + \text{HS} - \text{CoA} \longrightarrow \text{Enzyme} - \text{S} - \text{CoA} + \text{AMP}$$

$$\text{Enzyme} - \text{S} - \text{CoA} + CH_3COOH \longrightarrow CH_3 - CO - \text{S} - \text{CoA} + \text{Enzyme}$$

The summation of these reactions gives:

$$CH_3COOH + H - S - CoA + ATP \longrightarrow CH_3 - CO - S - CoA + AMP + PP$$

Thus it can be seen that ATP is essential in the synthesis of fatty acids from acetate. In this study the effects of nembutal anesthesia upon lipogenesis in-vivo are compared to the in-vitro effects cited above.

The fact that nembutal depresses the respiratory center directly, or indirectly by first depressing the cerebral cortex, made it of interest to analyze the expired air continually for $C^{14}O_2$ activity[16]. In the anesthetized I.P. rats, then, the time course of expired $C^{14}O_2$ was compared to the other I.P. injected animals that received no anesthesia. The time course of I.V. and I.P. injected animals was also compared. Thus the effect of anesthesia on the elimination of CO_2 and the turnover of the bicarbonate pool should yield information as to its influence upon respiration. Also the effect of different routes of tracer administration on $C^{14}O_2$ elimination should throw some light upon the relative availability of label to tissues when injected I.V. and I.P.

In an attempt to discover more about the high lipogenic activity of gut tissue, the intestinal tract was divided into six separate fractions. The stomach and esophagus, stomach contents, small intestine, small intestine contents, large intestine, and large intestine contents were examined for cholesterol and fatty acid C^{14} activity. The small intestine is very active in absorption of amino acids, sugars and fats. It is also active in esterifying cholesterol, phosphorylating sugars, excreting lipids; and it exerts selective absorption. It is thus important to know, because of the high metabolic activity of the intestine,

how much of the acetate incorporated into lipids in gut tissue is attributable to the small intestine alone. Cholesterol was isolated in the non-saponifiable material and then determined quantitatively by colorimetry. The percent of cholesterol found in each non-saponifiable fraction was calculated. A study of the factors that influenced the cholesterol content in different non-saponifiable fractions was studied.

II. METHODS

Many of the techniques used in the work being reported in this thesis have been described in publications from this laboratory. However, since a few modifications of the established techniques were developed, the previously reported procedures will be reviewed and the modifications presented.

A. Care and Feeding of Rats

Adult male rats of the Sprague-Dawley strain were trained-fed with Purina chow. The amount normally eaten by a rat in one day was determined to be 20 to 25 grams. To condition the animals to eat at specified times, the rats were fed 10-12 gm. of chow at 8 a.m. and again at 5 p.m. each day. Rats were started on trained feeding when they reached 100 gm. weight and were trained fed until they weighed approximately 220 gm. On the morning of the day of the experiment the rat was fed as usual at 8 a.m. Any remaining food was taken away at 9 a.m. and the animal was allowed to fast for one hour. The one hour fasting period was selected since during this period lipogenesis is at its maximum[10]. It was felt that if a significant change was effected in lipogenesis by our experimental methods, it could be best demonstrated in this period.

B. Anesthesia and Injection

Fifteen minutes before the hour fast was completed, the rat was injected intraperitoneally with nembutal, the dose being 3.5 mg. per 100 gm. body weight. At the completion of the one hour fast the rat was injected either intravenously or intraperitoneally with 0.5 ml. of

acetate-1-C^{14} solution, containing 1.35×10^6 c.p.m. when assayed as infinitely thick $BaCO_3$ with a Q gas counter*. When counted with a thin end window Geiger tube* the dose contained 0.57×10^6 c.p.m. This dose is equivalent to approximately 10 c.

C. Metabolism Period

After injection the rat was kept in a closed metabolism chamber for four hours, and the total CO_2 and $C^{14}O_2$ exhaled was collected in NaOH. The apparatus used is shown assembled in the photograph, Figure 1. The size and shape of chamber A permitted little body movement. When the rat recovered from the anesthesia it often struggled for a short period but then quieted down for the remainder of the metabolism period. At the end of the metabolism period, the Na_2CO_3-NaOH solution collected in absorber H was transferred to a 500 volumetric flask and brought up to volume with distilled water.

D. Injection Technique

In preliminary work, intravenous injection was attempted using the tail, jugular, or saphenous veins. The tail vein route was eliminated because we could not be sure that the full dose would enter the venous system. Griffith and Farris[18] reported that only 30% of tail vein injection attempts were quantitatively successful. Busch and Baltrush[13] found it necessary to subtract the amount of radioactivity left in the tail tissue after tail vein injection of acetate, from the total amount

*Research Equipment Co., Chicago.

*Tracerlab TGC-2 (1.2 mg./cm^2. mica window

Figure 1

Metabolism assembly for the collection of respiratory CO_2 and for the continuous recording of $C^{14}O_2$ concentrations

A. Soda lime tower

B. Flow meter

C. Animal chamber

D. Moisture trap

E. Geiger-Müller tube assembly

F. Flow meter

G. Fractional CO_2 collector

H. Bulk CO_2 collector

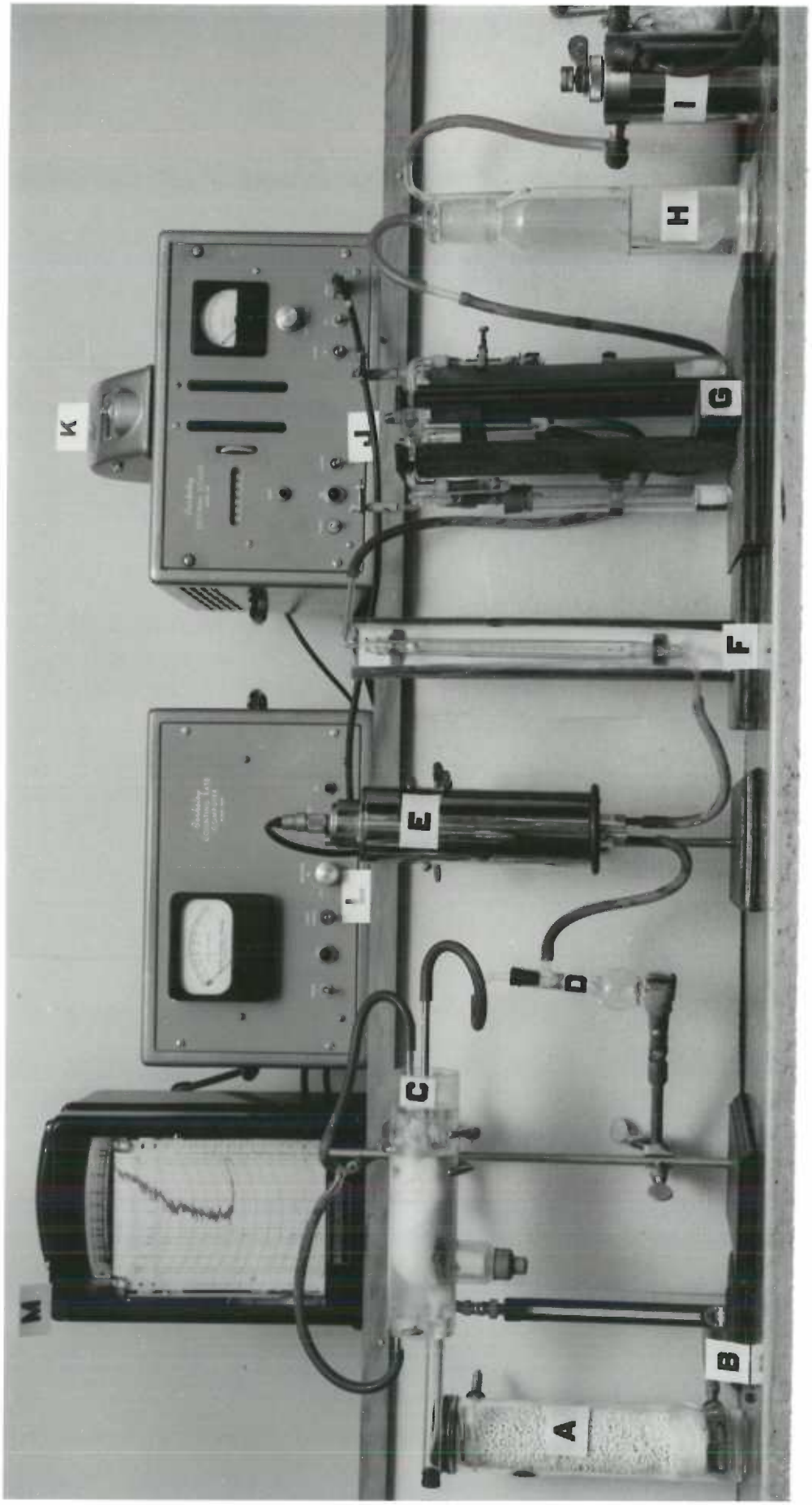
I. Cartesian manostat

J. Decimal scaler

K. Timer

L. Count rate computer

M. Esterline-Angus recorder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injected. These reports confirm our difficulty in getting 100% of the injected dose into the vein. The jugular vein injection was attempted, but without great success. However, the saphenous vein proved accessible and the following technique was developed:

1. The hair was clipped on the medial aspect of the hind leg.
2. The skin was picked up in a fold and cut across with scissors, very close to the medial malleolus.
3. The skin and subcutaneous tissue were pulled away from the muscle fascia for a distance of 1.5 cm. This revealed the junction of the saphenous and lateral planter veins.
4. The femoral vein at the groin was compressed with the index and middle fingers.
5. A 27 gauge, 1 inch needle was used and inserted into the junction of the saphenous and lateral planter veins. After aspiration of a small amount of blood the 0.5 ml. dose of acetate was quickly injected.
6. The vein was now compressed with a small square of saline wetted Gelfoam to prevent hemorrhage.
7. The skin flaps were approximated and the animal quickly put into the metabolism chamber.

E. Dissection and Digestion of Animals

After four hours in the metabolism chamber the rat was removed, quickly chloroformed and weighed. The rat was decapitated and dissected into gut, liver, carcass and skin. The tissues were put into previously prepared 20% alcoholic KOH and saponified by heating under reflux for four hours.

F. Isolation of Fatty Acids and Cholesterol

After reflux, each fraction was concentrated on a steam bath with the aid of an air stream, and the alcohol removed. Water was then added to the digest flasks to restore the original volume and the contents were transferred, hot, to separatory funnels. The gut and carcass fractions were filtered through glass wool into the funnels because of bone and other residues. To insure complete transfer, the digest flasks were washed three times with hot water, and twice with hot alcohol. Sufficient amounts of alcohol were used in the transfer to give a ratio of 5:1 of aqueous to alcohol phases. This ratio allowed maximum recovery of lipids with a minimum formation of emulsions.

The digest was extracted successively with four portions of petroleum ether (boiling point 30-60° C.). The petroleum ether extracts were pooled, washed with 1 N KOH, and then with water. The KOH and water washes were added to the aqueous digest. The pooled petroleum ether extracts were dried over Na_2SO_4. The alkaline water phase was acidified to Congo red with HCl, and four successive extractions again were made with petroleum ether. Ether extracts were pooled, washed with water, and then dried over Na_2SO_4.

The first ether extract contained the non-saponifiable (cholesterol) fraction, and the extract made after acidification contained the saponifiable fraction. The separation of lipids into these two fractions by the use of the same solvent is possible because the potassium soaps of the fatty acids are not soluble in petroleum ether, whereas the saponified sterols are soluble in this solvent. Upon acidification the fatty acids become soluble in petroleum ether and are removed by the solvent.

The ether extracts were now filtered into boiling flasks to remove the Na_2SO_4. Eight separate fractions were so obtained from each animal, representing the cholesterol and fatty acid fractions of gut, liver, skin, and carcass.

All eight fractions were next distilled to small volume and the last traces of petroleum ether removed by an air stream. The non-saponifiable extracts were transferred to volumetric flasks with four transfers of cold ethanol. Each transfer was first heated with the ethanol aliquot and then cooled and filtered. The filtrate was diluted to a standard volume with alcohol. Skin, carcass and gut extracts were made up to 100 ml., whereas liver was diluted only to 50 ml. Fatty acid fractions were transferred to weighed 250 ml. Erlenmeyer flasks by aliquots of acetone. Each aliquot of transfer solvent was first heated in the fatty acid flask and then cooled and filtered. About 200 ml. of acetone each were used to transfer carcass and skin fractions. Of this amount, 75 ml. were used in the first transfer. If these large volumes were not used, non-lipid residues were found in the fatty acid fractions. About 25 ml. of acetone were used in each gut and liver fatty acid transfer. The filtrate was evaporated and the amount of fatty acid determined by weighing.

G. Determination of Cholesterol

The colorimetric method used was a modification of the Schoenheimer-Sperry method, and was done as follows:

1. Two aliquots, generally of different sizes, were taken from each tissue fraction and pipetted into Coleman tubes. Sufficient volumes

were used so that one tube contained approximately 0.5 mg. and the other about 0.25 mg. of each fraction. These quantities of cholesterol gave optical densities in the most accurate range of the colorimeter. Two aliquots of a standard were also taken. The standard contained 50 mg. per 100 ml. so that each ml. contained 0.5 mg. of cholesterol. Thus, 1 ml. and 0.5 ml. aliquots were taken.

2. Cholesterol samples were now evaporated to dryness in a water bath with the aid of an air stream.

3. Tubes were next put in an 110° drying oven for one hour.

4. Two ml. of glacial acetic acid were added to each tube while hot, and the tubes lightly shaken to help dissolve the cholesterol. Two ml. of glacial acetic acid were also added to a blank tube.

5. Tubes were placed in a 25° C. water bath.

6. A reagent containing acetic anhydride and sulfuric acid in a ratio of 20:1 was prepared, and four ml. of this reagent were added to each tube ten minutes after preparation of the reagent.

7. Tubes were immediately placed in a light-tight water bath at 25° C.

8. The Coleman colorimeter was allowed to warm up for 15 minutes and then its response was checked with a standard Didymium filter.

9. The blank tube was used to set the instrument at zero density, the wave length being set at 640 mu.

10. Tubes were read at 40 minutes after the addition of the reagent and the optical density was recorded.

11. With the results obtained from the standards a curve was prepared on graph paper, and the amount of cholesterol in each tube was now

determined. From this figure the amount of cholesterol in each fraction was calculated.

H. Combustion Procedure

The apparatus and techniques established for the wet combustion of non-volatile samples by Claycomb, Hutchens and Van Bruggen[19] were employed. The method was as follows:

1. When cholesterol was combusted a volume of alcoholic solution containing approximately 7.5 mg.* of cholesterol was placed in a combustion tube and evaporated to dryness. The tube was then placed in an oven for one hour to remove traces of water.

2. In the combustion of the fatty acids, 10 mg.* samples were weighed in porcelain boats and these placed in combustion tubes.

3. Five ml. of Van Slyke-Folch solution were added to the proper side arm of the combustion tube. The equipment was arranged so that samples could be run in duplicate.

4. Six ml. of 0.5 N NaOH were placed in the absorption flasks.

5. High vacuum grease was applied to the glass joints and then the combustion tube and absorption flask assembly was evacuated by a water pump to about 20 mm. Hg.

6. The tubes were twisted or inverted so that the combustion solution mixed with the sample, and spontaneous oxidation was allowed to occur at room temperature for about 5 minutes.

7. The absorption flasks were cooled by ice water and the combustion tubes immersed in a hot wax bath (160° C.) and combustion carried

*The above amounts of lipids were chosen for they produce enough CO_2 to make infinitely thick $BaCO_3$ plates, i.e., 100 mg. in weight.

out for 20 minutes. The reaction was considered complete when the Hg. in the manometer arm returned to its previous setting.

I. Plating

1. The contents of the absorption flasks were transferred to centrifuge tubes and the $CO_3^=$ precipitated with an excess of $BaCl_2$-NH_4Cl reagent.

2. Equal aliquots (usually 10 ml.) of the NaOH-Na_2CO_3 solution from the collection of respiratory CO_2 were also put into centrifuge tubes and precipitated.

3. For optional precipitation conditions, the centrifuge tubes were warmed to 45-50° C. before the $BaCl_2$ reagent was added. After the addition of reagent the tubes remained at 50° C. for an additional 10 minutes to allow the precipitate to flocculate.

4. If the amount of $BaCO_3$ produced was low, carrier $BaCO_3$ was added at this point to insure an infinitely thick plate. One to two ml. of ether-alcohol were added and the tubes were centrifuged for 10 minutes at 2,000 RPM.

5. The supernatant fluid was aspirated and the precipitate was triturated.

6. The precipitate was now washed with 5 ml. H_2O, recentrifuged, and retriturated. This operation was done three times.

7. The precipitate was now washed with about 25 ml. of ether-alcohol (1:3) and centrifuged, and the supernatant was aspirated.

8. The precipitate was transferred to centrifuge cups containing tared aluminum discs. Not more than 15 ml. of ether-alcohol was used in the transfer.

9. The cups were centrifuged for 5 minutes at 2,000 RPM.

10. The supernatant ether-alcohol was aspirated and the cups were again centrifuged for 30 minutes; they were dried during this time with the aid of a heating apparatus in the centrifuge.

11. The samples were removed from the centrifuge cups and weighed to constant weight.

J. Counting[20,21,22,23]

The $BaCO_3$ samples from cholesterol and fatty acid combustions were of low enough activity to be counted in a gas flow counter using Q gas. The CO_2 plates were of higher activity and were best counted with the use of a Geiger-Müller end window tube. The background count and the activity of a standard polyethylene source were also determined. Most samples were counted for 30 minutes or until 6400 counts were obtained.

The standard had also been counted at the time that the radioactive sodium acetate had been assayed. The standard generally counted about 1300 c.p.m. with the Q gas counter and about 560 c.p.m. with the end window counter.

The efficiency of a counter depends upon geometric efficiency, back scatter correction, self absorption corrections, and upon ionization probability. The use of a standard as described above serves to minimize the corrections required. In radio-assays there is the possibility of two particles being emitted from the C^{14} at precisely the same time, causing an avalanche so that only one is counted; this phenomenon is called coincidence. It is necessary to correct for this only when over 5,000 c.p.m. are recorded.

After the counts of the $BaCO_3$ plate were obtained and the background was subtracted, the samples were corrected to infinite thickness. This was to account and correct for the absorption of beta particles occurring within the sample[20]. The self absorption correction was obtained by the following method. A series of increasing weight $BaCO_3$ plates was prepared, and the number of counts obtained from these plates was compared to the unit weight of the $BaCO_3$ plate. It was found that at a weight of 20 mg. per cm^2. and greater, all the plates gave a similar number of counts. After a thickness of 20 mg. per cm^2. was obtained the radiations of C^{14} below this thickness were absorbed in the sample itself and were not recorded. The planchets used in this laboratory were arbitrarily made 5 cm^2. in area. As infinite thickness is at 20 mg./cm^2., a 100 mg. plate is an infinitely thick plate. From the above series, a "G factor" was obtained. Using this "G factor" the value that a sample would count if it was infinitely thick could be obtained.

K. Recording of Data

The plate was corrected if it was either above or below infinite thickness in weight. For plates above infinite thickness the total plate count was calculated as follows:

$$\frac{\text{cts./min. - Bkg x Plate Weight in mg.}}{\text{Plate Weight at Infinite Thickness}} = \text{Total Plate Count}$$

For plate weights below infinite thickness the following formula was used:

$$\frac{\text{cts./min. - Bkg x Plate Weight in mg.}}{\text{G x Plate Weight at infinite thickness}} = \text{Total Plate Count}$$

The activity of cholesterol and fatty acids in each tissue was recorded as specific activity and as % incorporation. These figures were calculated by the following method:

$$\frac{\text{Total Plate Count}}{\text{Mg. Sample Combusted}} = \text{Counts/Minute/Mg.} = \text{Specific Activity (S.A.)}$$

$$\text{S.A.} \times \text{Total Amount of } \begin{cases}\text{Fatty Acid} \\ \text{Cholesterol}\end{cases} \text{ in Fraction} = \text{Total Activity in Fraction}$$

$$\frac{\text{Total Activity in Fraction}}{\text{Total Counts Injected}} \times 100\% = \% \text{ Incorporation}$$

With CO_2 the total activity in the CO_2 pool was calculated, and from this the % incorporation into CO_2 was determined.

L. Techniques used in Fractionated Gut Animals

1. The Fractionization of the Gut

In four animals the gut was dissected into six fractions. Two of these four rats were injected intraperitoneally and two were injected intravenously.

The gastrointestinal tracts of these animals were first separated into three parts, namely esophagus and stomach, small intestine, and large intestine; and each tissue, with contents, was weighed. The stomach was slit, the contents separated, and the two fractions were weighed. The small and large intestines were dissected into short sections; a syringe and water were used to force out the contents of these sections into digestion flasks. The tissues of small intestine and large intestine were also then weighed. The weights of the contents of these tissues were calculated.

The six fractions were now digested and extracted as previously explained; volumes of reagent proportional to the amount of substance were used in the digestion and extraction. As the amounts of fatty acid and cholesterol extracted were small, $BaCO_3$ was added as carrier at the combustion time to bring plates up to infinite thickness.

2. Analysis of Expired Air

In the fractionated gut animals the CO_2 given off in the expired air was continually analyzed for activity. To control the rate of flow of air through the metabolism chamber a Cartesian manostat, a vacuum regulating device, was used (see I in Figure 1, p. 12), and a water pump was connected in series with the manostat.

The $C^{14}O_2$ and CO_2 leaving the metabolism chamber entered a Tracerlab lead shield (E in Fig. 1) modified by Hutchens and Van Bruggen to allow direct counting of the activity in the air stream. A Geiger-Müller tube was mounted inside and near the end of the lead shield, and a small space was available for the air stream to sweep by the end window. The counts were recorded on a Model 100 Berkeley decimal scaler (J in Fig. 1). The scaler was in turn connected to a Berkeley Model 1600 count rate computer (L in Fig. 1), which transferred the impulses to a unit count basis, i.e. c.p.m. The pulses from the rate computer were transferred to an Esterline-Angus recorder (M in Fig. 1), a D.C. milliameter. An inkable slow moving graph provided a permanent record. The total accumulated number of counts was obtained from the Model 100 scaler at frequent intervals and recorded by hand. From this the time course of $C^{14}O_2$ expired was determined and recorded.

III. RESULTS

A. Incorporation of Acetate into Fatty Acids and Cholesterol in Anesthetized and Unanesthetized Rats

Since, as previously described, it was necessary to anesthetize rats to give an intravenous injection, the intraperitoneal route of acetate-1-C^{14} administration was employed with five controls, anesthetized with 3.5 mg. of nembutal per 100 gm. rat body weight. These anesthetized animals, intraperitoneally injected with tracer, were compared with four other rats which were not anesthetized and which received only the tracer acetate I.P. Another difference in the experimental conditions between these two groups was that the tracer dose of acetate-1-C^{14} was not the same; the anesthetized rats received 10 μc. of acetate, whereas the unanesthetized rats received 20 μc*. As no exact correlation is available between specific activity data and the size of dose administered, only % incorporation data will be compared, for this is independent of dose size.

In Table I are shown the % incorporations of acetate-1-C^{14} into cholesterol and fatty acid fractions in the four tissues of the two groups of rats. The results from the anesthetized rats were averaged for each tissue and compared to the average results of the unanesthetized animals. A statistical test of difference between the means was applied, using a 95% confidence limit. The "t", or test of significance values, were also determined. No significant difference was noted in the

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*20 microcuries (μc.) is equivalent to 2.6×10^6 c.p.m. when assayed as infinitely thick $BaCO_3$.

Table I

INCORPORATION OF ACETATE-1-C^{14} INTO TISSUE LIPIDS OF ANIMALS INJECTED INTRAPERITONEALLY WITH AND WITHOUT ANESTHESIA

A. Anesthetized Group

	Liver		Gut		Carcass		Skin	
Rat No.	F.A.*	Chol.‡	F.A.	Chol.	F.A.	Chol.	F.A.	Chol.
SV-4	.69	.09	3.40	.19	3.79	.25	.98	.12
SV-8	1.29	.26	5.60	.33	4.63	.28	1.82	.15
SV-9	.31	.16	1.43	.28	5.92	.20	.77	.13
SV-10	1.19	.11	1.17δ	.25δ	2.90	.19	1.24	.11
SV-12	.65	.10	.94δ	.10δ	4.47	.20	1.64	.11
Average	.81	.14	2.51	.23	4.34	.22	1.29	.12

B. Unanesthetized Group

N-10	.45	.14	7.89	.34	4.81	.21	1.66	.10
N-12	.92	.18	8.58	.46	3.74	.25	2.95	.11
N-13	.58	.12	1.74	.22	3.74	.19	1.15	.10
N-14	.42	.19	2.38	.68	1.36	.19	.57	.09
Average	.59	.16	5.15	.43	3.41	.21	1.58	.10
t	1.10	.47	1.05	1.85	1.19	.70	.54	1.26
Significant Difference: (α = .05)	No	No	No	No	No	No	No	No

*F.A. = Fatty Acid

‡Chol. = Cholesterol

δTotal of Gut Fractions

% incorporations of acetate into any of the tissues of the two groups. These results indicate that anesthesia had no significant effect on the % incorporation of acetate into fatty acids or cholesterol in the intact rat.

B. Comparison of I.P. with I.V. Injected Animals

As no significant difference was found between the groups reported above, all data of the I.P. animals were pooled. This pooling was done to give a larger number of animals in the I.P. sample and to give to the interpretation greater statistical security. These pooled data were compared to data from I.V. injected animals. In Table II are recorded the data for the % incorporation of acetate into fatty acid and cholesterol fractions of gut, liver, carcass, and skin tissues of the two groups. The statistical test of difference between the means was again employed at a 95% confidence level. The resulting "t" values for each tissue are listed in the next to the last line of figures at the bottom of Table II.

The results showed that the use of different routes of administration gave significant differences in the % incorporation of acetate in three of the tissue lipid fractions. In the fatty acid fractions of gut, there was a significant decrease in label incorporation from 3.68% to 1.55% when the I.V. route was used. Carcass and skin fatty acids showed no significant change. Liver fatty acid showed a significant increase in incorporation, i.e. from 0.71% to 1.13%, when the I.V. route was used. In the cholesterol fractions, only skin cholesterol incorporation changed significantly, increasing from 0.11% to 0.15% when the tracer was administered I.V.

Table II

COMPARISON OF % INCORPORATION OF I.P. AND I.V. INJECTED ANIMALS

I.P. Injected	Liver		Gut		Carcass		Skin	
	F.A.	Chol.	F.A.	Chol.	F.A.	Chol.	F.A.	Chol.
SV-4	.69	.09	3.40	.19	3.79	.25	.98	.12
SV-8	1.21	.26	5.60	.33	4.63	.28	1.82	.15
SV-9	.31	.16	1.43	.28	5.92	.20	.77	.13
SV-10	1.19	.11	1.17*	.25*	2.90	.19	1.24	.11
SV-12	.65	.10	.94*	.10*	4.47	.20	1.64	.11
N-10	.45	.14	7.39	.34	4.81	.21	1.66	.10
N-12	.92	.18	8.58	.46	3.74	.25	2.95	.11
N-13	.58	.12	1.74	.22	3.74	.19	1.15	.10
N-14	.42	.19	2.38	.68	1.36	.19	.57	.09
Average	.71	.15	3.68	.32	3.93	.22	1.42	.11
I.P. Injected								
SV-3	.76	.11	1.46	.24	2.51	.28	1.41	.17
SV-5	1.33	.18	1.27	.24	3.76	.22	1.78	.16
SV-6	1.76	.20	2.25	.26	6.70	.35	2.70	.16
SV-7	1.18	.26	1.83	.40	4.73	.51	2.53	.21
SV-11	1.07	.12	1.37*	.17*	4.23	.21	1.78	.10
SV-13	.65	.07	1.09*	.16*	3.55	.25	1.84	.10
Average	1.13	.16	1.55	.25	4.25	.30	2.00	.15
t	2.2	.30	6.28	.10	.5	1.7	1.87	2.2
Significant Difference: (α = .05)	Yes	No	Yes	No	No	No	No	Yes

F.A. = Fatty Acid
Chol. = Cholesterol

*Total of Gut Fractions

C. Comparison of Specific Activities of I.P. and I.V. Rats

As previously mentioned, since no correlation was available between the specific activity of the lipids and the size of dose injected, only the anesthetized I.P. and I.V. animals were compared, for these received the same dose of acetate-1-C^{14}. There was found (as shown in Table III) no significant difference between the specific activities of lipids of tissues from these animals. Specific activity figures may be misleading because of their extreme variation, due largely to the fact that the amount of lipid in the tissues of different animals varied considerably. This extreme variance was found even though the rats used were of the same strain, put on the same diet, kept under the same experimental conditions, and used at approximately the same weight, 220 gm. The extreme variance in specific activities made it difficult to show significant differences between groups. The % incorporation calculation helped correct for the divergence in fatty acid concentration, and these figures thus yield a more significant basis for comparison.

D. CO_2 Data

The CO_2 data from the five anesthetized I.P. injected rats and six I.V. injected rats are listed in Table IV. As high as 73% of the administered radioactivity was eliminated as $C^{14}O_2$ in the expired air during the four hour metabolism period. The % incorporation ranged from 56% to 73% in the eleven animals. The specific activity, expressed as counts per minute per millimol, ranged from 5730 to 8210. The total millimols of excreted CO_2 varied from 41.9 to 58. It can be clearly seen that there was no significant difference between any of these values, when different routes of administration of label were used.

Table III

COMPARISON OF SPECIFIC ACTIVITIES[δ]
OF I.P. AND I.V. INJECTED
ANESTHETIZED ANIMALS

Anesthetized	Liver		Gut		Carcass		Skin	
I.P. Injected	F.A.	Chol.	F.A.	Chol.	F.A.	Chol.	F.A.	Chol.
SV-4	27.22	49.87	31.97	47.02	5.4	17.42	2.28	10.25
SV-8	52.84	169.26	55.73	80.23	8.12	23.72	8.14	14.64
SV-9	15.21	101.08	21.04	74.95	11.97	23.76	2.49	20.14
SV-10	66.54	84.46	17.35[*]	73.92[*]	5.96	17.40	4.15	15.86
SV-12	30.00	56.73	11.53[‡]	30.00[‡]	6.16	18.75	4.23	14.19
Average	38.36	92.3	27.52	61.22	7.52	20.21	4.26	15.02
Anesthetized I.V. Injected								
SV-3	14.93	69.10	11.85	67.12	4.20	20.41	3.18	15.91
SV-5	51.57	109.86	13.20	64.29	4.50	15.62	3.83	14.31
SV-6	82.93	142.23	17.66	56.19	9.84	26.64	8.15	18.84
SV-7	53.25	174.90	23.32	106.80	9.62	33.07	8.59	20.64
SV-11	49.40	76.0	18.33[*]	42.28[*]	9.21	22.18	8.30	14.03
SV-13	29.63	48.59	17.12[‡]	48.16[‡]	5.58	19.96	5.64	10.65
Average	46.95	103.45	16.91	64.14	7.16	22.98	6.28	15.70
t	.66	.30	1.39	.19	.14	.90	1.4	.3
Significant Difference	No	No	No	No	No	No	No	No

[δ]Specific Activity = S.A. = c.p.m./mg. lipid
[‡]Total of Gut Fractions

F.A. = Fatty Acid
Chol. = Cholesterol
I.P. = Intraperitoneal
I.V. = Intravenous

Table IV

DATA ON CARBON DIOXIDE COLLECTED
FROM THE FOUR HOUR EXPERIMENTAL PERIOD

I.P. Injected	Total Millimoles CO_2	Specific Activity a Counts/Min/mM x 10	% Incorporation of Acetate
SV-4	58.0	66.0	68.7
SV-8	56.0	67.2	67.4
SV-9	43.7	81.2	60.0
SV-10	46.7	68.3	58.7
SV-12	44.2	75.4	62.9
Average	49.7	71.6	63.5
I.V. Injected			
SV-3	57.3	72.0	73.6
SV-5	55.3	72.0	71.0
SV-6	49.0	75.4	66.0
SV-7	47.9	76.8	65.8
SV-11	56.6	57.3	61.7
SV-13	41.9	82.1	63.0
Average	51.3	72.6	66.9

E. Fractionated Gut

In Table V the results obtained on the fatty acid fractions of the fractionated gut animals are listed, and in Table VI the cholesterol values are recorded. Rats SV-10 and SV-12 were I.P. injected, while SV-11 and SV-13 were I.V. injected animals. There was found to be no significant difference between the specific activities, % incorporations, tissue weights, or fatty acid or cholesterol weights of these two groups; and, as a result, the animals were grouped together. It was interesting to note that stomach contents showed no measurable incorporation of acetate label into fatty acid or cholesterol. This finding enables us to have confidence in the extraction and transferring techniques, for no C^{14} contamination was present.

As the small intestine is very active metabolically, it might be expected to have considerable ability to incorporate acetate into fatty acids and cholesterol. This proved to be true, for almost twice the incorporation of acetate (0.74%) into fatty acid occurred in the small intestine tissue as compared with the value for all other gut fatty acid fractions combined (0.41%).

1. Lipogenesis

The largest amount of fatty acid found in the three gut tissue fractions, 336 mg., was found in small intestine tissue, although it was not the heaviest tissue. It was also interesting to note that small intestine had approximately the same cholesterol concentration as liver, i.e., 2.2 mg./gm. tissue. Small intestine and liver were of about the same weight and were comparable as to their lipogenic activity.

Table V

FRACTIONATED GUT FATTY ACID DATA

Tissue	Weight (gm.)	Fatty Acid Weight (mg.)	Mg. of F.A./ gm. Tissue	Specific Activity	% Incorporation
Stomach Tissue					
SV-10	2.53	161.0	63.6	12.0	.15
SV-11	2.30	106.4	46.3	20.0	.16
SV-12	2.46	248.3	100.9	8.4	.16
SV-13	1.96	71.1	36.3	13.7	.07
Average	2.31	146.7	61.78	13.5	.14
Stomach Contents					
SV-10	8.65	205.5	23.76	0	0
SV-11	12.66	300.8	23.76	0	0
SV-12	13.86	82.0	5.92	0	0
SV-13	8.08	176.0	21.78	0	0
Average	10.81	191.1	18.80	0	0
Small Intestine Tissue					
SV-10	8.84	368.2	41.65	31.31	.84
SV-11	10.19	469.5	46.07	28.98	1.00
SV-12	6.81	208.8	30.66	25.65	.39
SV-13	8.22	299.1	36.39	32.33	.71
Average	8.51	336.4	38.69	29.57	.74
Small Intestine Contents					
SV-10	3.57	32.6	9.13	6.19	.02
SV-11	3.36	20.7	6.16	6.02	.01
SV-12	3.63	19.3	5.31	7.61	.01
SV-13	.23	14.8	64.34	13.43	.02
Average	2.70	21.9	21.24	8.31	.01
Large Intestine Tissue					
SV-10	2.75	128.5	46.73	17.14	.16
SV-11	2.49	70.5	28.31	19.56	.19
SV-12	3.17	517.0	162.83	9.78	.37
SV-13	4.14	274.0	66.18	13.58	.28
Average	3.14	247.5	76.01	15.02	.25
Large Intestine Contents					
SV-10	4.73	25.4	5.37	4.91	.01
SV-11	5.41	41.5	7.67	3.06	.01
SV-12	4.56	27.8	6.09	2.56	.01
SV-13	4.21	27.7	6.58	5.04	.01
Average	4.73	30.6	6.43	3.89	.01

Table VI

FRACTIONATED GUT CHOLESTEROL DATA

Tissue	Weight (gm.)	Mg/Chol. per Gm. Tissue	Non-saponifiable Fraction (mg.)	Mg.Chol. Color Determined	% Chol. in non-saponifiable	S.A.	% Incorporation
Stomach Tissue							
SV-10	2.53	2.97	10.2	7.51	74	24.6	.01
SV-11	2.30	3.00	14.5	6.92	48	30.1	.02
SV-12	2.46	2.72	12.1	6.69	55	25.5	.01
SV-13	1.96	3.50	12.1	6.88	57	22.9	.01
Average	2.31	3.05	12.2	7.00	58.5	25.7	.01
Stomach Contents							
SV-10	8.65	.69	13.5	5.95	44	0	0
SV-11	12.66	.69	19.1	8.73	46	1.69	0
SV-12	13.86	1.08	23.7	14.90	63	1.30	0
SV-13	8.08	.88	15.5	7.13	46	2.06	0
Average	10.8	.84	18.0	9.18	50	1.26	0
Small Intestine Tissue							
SV-10	8.84	2.32	23.4	20.48	87	131.6	.20
SV-11	10.19	1.92	24.7	19.52	78	75.9	.11
SV-12	6.81	2.54	23.0	17.30	75	63.9	.08
SV-13	8.22	2.30	23.9	18.98	79	88.0	.12
Average	8.51	2.27	23.8	19.07	80	89.8	.13
Small Intestine Contents							
SV-10	3.57	.84	8.4	3.0	36	76.9	.02
SV-11	3.36	.57	9.6	1.9	20	31.9	.01
SV-12	3.63	.32	5.8	1.16	20	47.1	0
SV-13	.23	7.39	5.4	1.70	32	68.2	.01
Average	2.70	2.28	7.3	1.94	27	55.9	.01
Large Intestine Tissue							
SV-10	2.75	1.91	18.3	5.26	27	29.2	.01
SV-11	2.49	2.30	15.5	5.72	37	35.0	.02
SV-12	3.17	1.87	12.5	5.92	47	23.5	.01
SV-13	4.14	1.68	11.4	6.95	61	25.3	.01
Average	3.14	1.94	14.4	5.96	43	28.3	.01
Large Intestine Contents							
SV-10	4.73	.84	10.0	3.95	39	37.7	.01
SV-11	5.41	1.15	22.1	6.20	28	17.0	.01
SV-12	4.56	1.12	16.5	5.10	30	8.7	0
SV-13	4.21	1.10	16.1	4.65	29	20.9	.01
Average	4.73	1.05	16.2	4.98	32	21.1	.01

2. The Non-Saponifiable Fraction

The percent of cholesterol in each non-saponifiable fraction was determined. The highest was in the small intestine tissue, this being 80% cholesterol. Only 27% of the non-saponifiable fraction of small intestine contents was cholesterol. The large intestine non-saponifiable fraction contained 32% cholesterol. The % incorporation into cholesterol was highest in small intestine tissue, an average of 0.13% of the label being incorporated, whereas less than 0.01% of the injected acetate was incorporated into all other cholesterol fractions combined.

It was noteworthy that fatty acid synthesis in the large intestine tissue (based on % incorporation) was the next highest, being one-third of the small intestine activity. However, the ability to synthesize cholesterol in the large intestine was only one-thirteenth that of the small intestine tissue.

3. Carbon Dioxide Data

In Figure 2 the time course of $C^{14}O_2$ elimination in the expired air is shown. Three different groups of rats were compared: (1) I.P. injected, anesthetized rats; (2) I.P. injected, unanesthetized rats; and (3) I.V. injected, anesthetized rats. The rats recovered from the nembutal anesthesia approximately 50 minutes after the injection of acetate was given and, thus, 65 minutes after the administration of nembutal.

When the acetate-1-C^{14} was given by the I.V. route, it was quickly converted to CO_2; the concentration of $C^{14}O_2$ in respired air reached a maximum in only four minutes. This concentration gradually decreased

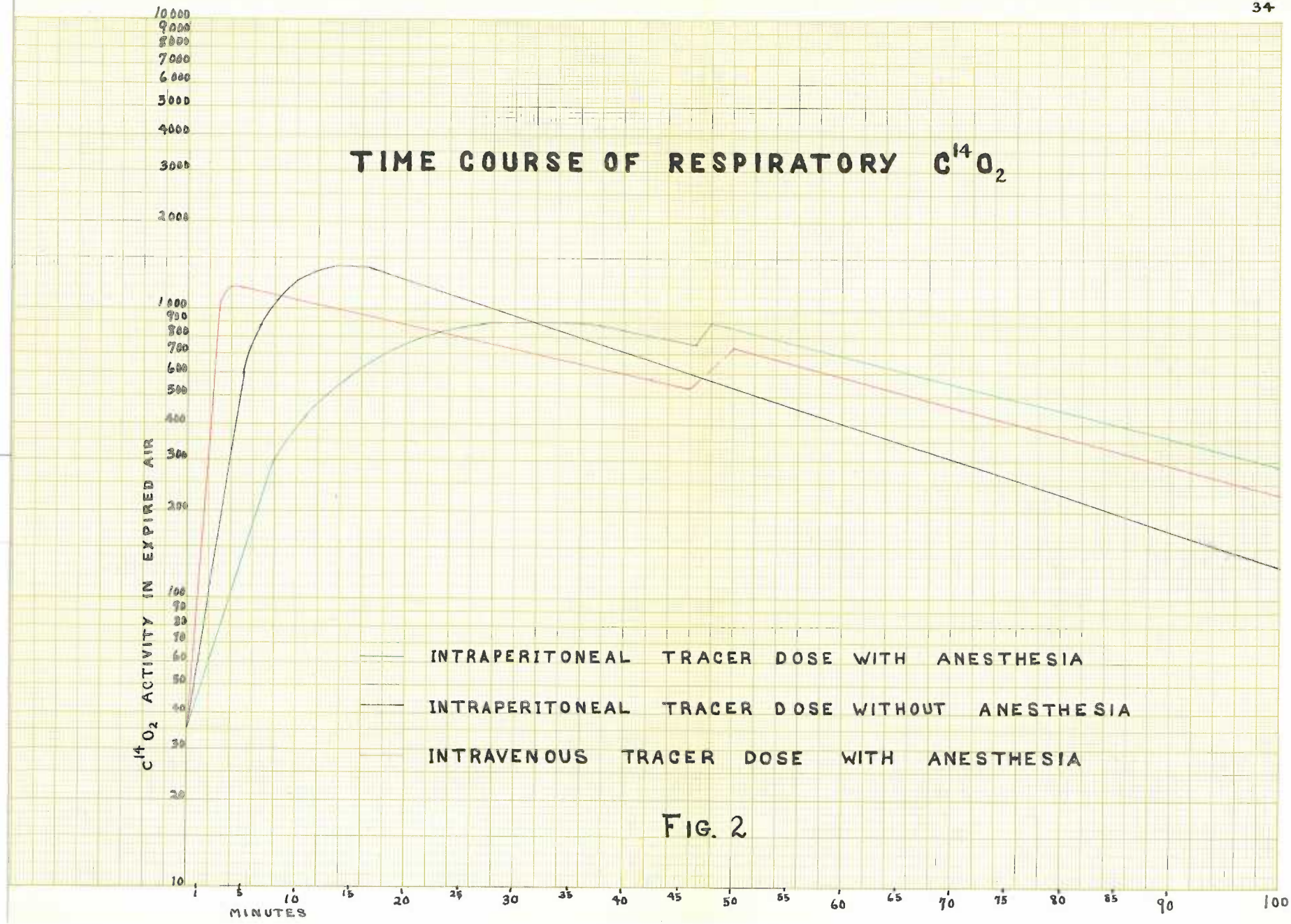
TIME COURSE OF RESPIRATORY $C^{14}O_2$
$C^{14}O_2$ ACTIVITY IN EXPIRED AIR
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
900
800
700
600
500
400
300
200
100
90
80
70
60
50
40
30
20
10
MINUTES
1
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
100
INTRAPERITONEAL TRACER DOSE WITH ANESTHESIA
INTRAPERITONEAL TRACER DOSE WITHOUT ANESTHESIA
INTRAVENOUS TRACER DOSE WITH ANESTHESIA

FIG. 2

until the animal came out of anesthesia. At this point the concentration of $C^{14}O_2$ abruptly increased and a new excretion rate was developed.

When the dose was given I.P. to the anesthetized rats a gradual increase of $C^{14}O_2$ activity occurred for 30 minutes. From 30 to 34 minutes after the injection, the $C^{14}O_2$ eliminated was almost constant. From 34 to 51 minutes there was a gradual decline in $C^{14}O_2$ concentration. The animal at this time recovered from the anesthesia, and again an abrupt increase in activity was noted. This was followed by a gradual decrease for the remainder of the experiment.

The I.P. non-anesthetized rat expired the highest activity 14 minutes after injection. The activity in the exhaled air now decreased at a regular rate for the remainder of the metabolism period.

IV. DISCUSSION

A. Effect of Nembutal Anesthesia on Lipogenesis

The fact that nembutal anesthesia produced no significant change in the % incorporation of acetate-1-C^{14} into lipids is significant. Brody and Bain[17] showed that commonly used barbiturates inhibited phosphate uptake of liver slices when pyruvate was the substrate. From their in-vitro studies the authors concluded that barbiturates uncouple phosphorylation from oxidation.

The results from this experiment are not in agreement with the in-vitro report of Brody and Bain, but they do agree with the theories stated by Krantz and Carr[24]. These latter authors stated that barbiturates had no effect on liver or gastrointestinal function when used in normal therapeutic doses. Our own experiments demonstrated that liver tissue showed a slightly greater incorporation of acetate into fatty acids when under the influence of anesthesia. The difference was not statistically significant but showed that nembutal did not inhibit lipogenesis.

Additional physiological effects of nembutal as an anesthetic are given below. Drill has reported that the cardiovascular system is not significantly affected by the administration of the usual soporific doses of the barbiturates[25]. Also in therapeutic doses the barbiturates have no direct effect on the myocardium and produce no significant electrocardiographic abnormalities. There is no significant direct effect on the gastrointestinal tract except a secondary effect to depress the nervous system. As to the kidney[26], the glomerular filtration rate is

not changed; however, renal plasma flow is decreased slightly. The in-vivo results of our own study are in direct agreement with those of Drill, Krantz and Carr.

If barbiturates inhibit inorganic phosphate uptake, the synthesis of fatty acids and cholesterol would be decreased. Lehninger and Kennedy[27], in their studies on the mechanism of fatty acid oxidation in intact mitonchondria, found that neither acetic acid, nor any other two carbon compounds behave as intermediates in the reactions of enzymatic fatty acid oxidation. It has also been shown[28] that acetate can be biologically activated and will then act as an intermediate having the properties of active acetate. It was found that acetate is activated by an enzymatic reaction requiring ATP and a new coenzyme, Coenzyme A. Fatty acid is also activated by the formation of a fatty acid-CoA complex. This activated form was shown to be oxidized in the breakdown of fatty acids to yield acetyl CoA and a fatty acid-CoA compound now shortened by two carbon atoms. This oxidation continues through seven or eight cycles in the case of the 16 or 18 carbon acids to yield all acetyl CoA molecules[29].

All the reactions of fatty acid oxidation were shown to be reversible, so that the same systems can readily account for the reactions of fatty acid synthesis, starting from acetyl CoA. Stansley and Beinert[30] have shown that the purified enzymes of the oxidative cycle can convert isotopically labeled acetyl CoA to butyryl CoA.

The oxidation of fatty acids is important as a source of energy and the synthesis of fatty acids is important as a means of storing energy for future use. It appears clear that acetyl CoA is an obligatory

intermediate in the synthesis of fatty acids.

What have the mechanisms of oxidation and synthesis of fatty acids to do with nembutal anesthesia? It was shown in-vitro that nembutal inhibits inorganic phosphate uptake. In addition, activation of acetate and fatty acids is necessary for oxidation and synthesis of fatty acids. However, phosphorylation is necessary for coenzyme A to react and form acetyl-CoA. Nembutal then should inhibit the oxidation and synthesis of fats and cholesterol in-vitro. However, our in-vivo results showed no effects on lipogenesis.

That neurons have a definite sensitivity to barbiturates has been shown. This may be the explanation of the different results found between the in-vivo and in-vitro techniques. In in-vivo studies this chemical attraction of neurons in the central nervous system might have been such that the rat would expire from depression of the respiratory center before high liver or gut tissue levels of drug could be attained. Brody and Bain[17] in liver homogenates used nembutal concentrations ranging from 1×10^{-5} to 75×10^{-5} M., and found that phosphorylation was inhibited. In our studies 3.5 mg. of nembutal per 100 gm. of rat were injected. The liver of the rat weighed approximately 10 gm. If we assumed universal distribution of nembutal, this would result in a nembutal concentration in liver of 14×10^{-5} M. However, it is doubtful that the concentration of nembutal reached this level in-vivo. In in-vitro at this concentration[17] oxygen uptake was not inhibited but inorganic phosphate uptake was inhibited. From our in-vivo study it is concluded that nembutal anesthesia has no significant effect on lipogenesis in the rat during the four hour metabolism period.

B. Effect of Route of Administration on Lipogenesis

Our results show that liver fatty acid activity and skin cholesterol activity increase significantly when the tracer acetate is administered I.V. rather than I.P. However, gut fatty acid % incorporation of acetate decreases significantly when the I.V. route is used. The higher value for liver by the I.V. route was not expected. It was anticipated that the I.P. tracer dose would distribute itself in the peritoneal fluid and then drain into the liver portal system. If this were true, then the liver would be expected to have ready access to the acetate activity and result in a higher % incorporation than if the dose of acetate were injected I.V. The lower % incorporation shown by the I.P. route might cast suspicion on the supposition that the peritoneum is drained by the hepatic portal route. Indeed the anatomists are not in general agreement on the matter of peritoneal drainage. One possibility is that the peritoneum is drained by the lymphatic system. The thoracic duct in the rat runs along the superior vena cava and the left innominate to drain into the venous system at the jugulo-subclavian[31]. The C^{14} activity could then bypass the liver and reach the general circulation by the thoracic duct directly.

The increased activity of gut tissue in I.P. injected rats was possibly contributed to by a direct utilization of the acetate by gut tissue. This seemed a plausible explanation at first, for the half time of peritoneal drainage was established to be one minute, and it was shown that acetate was extremely labile. If the gut had absorbed an amount of the tracer dose directly, a higher % incorporation would have resulted than if the acetate had to go first through the general circulation before

reaching the gut tissue. The higher activity of skin cholesterol by the I.V. route might then be due to gut tissue not having had an opportunity for direct absorption. When the dose was given I.V., more activity was available for utilization by the other tissues. The increase seen in incorporation from the I.V. dose was 0.04%, increasing from 0.11% to 0.15%. However, if a lessened trapping of acetate by gut was responsible for this, other tissues should have also shown an increase by the I.V. route. The data indicates that this did happen in most tissues, excluding, of course, gut tissue. The increase was, however, only statistically significant in liver fatty acid and skin cholesterol.

The average total of the acetate activity incorporated into all tissues when the I.V. route was employed was 9.77%. When the I.P. route was used, it was 9.67%. Thus, approximately the same amounts of acetate were incorporated by the different routes. The only difference was a redistribution of the label by the tissues. The difference in % incorporation in gut tissue between I.V. and I.P. injection was compared with that difference in the other tissues. Gut decreased an average of 0.96% incorporation when the I.V. route was used. The average increase in the other tissues was 1.04% when the rats were injected I.V. It must, therefore, be considered a possibility that the activity which the gut lost by the I.V. route was gained by the other tissues. The above comparison was made on data from anesthetized animals.

C. Effect of the Route of Administration upon Fractionated Gut

In the tissues of the four rats that had their gastrointestinal tracts fractionated, no difference was found in % incorporation between

the I.V. and I.P. injected animals. As shown in Table II, the value for total gut tissue of all four animals was low in comparison to that of the non-fractionated rats. The two I.P. fractionated rats showed especially low % incorporations when compared to the other I.P. injected rats. Since only gut tissue showed this lowered activity when the gastrointestinal tract was fractionated, it became obvious that an explanation of this phenomenon was desirable. It is possible that in the division of gut into six fractions and with the subsequent extraction some activity was lost. However, this seems improbable since the amount of cholesterol extracted from the three gut tissues was very constant (see Table VI). If activity were lost, one would expect to find a variance in the amount of cholesterol in the particular tissue, for tissue cholesterol has been shown in the past to be quite constant.

When the fatty acid fractions are compared (Table V), it is seen that the amount of fatty acid and mg. per gm. of fatty acid vary widely for stomach, small intestine, and large intestine tissue. The least variance is found in small intestine tissue. In the previous separation of gut tissue from the animal, the peritoneal fat was not carefully dissected away from gut tissue and thus the amount of peritoneal fat included in gut tissue varied from dissection to dissection.

It is possible that peritoneal fat had an influence on the % incorporation of acetate into the tissue, for it is apparent from the fractionated gut studies that the gut tissue itself did not directly adsorb the I.P. injected acetate. In the fractionated gut, less peritoneal fat was included with gut tissue. Then if the peritoneal fat had

incorporated acetate directly it could have had an influence on gut % incorporation.

D. Possible Effect of Peritoneal Fat on Results

Hausberger, Milstein and Rutman[32] demonstrated that adipose tissue participates in lipogenesis from glucose. They used uniformly labeled glucose C^{14} and found that both liver and adipose tissue have similar capacities for the oxidation of glucose. It was suggested that a greater % of the energy derived from the oxidation of glucose in adipose tissue is channeled into fatty acid formation than is the case in liver tissue. They related lipogenesis to the nitrogen content of the tissue and reported that adipose tissue is 500 times more active than liver on this basis. In-vitro work on incorporation of acetate-1-C^{14} into lipids by slices of adipose tissue was reported by Feller[33]. He found that adipose tissue converted 2.5% of the radioactivity supplied as acetate-1-C^{14} to fatty acids. This was about twice the amount of the substrate converted to fatty acids by liver tissue. He also found that adipose tissue converts only negligible amounts of acetate into the cholesterol-containing non-saponifiable lipids. In in-vivo studies, Lepkovsky, Lemon, Chari-Briton and Dimick[34] reported that adipose tissue is of lower activity than liver in the incorporation of labeled acetate in fasting rats. However, 5 hours after feeding the activity of adipose tissue increased 20 to 40 times, whereas liver only increased 2 to 6 times. All our animals were sacrificed five hours post-prandially but received the labeled acetate one hour post-prandial.

Also, Masoro, Chaikoff and Daubin[35] demonstrated that functionally liverless rats deprived of their intestines synthesized the same amount

of fatty acids from radioglucose as did intact controls, the C^{14} glucose being injected subcutaneously. It was assumed that adipose tissue is capable of forming fatty acids in-vivo as well as in-vitro. As skeletal muscle and kidney are not very active in vitro[36], Chernik, Masoro, and Chaikoff concluded that adipose tissue has a high lipogenic activity in-vivo.

The total gut fractions of the four fractionated gut animals had a constant concentration of fatty acid expressed in mg. per gram of tissue, this being approximately 30 mg. per gm. in all four animals. In the rats that did not have their gut fractionated and were injected I.P., the average was 44.7 mg. per gram of gut tissue. This is an average of 15 mg. per gram more fatty acid. The average weight of total gut tissue was 31 grams. Then the average amount of fatty acid found additionally in the I.P. injected, fractionated animals was:

$$31 \times 15 = 465 \text{ mg. of fatty acid}$$

The I.V. injected, non-fractionated rats had an average of 51 mg. of fatty acid per gram of gut tissue. In the fractionated gut animals the % of acetate fixed into fatty acid did not vary from I.P. to I.V. injected animals. Also, in the small intestine, which had by far the greatest activity, there was no difference between the I.P. and I.V. injected rats as to % label incorporation into fatty acids. If any adsorption of label by gut tissue occurred, a difference would have been in evidence here. From the results that adipose tissue has high ability to incorporate acetate-1-C^{14} into fatty acids, and since less fatty acid was found in fractionated gut tissue, there is the definite possibility that peritoneal

or mesenteric fat made the difference between the I.P. and I.V. injected rats as to % incorporation.

The average specific activity of fatty acids of liver tissue in our eleven animals was 43. If, as Feller[33] indicated, adipose tissue is twice as active as liver, it could have a specific activity of 86. Now an average of 465 mg. more of fatty acid was found in non-fractionated, I.P. injected animals. Thus the following calculation can be made:

$$\frac{\text{Specific activity (86)} \times \text{mg. of fatty acid (465)} \times 100\%}{\text{Total activity injected } (1.37 \times 10^6)} = 3\%$$

Therefore if adipose tissue in the peritoneum could directly incorporate radioactive acetate, this might account for the higher % incorporation found by the intraperitoneal route. At the same time by I.V. injection more labeled acetate would have been available for liver to incorporate into fatty acid. Gut cholesterol showed no change when a different route of injection was used. Feller[33] reported no in-vitro incorporation of labeled acetate into cholesterol by adipose tissue. Therefore we would not expect cholesterol to show a change.

Of course, the proof for the above explanation could be forthcoming from the following experiment. First, inject trained fed Sprague-Dawley rats I.P. and I.V.; strip all peritoneal fat from both animals and extract the fatty acids and cholesterol by methods that have been described, and determine the % incorporation into fatty acids and cholesterol. This would show if peritoneal fat can incorporate acetate-1-C^{14} into fatty acid and/or cholesterol. Also, if the peritoneal fat adsorbed labeled acetate directly upon I.P. injection, it would be more clearly shown by the contemplated experiment. When the label is injected I.V., then the

peritoneal fat would have to await delivery of label by the regular arterial supply before it had access to the acetate.

The half time for blood acetate reported by Busch and Baltrush[13] was 18 seconds. Van Bruggen and Hutchens[12], also Ciaranfi[15] and Harper[14] from their various separate studies reported a half life of 5-10 minutes for acetate. If peritoneal fat can absorb acetate directly and the half time for peritoneal drainage is one minute, then the 18 seconds reported by Busch seems too rapid. The peritoneal fat with its reported high lipogenic ability, with an acetate half life of 18 seconds, would probably have incorporated more of the tracer acetate.

E. Specific Activity

The specific activities as reported in Table III were not used directly to indicate lipogenic activity of various tissues. As mentioned before, the extreme variance found in specific activity is partly eliminated when % incorporation is determined. Many laboratories report their results as specific activity. Because of the large variation in specific activities, the differences in activity of various tissues is quite large. Specific activity data can serve as a guide in reaching conclusions, but this measurement is not adequate for the complete evaluation of a metabolic process.

F. A Detailed Analysis of Gut Contents

A lipid analysis was made on the Purina rat chow used in our feeding experiments. The total fatty acid content was found to be approximately 4.7%, whereas the cholesterol content was 0.12% Five hours before

sacrificing, the rats had been fed 2 pellets of chow, which was a total of about 12 grams of food. The meal, then, contained about:

0.047 x 12,000 mg. = 564 mg. of fatty acid, and

0.0012 x 12,000 mg. = 14.4 mg. of cholesterol.

In Tables V and VI are listed the analyses recorded for the gastrointestinal tracts that were fractionally analyzed. It was observed that five hours postprandially about one-third of the ingested fatty acid was still left in the stomach. Of the approximately 564 mg. of fatty acid taken orally in the food, an average of 320 mg. had left the stomach. Around 30 mg. of fatty acid were found in the large intestine. There was an average of 22 mg. of fatty acid still in the small intestine, some contents of which would likely be absorbed. There was also the possibility that the large intestine contained fatty acid in the feces from the previous day's food. It was thus clear that the ingested fatty acids were absorbed at a high rate. The rat has been reported to compare favorably with man in its ability to utilize high melting point fats[37]. Evans and Lepkovsky[38] showed that in rats most fats are about 96% absorbed, but that palmitic acid is only 74% absorbed. Our results are in agreement with these findings, for our rats demonstrated a high digestibility coefficient. The digestibility coefficient is that per cent of fat ingested that is absorbed.

Of the approximately 14.4 mg. of cholesterol ingested, an average of 9.2 mg. was found in the stomach contents; an average of 2.0 mg. was found in the small intestine contents; and an average of 5 mg. in the large intestine contents. These values for gut cholesterol total 16.2 mg., and thus there appears to be more cholesterol in the gut than the

amount ingested. This is not incongruous, as Siperstein and Chaikoff[39] have shown that cholesterol is continually eliminated through the intestinal wall and in the bile. It is likely that most of the 5 mg. of cholesterol isolated from the large intestine contents was an accumulation of previous cholesterol excretion. Also, it has been shown by Siperstein and Chaikoff that about 20% of cholesterol ingested is not absorbed. The large amount of cholesterol found in stomach contents (approximately 63% of the amount ingested) did not correlate with 33% of ingested fatty acids that were found. It is possible that cholesterol may leave the stomach at a slower rate.

The non-saponifiable fractions of the six gut fractions were analyzed for their cholesterol contents. The stomach contents contained 50% cholesterol in the non-saponifiable fraction. This weight difference is possibly due to plant sterols. In the small intestine contents, the cholesterol content of the non-saponifiable fraction was only 27%. This is not unexpected, for the intestine was absorbing cholesterol, whereas plant sterols were not absorbed. In the small intestine bacterial action possibly transformed some cholesterol into coprosterol. This would reduce the per cent of cholesterol in the non-saponifiable fraction. The cholesterol content of the large intestine non-saponifiable fraction was slightly increased to 32%. This was probably due to the small accumulation of cholesterol secreted through the intestinal wall and also eliminated in the bile. About 10% of cholesterol absorbed is excreted through the intestinal wall. The non-saponifiable fraction of the small intestine tissue contained about 80% cholesterol. This was interesting for it was much higher than that of stomach tissue (58%) or large intestine tissue (43%).

The % incorporation of acetate into cholesterol of small intestine was three times greater than in all other tissues of gut combined. It is noteworthy that small intestine tissue and liver weighed about the same, i.e., 8-9 grams. Both had the same cholesterol content of 19 to 20 mgs., and both showed the same incorporation of acetate into cholesterol (0.13 to 0.14%). This demonstrated that small intestine tissue is of the same high metabolic activity as liver tissue in the metabolism of cholesterol. Small intestine tissue is also very active in the synthesis of fatty acids, the % incorporation found being 0.79%. The remainder of gut incorporated 0.41%; thus again small intestine was most active of all gut tissues studied. A comparison of liver and small intestine tissue again was carried out. This comparison was considered important as gut had been reported to be of higher lipogenic activity than liver. This comparison was made more significant by the removal of peritoneal fat from the small intestine tissue. The average % incorporation of acetate into fatty acid in liver was 0.87%. For small intestine it was 0.74%. This demonstrated liver to be slightly more active than small intestine in this respect. If all gut tissue were included, the incorporation by gut would be higher than that of liver, namely 1.14 to 0.87%. As previously discussed, portions of the gastrointestinal tract may have had varying amounts of high lipogenically active peritoneal fat present.

Large intestine tissue gave about one-third the % incorporation into fatty acid as shown by small intestine. However, large intestine tissue had essentially zero per cent incorporation of acetate into cholesterol. Stomach tissue is similar to large intestine tissue in fatty acid and cholesterol synthesis; both incorporated acetate into

fatty acid to a nominal degree, but practically no activity was found in cholesterol.

The effect of nembutal on the emptying time of the stomach could not be definitely determined. As two-thirds of the fatty acids had left the stomach in a five hour period, we can surmise an approximate emptying time of 7.5 hours. In humans, ordinarily, 5-8 hours are required for a meal to leave the stomach[40]. Drill[25], in addition, reports no direct effect of hypnotic doses of barbiturates on the gastrointestinal tract. The normal figure for the emptying time of the rat stomach is not known, and the effect nembutal has on the emptying time was therefore not determinable.

G. $C^{14}O_2$ Time Course Study

In Figure 2 the elimination of $C^{14}O_2$ over a two hour period is shown for anesthetized and unanesthetized rats, injected I.P. (see page 34). The time course of respiratory $C^{14}O_2$ for anesthetized I.V. injected rats is also compared. The $C^{14}O_2$ reached a peak in the unanesthetized I.P. animal in 14 minutes, and then gradually decreased at a fairly rapid, uniform rate. However, when nembutal was administered to the I.P. rat, the C^{14} of acetate was again initially fairly rapidly expired as $C^{14}O_2$, but at a slower rate than the unanesthetized I.P. rat. The peak elimination was maintained for from 28 to 36 minutes. The anesthetized I.P. rat gave off more activity after reaching its maximum than the unanesthetized I.P. rat for the remainder of the experiment.

It is of interest to inquire as to the mode of action of nembutal in inhibiting the $C^{14}O_2$ concentration of the respired air of the anesthetized

rat. The fate of a tracer dose of acetate will be followed (to the best of our knowledge) from the time of injection to the time of its elimination as $C^{14}O_2$, in an attempt to decide the role of the anesthetic agent in its metabolism. The time required for the label to leave the peritoneal space is not great. Krantz and Carr[24] reported that therapeutic doses of barbiturates do not noticeably affect the circulation. The uptake of the label by the cells did not appear to be affected. This was shown by the I.V. injected acetate in the anesthetized rat. There was, in this case, an immediate elimination of $C^{14}O_2$, reaching a maximum in only three minutes. If the metabolism of acetate were inhibited by nembutal, a more gradual uptake of labeled acetate and elimination of $C^{14}O_2$ would be expected. Thus the metabolism of acetate to CO_2 in the cell was not noticeably affected by nembutal. A final obvious possible factor in the slow release of $C^{14}O_2$ is the elimination of $C^{14}O_2$ from the bicarbonate pool of the blood. Van Bruggen, in an unpublished study, has found that when radioactive bicarbonate is injected into an animal the turnover time of the bicarbonate pool is reduced by nembutal. The present experiment gave the same result. When the animal awoke, usually about 50 minutes after the injection of acetate and 65 minutes after the injection of nembutal, an increase in bicarbonate turnover was immediately noticed. In the anesthetized I.V. rat the half time of bicarbonate turnover was 39 minutes. When the animal awoke it immediately decreased to 32 minutes. In the I.P. anesthetized rat the half time of bicarbonate turnover was 38 minutes, but it decreased to 31 minutes when the animal awoke. This demonstrated that the respiratory depression inhibits the bicarbonate pool turnover. In the anesthetized and unanesthetized I.P. rats, although

the time course of $C^{14}O_2$ eliminated was different, the total amount of activity given off in four hours proved to be the same. This again showed that nembutal used in-vivo, in usual hypnotic doses, had no apparent inhibition of the activation of acetic acid. This is in accord with Drill[25], who reported that hypnotic doses of barbiturates produce only a slight depression of the respiration, similar to that of sleep. However, with larger doses, there occurred a progressive increase in respiratory depression, with rate, depth, and minute volume being affected.

In this study nembutal had no effect on the lipogenic ability of liver, gut, carcass or skin tissue. Nembutal had an effect on respiration, as evidenced by inhibiting the turnover of the bicarbonate pool. In agreement with Drill[25], and Krantz and Carr[24], it was shown that nembutal in hypnotic doses shows significant effects on respiration only.

It is realized that a complete knowledge of the metabolic events occurring during a time course study of acetate metabolism is impossible. The activity of the $C^{14}O_2$ curves reflects complex metabolic pathways of the label. The above discussion of the "turnover" of the bicarbonate pool represents only a general and superficial analysis of the data obtained.

V. CONCLUSIONS

From the data presented in this thesis it can be concluded that:

1. Nembutal anesthesia has no significant effect upon lipogenesis when acetate label incorporation into liver, gut, carcass, and skin lipids is used as the criterion.

2. Nembutal, in addition, was found to have no measurable effect upon acetate metabolism in-vivo, as measured by $C^{14}O_2$ time course studies. The known effect of anesthesia upon respiration was confirmed, for Nembutal anesthesia did increase the turnover time of the plasma bicarbonate pool.

3. The route of tracer administration (I.V. as contrasted to I.P.) was shown to have a real effect on lipid labelling. The fatty acid and cholesterol fractions of liver, carcass and skin showed an increased incorporation of acetate-1-C^{14} when the dose was given I.V. Gut fatty acids and cholesterol both showed a decreased incorporation of label. Only the changes seen for liver fatty acid, skin cholesterol, and gut fatty acid were statistically significant.

4. The changes seen in the liver lipid incorporation when the label was given I.V. rather than I.P. casts suspicion upon the portal system as the main route of peritoneal drainage.

5. The role of peritoneal fat in the incorporation of acetate label into "gut tissue" lipids has been discussed, and its potential large contribution to lipid labeling reaffirmed.

6. A comparison was made of the lipogenic activity of stomach, small intestine, and large intestine tissue. Marked differences were seen,

both in lipid content and in lipogenic ability of these tissues.

7. The amounts and C^{14} content of the fatty acid and cholesterol fractions found in the gastrointestinal tract content were determined. A consideration of the data so obtained showed that 85% of the dietary fatty acids had been absorbed in the four hour period.

BIBLIOGRAPHY

1. Rittenberg, D., and Block, K. The utilization of acetic acid for fatty acid synthesis. J. Biol. Chem., 154:311-312 (1944).

2. West, E.S., and Todd, W. R. Textbook of Biochemistry, 2nd ed., 903, MacMillan Co., New York, 1955.

3. Rittenberg, D., Schoenheimer, R. Deuterium as an indicator in the study of intermediary metabolism. J. Biol.Chem., 121:235-253 (1937).

4. Block, K., and Rittenberg, D. The utilization of acetic acid for cholesterol formation. J. Biol. Chem., 145:652, 1942.

5. Cornforth, J.W., Hunter, G.D., Popjak, G. Studies of cholesterol biosynthesis. Distribution of acetate carbon in the ring structure. Biochem. J., 54:597-601, 1953.

6. Wuersch, J., Huang, R.L., Block, K. The origin of the iso-octyl side chain of cholesterol. J. Biol. Chem., 195:439-446, 1952.

7. Hutchens, T.T., Van Bruggen, J.T., West, E.S. Fatty acid and cholesterol synthesis rates in the intact rat. Arch. Biochem. & Biophysics., 52 (No. 1):261-268, 1954.

8. Lipmann, F., Klein, H.P. The relationship of CoA to lipid synthesis, II. Experiments with rat liver. J. Biol. Chem., 203:101-108, 1953.

9. Van Bruggen, J.T., Hutchens, T.T., Claycomb, C.K., West, E.S. Time course of lipid labeling in the intact mouse and rat. J. Biol. Chem., 200 (No. 1):31-37, 1953.

10. Van Bruggen, J.T., Hutchens, T.T., Claycomb, C.K., Cathey, W.J., West, E.S. The effect of fasting upon lipogenesis in the intact rat. J. Biol. Chem., 196:390-394, 1952.

11. Hutchens, T.T., Van Bruggen, J.T., Cockburn, R.M., West, E.S. The effect of fasting upon tissue lipogenesis in the intact rat. J. Biol. Chem., 208:115-122, 1954.

12. Hutchens, T.T., Van Bruggen, J.T., Kinetic aspects of acetate and lipid metabolism in the intact rat. Fed. Proc. 235, 1952.

13. Busch, H., Baltrush, H.A. Rates of metabolism of acetate-1-C^{14} in tissues in vivo. Cancer Research, vol. 14, No. 6:448-55, 1954.

14. Harper, P.V., Neal, W.B., Hlavacek, G.R. Lipid synthesis and transport in the dog. Metabolism, 11:69-80, 1953.

15. Ciaranfi, E., and Formesu, A. Time course of injected acetate in normal and depancreatized dogs. Biochem. J., 57:171-175, 1954.

16. Drill, V.A. Pharmacology in Medicine, pp. 14/5-14/8. McGraw Hill Book Co., New York, 1954.

17. Brody, T., and Bain, J. Effect of barbiturates on oxidative phosphorylation. Proc. Soc. Exptl. Biol. Med., 77:50-53, 1951.

18. Griffith, J.Q., and Farris, E.J. The Rat in Laboratory Investigation, ed. 2., pp. 268-289, J. P. Lippincott Co., Philadelphia, 1949.

19. Claycomb, C.K., Hutchens, T.T., Van Bruggen, J.T. Techniques in the use of C^{14} as a tracer. Nucleonics 7:38-48, 1950.

20. Calvin, M., Isotopic Carbon, pp. 283-318. Wiley & Sons, New York, 1949.

21. Siri, W. E. Isotopic Tracers and Nuclear Radiations, ed. 1., pp. 304-402. McGraw Hill, New York, 1949.

22. Kamen, M. Radioactive Tracers in Biology, 1:172-183. Academic Press, New York, 1947.

23. Freedlander, G., and Kennedy, J. W. Introduction to Radiochemistry, pp. 188-190 and 227-235. John Wiley, New York, 1949.

24. Krantz, J.C., Carr, C.J. The Pharmacologic Principles of Medical Practice, ed. 3, pp. 540-545. Williams & Wilkins Co., Baltimore, 1954.

25. Drill, V.A. Pharmacology in Medicine, p. 14/6. McGraw Hill Book Co., New York, 1954.

26. Glauser, K.F., Selkurt, E.E. Effect of barbiturates on renal function in the dog. Am. J. Physiol., 168:469-479, 1952.

27. Lehninger, A.L., and Kennedy, E.P. Phosphorus Metabolism, Vol. II, pp. 253-279, Johns Hopkins Press, Baltimore, 1952.

28. Lipmann, F. Biosynthetic mechanisms. The Harvey Lectures, Vol. 44, Series XLIV, pp. 99-123. Charles C. Thomas, Springfield, Illinois, 1948-49.

29. Green, D.E. Oxidation and synthesis of fatty acids in soluble enzyme systems of animal tissues. Clinical Chem., vol. 1, No. 1, pp. 53-67, 1955.

30. Stansly, P., and Beinert, H. Synthesis of butyryl-coenzyme A by reversal of the oxidative pathway. Biochem & Biophysics Acta, 11:600-601, 1953.

31. Green, E.C. Anatomy of the Rat, Vol. XXVII, p. 239 and p. 330. Haddon Craftsmen, Inc., Camden, New Jersey, 1935.

32. Hausberger, F.X., Milstein, S.W., Rutman, R.J. The influence of insulin on glucose utilization in adipose and hepatic tissues in-vitro. J. Biol. Chem., 208:431-438, 1954.

33. Feller, D.D. Incorporation of acetate carbon into lipids by slices of adipose tissue. J. Biol. Chem., 206:171-180, 1954.

34. Lepkovsky, S., Lemon, R., Chari-Briton, A., Dimick, M. Lipogenesis of tissues as affected by ingestion of food and water. Fed. Proc., vol. 14, no. 786, p. 243, March 1955.

35. Masoro, E.J., Chaikoff, I.L., Dauben, W.G. Lipogenesis from glucose in the normal and liverless animal as studied with C^{14} labeled glucose. J. Biol Chem., 179:1117-1125, 1949.

36. Chernick, S.S., Masoro, E.J., Chaikoff, I.L. Conversion of C^{14} labeled glucose to fatty acids. Proc. Soc. Exptl. Biol. Med., vol. 73, no. 3, pp. 348-352, 1950.

37. West, E.S., and Todd, W.R. Textbook of Biochemistry, ed. 2, p. 1179. Macmillan Co., New York, 1955.

38. Evans, H., Lepkovsky, S. The sparing action of fat on vitamin B. J. Biol. Chem., 96:179-184, 1932.

39. Siperstein, M.D., Chaikoff, I.L. Excretions of carbons 4 and 26 in feces, urine, and bile. J. Biol. Chem., 198:93-103, 1952.

40. West, E.S. and Todd, W.R. Textbook of Biochemistry, ed. 2., p. 492. Macmillan Co., New York, 1955.

determined. From this figure the amount of cholesterol in each fraction was calculated.

H. Combustion Procedure

The apparatus and techniques established for the wet combustion of non-volatile samples by Claycomb, Hutchens and Van Bruggen[19] were employed. The method was as follows:

1. When cholesterol was combusted a volume of alcoholic solution containing approximately 7.5 mg.* of cholesterol was placed in a combustion tube and evaporated to dryness. The tube was then placed in an oven for one hour to remove traces of water.

2. In the combustion of the fatty acids, 10 mg.* samples were weighed in porcelain boats and these placed in combustion tubes.

3. Five ml. of Van Slyke-Folch solution were added to the proper side arm of the combustion tube. The equipment was arranged so that samples could be run in duplicate.

4. Six ml. of 0.5 N NaOH were placed in the absorption flasks.

5. High vacuum grease was applied to the glass joints and then the combustion tube and absorption flask assembly was evacuated by a water pump to about 20 mm. Hg.

6. The tubes were twisted or inverted so that the combustion solution mixed with the sample, and spontaneous oxidation was allowed to occur at room temperature for about 5 minutes.

7. The absorption flasks were cooled by ice water and the combustion tubes immersed in a hot wax bath (160° C.) and combustion carried

*The above amounts of lipids were chosen for they produce enough CO_2 to make infinitely thick $BaCO_3$ plates, i.e., 100 mg. in weight.

out for 20 minutes. The reaction was considered complete when the Hg. in the manometer arm returned to its previous setting.

I. Plating

1. The contents of the absorption flasks were transferred to centrifuge tubes and the $CO_3^=$ precipitated with an excess of $BaCl_2$-NH_4Cl reagent.

2. Equal aliquots (usually 10 ml.) of the NaOH-Na_2CO_3 solution from the collection of respiratory CO_2 were also put into centrifuge tubes and precipitated.

3. For optional precipitation conditions, the centrifuge tubes were warmed to 45-50° C. before the $BaCl_2$ reagent was added. After the addition of reagent the tubes remained at 50° C. for an additional 10 minutes to allow the precipitate to flocculate.

4. If the amount of $BaCO_3$ produced was low, carrier $BaCO_3$ was added at this point to insure an infinitely thick plate. One to two ml. of ether-alcohol were added and the tubes were centrifuged for 10 minutes at 2,000 RPM.

5. The supernatant fluid was aspirated and the precipitate was triturated.

6. The precipitate was now washed with 5 ml. H_2O, recentrifuged, and retriturated. This operation was done three times.

7. The precipitate was now washed with about 25 ml. of ether-alcohol (1:3) and centrifuged, and the supernatant was aspirated.

8. The precipitate was transferred to centrifuge cups containing tared aluminum discs. Not more than 15 ml. of ether-alcohol was used in the transfer.