LIPOGENESIS

OF THE

INTACT ALLOXAN-DIABETIC RAT

by

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A THESIS

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TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

This thesis presents a study of some phases of lipid metabolism in the alloxan-diabetic rat, acetate-1-C^{14} being used as the "tracer-tool".

Diabetes is a disease in which the utilization of carbohydrate is impaired. In this disease state, subjects preferentially metabolize fat for energy production and lose much of the ingested sources of energy from their bodies as urinary glucose and ketone bodies. Heredity and obesity are known to be important factors in the development of this disease. The etiology of diabetes is still unknown.

Approximately 1,000,000 persons (1) in the United States have been diagnosed as diabetic, and it is estimated that there are at least another million undiagnosed diabetic cases in this country (2). Approximately 60,000 new cases are diagnosed annually. About 39,000 deaths in 1948 were classified as due to diabetes, which made the death rate of diabetics 26.4 per 100,000 population (3).

Recent statistics on mortality from diabetes show that the disease causes more deaths in the female than in the male (4). This sex difference may be due to be the influence of endocrine factors outside the pancreas. Another characteristic of diabetes mortality is the rise with age. The cause of death in the diabetic group is predominantly arteriosclerotic lesions of the vascular system.

Root and co-authors (5) have studied vascular lesions in the kidney, retinae, and peripheral vessels of 282 patients who first developed diabetes when they were between 15 and 30 years of age. These authors found that the arteriosclerotic lesions increased in these patients with the duration and severity of diabetes. Clinically, the incidence of atherosclerosis

was higher when hypercholesteremia was present. A disturbance in cholesterol metabolism may be related to the incidence of atherosclerosis, but a direct proof of this is lacking. In animal experiments in which vascular lesions are produced from the ingestion of excess cholesterol, a relationship between hypercholesteremia and atherosclerosis is clearly demonstrable.

Pathologically, the disease of diabetes is not an easily definable state of metabolic derangement. It is definitely established that the beta cells of the islands of Langerhans in the pancreas produce insulin. Primary pathological changes are often found in the islands of Langerhans, but in 20% of diabetic cases, no demonstrable pancreatic changes can be shown by the most modern histological techniques (6,7). In the great majority of human diabetics, very few beta cells are destroyed, but they are frequently granulated which suggest that they are injured to some degree. Pituitary growth hormone may nullify the effect, or prevent the formation, of insulin by acting directly upon the beta cells or acting at the hexokinase level. The adrenal steroids promote glyconeogenesis and may cause hyperglycemia. The recent work of Bornstein and Park (7a) indicates that through the combined actions of the growth hormone and an adrenal cortical hormone, a substance is formed that inhibits glucose utilization. This substance is associated with the lipoproteins of blood and its effect can be reversed by insulin. This material may be the true inhibitor of the hexokinase reaction. Glyconeogenesis is promoted by adrenocorticotropic and hypophyseal hormones through the adrenal glands. These agents which promote hyperglycemia and agents which are antagonistic to insulin action may cause stimulation and proliferation of the beta cells in the pancreas. In excess, such stimulation may exhaust the beta cells.

Experimental diabetes was produced by pancreatectomy by Mehring, Minkowski, and Allen; by treatment with anterior pituitary extract by Houssay, Brasotti, and Rietti and Evans et al; and by the use of chemical agents, alloxan, etc., by Dunn, Sheehan, and McLetchie (8). Alloxan has a specific necrosing or killing action on pancreatic beta cells but may also affect the epithelium of the renal tubules when injected into rabbits and rats (9,10,11).

Since an understanding of the metabolic defects that occur in diabetes involves an understanding of lipid metabolism, the following section is presented as a review of some of the more important phases of lipid metabolism.

I. HISTORICAL BACKGROUND

INTERMEDIARY METABOLISM OF FATTY ACIDS

Only small amounts of fatty acids are present in the body in the free state; rather, they exist in combination with glycerol, cholesterol, carbohydrates, and proteins. Of these, the glycerides are predominant. A constant amount of these lipids occurs in the tissues as essential constituents of the cell structure. Much of the glycerides is stored as reserve food in the various depots of the animal body. The amount of fat in the depots is subject to wide variations. However, the kind of fatty acids forming these lipids is constant and characteristic in a given species on a uniform diet. The quantitative fatty acid distribution in the tissues of normal rats is widely variable. In an animal subjected to starvation, or in an uncontrolled diabetic, the depot fat may be depleted to a very small value.

The presence of a marked impairment of fat synthesis in diabetic animals was first shown by Stetten and Boxer (12, 13) who administered deuterium labeled glucose to alloxan-diabetic rats. These workers found that the utilization of glucose for fatty acid synthesis in diabetic rats was reduced to 5% of the normal. Brady and Gurin (14) observed that utilization of acetate and octanoate for long chain fatty acid synthesis also was markedly reduced in liver slices from alloxan-diabetic rats. This defect in fatty acid formation in diabetic animals is related to their inability, or drastically reduced ability, to utilize carbohydrate precursors for fatty acid synthesis. The similarity of the diabetic state to that of the fasted state was noted by several authors (15,16,17,18).

Masoro et al (19) found that glucose utilization was depressed in fasted normal animals and in the normal animals fed a diet containing

little or no carbohydrate. Semi-starved human subjects studied by Chambers (20) and Lundeback (21) showed similarly depressed glucose oxidation, depressed glucogenesis and increased glyconeogenesis. When glucose was given to such fasted subjects, hyperglycemia and glucosuria resulted. A term, "hunger diabetes", is given to such a nutritional state because of its similarity to diabetes. Boxer and Stetten (22) reported reduction in deposition of newly synthesized fatty acids in rats on restricted food intake. Further evidence was recently reported by Van Bruggen and coworkers (15) that rats fasted 120 hours lost about 25% body weight, 75% of their fatty acids, and much of their ability to form new fatty acids. Lyon, Masari, and Chaikoff (17) found decreased fatty acid formation in liver slices from fasted rats.

Chernick and Chaikoff (23) reported that livers of both alloxan-diabetic rats and fasted rats regained the capacity to synthesize long chain fatty acids when these animals were pretreated with insulin and carbohydrate for 48 hours. Krahl and Cori (24) reported that the rate of glucose utilization by diaphragms from diabetic rats varied inversely with the severity of the diabetes. Adrenalectomy of such severely diabetic rats was followed by a fall in blood sugar and a return to normal of the rate of glucose utilization by the diaphragms. Insulin did not restore the glucose utilization of diabetic diaphragm to normal. Broh-Kahn and Mirsky (25) reported that muscle extract of alloxan-diabetic rats displayed hexokinase activity comparable with that found in the normal rat. Furthermore, the addition of insulin failed to increase the activity of either "normal" or "diabetic" extracts. These findings appear to support these authors' idea that inhibition of hexokinase activity is not necessarily the only cause for the disturbed glucose metabolism in diabetes.

Brady and Gurin (26) reported that liver slices of normal and fasted cats converted appreciable amounts of radioactive acetate to long chain fatty acids. Addition of insulin to the media containing these tissues enhanced the synthesis significantly. In contrast, liver slices from depancreatized cats converted only minimal amounts of acetate to fatty acids. Addition of insulin to the incubation media showed no effect. Houssay cats, on the other hand, readily incorporated labeled acetate to long chain fatty acids. Gurin (27) obtained similar results from alloxan treated rats. Liver slices of hypophysectomized animals synthesized fatty acids to a greater extent than did the liver of intact animals. Surgical removal of both hypophysis and pancreas from animals did not influence the rate of fat synthesis, but addition of purified growth hormone or cortisone to the media lowered the fat formation. Gurin believes that these results indicate that neither hormones of the pituitary nor insulin is needed directly for fat synthesis. The pituitary secretion may contain a principal which inhibits fatty acid formation. It is possible that in the diabetic state the normal hormonal balance is disturbed, and conversion of C_2 fragments to fatty acid is blocked (28).

In 1937 Schoenheimer and Rittenberg (29) discovered that when the body water of rats was enriched with heavy water (D_2O), fatty acids, among other body constituents, became labeled with deuterium. These investigators were able to increase the amount of deuterium in the fatty acid up to 50% of the deuterium concentration in the body water. The isotope, stably incorporated into the fatty acids of these animals, was convincing proof of the synthesis of new fatty acids in the body. This finding led the way for intensive research in the field of lipid synthesis and metabolism.

About fifty years ago, Knoop (30) advanced a concept that fatty acids are degraded through beta oxidation and that the synthesis of fatty acids proceeds by the reversal of the catabolic processes. However, the direct proof of this concept was lacking until recently. Correctness of Knoop's principle is now recognized, and the intermediary steps involved in the synthesis of fatty acids are better understood.

Block (31) advocated that any scheme proposed as a mechanism of fatty acid synthesis must account for the presence of an even number of carbon atoms in naturally occurring fatty acids of series C_2-C_{24}, if milk fatty acids also are included. Condensation of acetic acid or of closely related 2 carbon compounds in stepwise elongation to form long chain fatty acids is generally accepted as the mechanism of fatty acid synthesis. The proof of this process is available from the reports of many laboratories. Stetten and Schoenheimer (32) reported that deuteriopalmitic acid elongated to deuterio stearic acid. Rittenberg and Block (33) by feeding acetate labeled with deuterium and C^{13} to rats and mice, demonstrated that the acetate is utilized in the formation of higher fatty acids. The degradation of such fatty acids suggested that the carbon atoms of acetate were uniformly distributed along the fatty acid chain.

More recently, using C^{13} labeled palmitic acid and 1-C^{14} acetate in the media, Zabin (34) studied fatty acid synthesis in liver slices from fasted rats. This author found that stearic acid isolated from the liver slices contained almost all of the C^{14} carbon in the carboxyl position.

Popják and collaborators (35) reported that in mammary glands of ruminants, acetate is a major source of the carbon chains for fatty acid formation, and that milk short chain acids arise not by degradation of fats but are intermediates in the formation of long chain acids by

stepwise chain elongation. Popjack reported that the mechanism of milk fat formation is basically similar to that of other fats in the animal and that the process of rapid secretion of milk by the mammary gland has the effect of causing an accumulation of the intermediary products of synthesis. Anker (36) fed 1-C^{14} myristic acid to rats and isolated palmitic acid from their carcasses. The rats synthesized palmitic acid by the condensation of the methyl carbon of a 2 carbon compound with the carboxyl carbon of a long chain fatty acid. Stadman (37) reported that in the organisms, Clostridium kluyveri, the caproic acid chain is formed by condensation of the carboxyl-carbon atom of butyric acid with the methyl carbon atom of ethanol. A general scheme of the mechanism of fatty acid formation in which acetate or active acetyl compounds are used as the building blocks in the synthesis of fatty acids was proposed upon the basis of the above findings.

Acetate arises directly from the beta oxidation of fatty acids, from a number of amino acids and indirectly from carbohydrate sources through pyruvate. The magnitude of C_2 formation, in rats, was estimated (38) by the acetylation of foreign amines as 15 to 20 mM of acetic acid formed per 100 grams of rat tissue per day. Anker (39) demonstrated the importance of pyruvate as a source of acetate in the normal rat.

Zabin (34) reported that stearic acids isolated from the liver slices of fasted rats showed an asymetrical distribution of isotope. He believed that unequal distribution of acetate carbon in fatty acids would result whenever the rate of fat synthesis was slow. This author stated that under a condition of impaired fat synthesis, higher fatty acids are formed primarily by the elongation of the lower homologue, and the rate of this reaction is much slower than the formation of a long chain fatty acid by

by total synthesis from 2 carbon units.

Recent advances in enzyme studies of fatty acid metabolism opened new approaches to this problem. The work of Barker and coworkers (40) demonstrated that the processes of fatty acid synthesis and oxidative degradation in cell-free extracts of Clostridium kluyveri are complex reactions involving many steps. In the synthesis the net result is a condensation of the methyl carbon of C_2 compounds with the carboxyl carbon of a preformed fatty acid to produce a fatty acid with two more carbons. They were unable to produce caproic acid by a condensation of two molecules of propionic acid.

Green and coworkers (41) reported from their findings that the enzymes which affect fatty acid oxidation and synthesis can be isolated and studied in vitro. These authors believe that the cells of all animal tissues contain thousands of specific enzyme systems. These enzyme systems are so integrated structurally and functionally that the complex acts as a unit. The structural unit of this complex is the mitrochondrion, and Co A appears to hold a key position. According to the evidence available, the synthesis of fatty acids in vitro is a reversal of the catabolic process.

INTERMEDIARY METABOLISM OF CHOLESTEROL

Cholesterol is an essential constituent of the cell structure. It occurs in all tissues and tissue fluids of the animal body. Normally, the amount of this lipid in each tissue is fairly constant. The animal forms cholesterol from metabolites of small molecular size, principally acetate. The principal site of the formation of this lipid has been thought to be the liver, but the synthesis is known to occur in other tissues and organs as well (42).

It was shown recently that in all tissues, except in the brain and the spinal cord, continual regeneration of cholesterol occurs. The brain and the spinal cord themselves are the sites of active cholesterol synthesis in the early stages of an animal's development. Cholesterol synthesis may be accelerated in the diabetic state for hypercholesterolemia is often associated with prolonged severe diabetes.

The use of a tracer technique for the study of cholesterol formation dates back to the classical work of Rittenberg and Schoenheimer (29). These authors noted that deuterium was stably incorporated into the cholesterol molecule. Rittenberg and Block (43) demonstrated that the synthesis in surviving rat liver slices proceeded without an appreciable change in the total cholesterol content of the tissue. They showed that when liver slices were incubated with a media containing radioactive acetate, or precursors of acetate, the cholesterol isolated several hours later showed radioactivity. Chemical degradation demonstrated that the majority, if not all the carbon atoms, of the steroid structure were contributed by acetic acid (44). Acetoacetic acid, short chain fatty acids, certain amino acids, and pyruvate were readily incorporated into the cholesterol molecule presumably as two-carbon units. In the three or more carbon precursors, the preliminary fragmentation of precursors into two-carbon units occurred prior to their incorporation into the steroid molecule.

Zabin and Block (45) found that isovalerate was degraded into two types of C_2 units. One of these C_2 units resembled acetate, while the other unit, derived from the isopropyl portion of isovalerate, was a more efficient carbon source for cholesterol synthesis. They suggested that acetoacetate or a similar C_4 compound is formed in vivo from the isopropyl group of isovaleric acid by the addition of a C_1 compound. Brady and

associates (46) assumed that aldol may be cleaved by liver slices into two carbon fragments by way of beta hydroxybutyric acid or acetoacetic acid. These authors found that aldol was more efficiently incorporated into cholesterol than into the long chain fatty acids.

Little and Block (44) studied the incorporations of acetate-1-C^{14} and acetate-2-C^{14}, as well as acetate labeled with C^{13} and C^{14}. They found that the methyl carbon was incorporated into positions 18, 19, 26, and 27, and probably, 17, while the carboxyl carbon entered into carbon atom 25, and probably, 10, of the cholesterol molecule. Some decarboxylation of acetate must have occurred in the process since the ratio, CH_3 to COOH carbons incorporated into cholesterol, was found to be 1.27. These authors noted that more carboxyl carbon of acetate was incorporated into the nucleus than into the side chain of cholesterol. The ratio of CH_3/COOH incorporated into the nucleus was 1.1, while the corresponding value for the side chain was 1.67.

The mechanism of cholesterol formation is not well understood. Block et al (47) observed that the synthesis of cholesterol was completely inhibited in liver slices when the organization of the liver tissue was destroyed by grinding or homogenizing the cells. These authors believed that the synthesis required a higher organization of the enzyme system in space.

Formation of cholesterol from the long chain hydrocarbon squalene was reported by Block (48). In such reactions the condensation of two carbon units was followed by dehydration, rearrangement, and ring formation. Squalene is found in small quantity in the liver, and when C^{14} labeled squalene was administered to a rat, labeled cholesterol was obtained. The literature contains few reports on lipogenesis on intact

diabetic rats. Most of the information on cholesterol synthesis was obtained by the liver slice technique, but the results obtained were not consistent. Brady and Gurin (28) found little impairment in the cholesterol synthesis from acetate while Hotta and Chaikoff (48a) found a 10-fold increase in cholesterol specific activity and stated that this implies a definite increase in cholesterol formation in the livers of alloxan-diabetic rats. Because of a possible relation to atherosclerosis, more information on lipogenesis, especially cholesterol synthesis, in various tissues of the intact diabetic rat is desirable.

KETONE BODIES

Due to the presence of metabolic blocks, diabetics are predisposed to the development of ketosis. Superimposition of ketosis, induced by insulin hypoglycemia upon such predisposition, tends to cause severe ketosis, acidosis, and potassium depletion.

In laboratory animals, the injection of alloxan has the initial effect of releasing an abnormally large quantity of insulin. The severe hypoglycemic shock that follows often causes coma and death of the animals unless glucose is given. Beatty and West (49) reported that alloxan-diabetic rats showed severe ketosis during the first week following alloxan injection. The degree of ketosis dropped to a lower steady level after the second week. At the terminal stage the ketosis again rose to a higher level.

Deuel (50) observed that glucose in small quantity is capable of reducing ketonuria in rats. MacKay and associates (51) found that succinic acid, as well as citric acid, reduced ketonuria in their rats, and Beatty and West (52) reported that substances related to the tricarboxylic

acid cycle diminished ketosis in their rats. It is well known that ketosis develops in starvation, diabetes, and thiamin deficiency in some species. In these conditions the animal's carbohydrate metabolism is diminished and fat catabolism is increased.

Weinhouse and associates (53) reported that acetoacetate is formed and oxidized by the liver, the kidney, and other tissues. These authors found that the kidney oxidized acetoacetate ten times more efficiently than the liver tissue. Ketone body production is at a minimal rate under normal dietary conditions, because the acetyl groups of intermediary metabolism condense preferentially with oxaloacetate to enter the citric acid cycle for complete oxidation. Ketogenesis is assumed to become prominent only when the supply of oxaloacetate is depleted to such an extent that acetyl groups undergo self condensation, predominantly in the liver.

Brady and Gurin (28) believe that every isolated tissue capable of the synthesis of cholesterol also has the ability to form acetoacetate; the amount accumulated, however, may be very small.

A possible role that ketosis plays in the etiology and progress of diabetes may be surmised from the work of Nath and associates (54). They daily injected gradually increasing doses of sodium beta hydroxybutyrate and acetoacetate into rabbits for 130 days. These substances caused the onset of diabetic symptoms in normal rabbits. Their histological studies on the pancreas of these rabbits showed that ketone bodies first stimulated the beta cells and later exhausted them.

Stadman and associates (55) reported a mechanism of Co A-catalyzed acetoacetate synthesis from acetate and ATP in a pigeon liver extract. Their findings showed that 16,000 calories are required for the condensation of two acetates to form acetoacetate. One energy-rich phosphate

bond carried sufficient energy for such condensation. These authors observed that the reactions proceed as follows:

$$\text{acetic acid} + \text{ATP} \xleftarrow{\text{enzyme}} \text{acetyl}\sim PO_4 + \text{ADP}$$

$$2\ \text{acetyl}\sim PO_4 + 2\ \text{Co A} \xrightarrow{\text{transacetylase}} 2\ \text{acetyl-Co A} + 2\ PO_4$$

$$2\ \text{acetyl-Co A} \xrightarrow{\text{liver enzyme}} \text{acetoacetate} + 2\ \text{Co A}$$

In confirmation they found that for every acetoacetate formed, about two moles of acetyl phosphate disappeared from the media.

These findings indicate that ketosis is an important intermediary metabolic process in an alloxan-diabetic rat.

II. EXPERIMENTAL METHOD

A. Acetate-1-C^{14}

Carboxyl-labeled acetate was synthesized from $BaC^{14}O_3$ in semi-micro quantities by the carbonation of a methyl Grignard reagent. Almost theoretical yields of C^{14} acetate were obtained according to the method of Van Bruggen et al (56). The samples of tagged acetate were pooled, assayed for radioactivity, and diluted so that 1 ml. of the final sample assayed approximately 1×10^6 counts per minute as an infinitely thick $BaCO_3$ sample, counted under a thin end-window tube.

B. Alloxan monohydrate

Alloxan monohydrate was twice recrystallized according to Speer et al (57). The method consists of dissolving the sample in a minimal quantity of hot water and then adding glacial acetic acid in a ratio of one gram of alloxan monohydrate to 10 ml. of acetic acid. This solution was allowed to cool in a refrigerator, and the crystals were harvested by filtration through a sintered glass funnel. The crystals were washed twice with ethyl ether and allowed to dry overnight over calcium chloride and solid sodium hydroxide under vacuum. The twice recrystallized alloxan monohydrate was colorless, very soluble in water, and decomposed without melting at 170°-175° C.

C. Alloxan-Diabetic Rats

Healthy, virgin female rats of the Sprague-Dawley strain, weighing approximately 100 gms., were selected. Female rats were used because this sex is more susceptible to the effects of alloxan than male rats (58).

1. Maintenance of Diabetic Rats

Alloxan diabetic rats are susceptible to various respiratory, as well as gastro-intestinal diseases. Therefore, these rats were isolated from the rat colony, housed in clean, standard wire cages, and placed in specially vented boxes (59). An excess of Purina Laboratory Chow and water was always maintained. The progress of the disease was checked periodically by the body weight and determinations of the blood and urine sugar levels.

2. Blood Sugar

Tail blood was obtained by warming the tail for one minute in warm water, drying it thoroughly, and cutting the tip by a single stroke of a scapel. Six to eight drops of blood were collected on a spot plate containing potassium oxalate crystals.

3. Urine Sugar

The alloxan-diabetic rats were placed in stainless steel metabolism cages and a twenty-four hour urine sample collected under toluene. The total urine output was diluted to one liter. A 1 ml. aliquot was diluted again to 100 ml., and 1 or 2 ml. samples from this dilution were taken for analysis.

4. Sugar Determination

The determinations of blood and urine sugar samples were made according to Somogyi and Nelson (60-63) on a Model 14 Spectrophotometer.

5. Carbon dioxide

The total collection of CO_2 (in NaOH) of each experiment was transferred with washing into a 500 ml. volumetric flask and diluted to the mark. Duplicate samples of aliquots were titrated to the phenolphthalein end point for the total CO_2 determination.

Milliequivalents of CO_2 in the samples were calculated from the following reactions:

$$2\ NaOH + CO_2 \longrightarrow Na_2CO_3 + H_2O$$

$$Na_2CO_3 + HCl \longrightarrow NaHCO_3 + NaCl$$

The radioactivity of the CO_2 was calculated from the activity of a $BaCO_3$ plate (infinite thickness) prepared from one-half to one ml. aliquots and a sufficient amount (0.75 millimole) of inert Na_2CO_3.

D. Apparatus and Technique for Wet Combustion of Fatty Acids and Cholesterol (56a)

The combustion apparatus consists of five components: an oxidation flask with oxidant reservoir, a delivery or adapter tube, an absorption flask, a mercury manometer, and an evaporation sleeve. A constant temperature bath maintained at 160° C. was used to heat the oxidation flask during the combustion period. Fisher Company's Bath Wax was a satisfactory bath fluid at this temperature.

The reagents required for oxidation are:

1. Van Slyke-Folch wet oxidation mixture without potassium iodate
2. Standard CO_2-free NaOH 0.5 N
3. Standard HCl, about 0.2 N
4. Standard Na_2CO_3, about 0.75 N

The glass to glass joints of chemically clean and thoroughly dry glass equipment were lightly lubricated with Dow-Corning High-Vacuum Silicone Lubricant.

Aliquots of cholesterol solutions, containing about 10 mg. of cholesterol, were pipetted directly into the bulbs of the oxidation flasks. The evaporation sleeves were attached, and the alcohol was driven off from the

samples with an air stream. When cholesterol residues were dry, acetone was added to each flask to concentrate the cholesterol in the bottom of the flask for combustion.

The weighed fatty acid sample was warmed and mixed well with a spatula. 10 mgs. fatty acid samples of each tissue were weighed accurately in porcelain boats and transferred carefully into the bulbs of the oxidation flasks.

With a bent-tip pipette, 5 ml. of Van Slyke-Folch reagent were carefully introduced into the oxidant reservoir of the oxidation flasks containing cholesterol or fatty acid samples. 5 ml. of CO_2-free NaOH were pipetted into the absorption flasks. The oxidation flask, the absorption flask, and the manometer were fitted to the adapter. This apparatus was then secured to the stand. The system was evacuated to 20 m.m. of mercury, and the stopcocks were closed. Any air leak was detected by changing manometer readings.

The oxidation flask was rotated 180 degrees to empty the contents of the oxidant reservoir into the bulb of the oxidation flask. Fifteen minutes were allowed for the initial reaction to subside, then the bulb of the oxidation flask was immersed in the 160° bath. The absorber flask was cooled in an ice bath during the oxidation and cooling period. Fifteen minutes were allowed to oxidize each sample. If no leakage had occurred, the manometer reading returned to 20-30 m.m. of mercury at the completion of the oxidation. Allowing ten minutes for cooling, the air was admitted slowly from the stopcock, and the absorber flask was removed. The contents of the absorber flask were titrated with 0.2 N standard HCl to a definite phenol phthalein pink. After titration, the solution was

made alkaline with 3 ml. of 0.5 N NaOH and then transferred to a 40 ml. heavy walled, round bottom tube.

0.75 mM or more of CO_2 will produce an infinite thickness plate, 20 mg/cm^2. If the titration value is less than 0.75 mM of CO_2, a quantity of standard Na_2CO_3 is added to produce a plate of infinite thickness.

E. Preparation of $BaCO_3$ Plate

$BaCO_3$ plates were prepared according to Hutchens et al (56b). The entire alkaline carbonate solution from combustion was used for the $BaCO_3$ plates. Aliquots of $NaOH$-Na_2CO_3 solutions from the respiration train were also plated. The apparatus required is as follows:

1. International centrifuge with 250 ml. trunion cups
2. Dural plating cup assembly and an adapter
3. Aluminum plates (1¼ inches in diameter)
4. Filter paper (1¼ inches in diameter)

The following reagents are required:

1. 0.5 N $BaCl_2$ in 0.4 N NH_4Cl
2. Alcohol-ether mixture 3:1

To the alkaline carbonate solution, containing about 0.75 mM of CO_2, three or four drops of phenolphthalein indicator was added. The tubes containing this mixture were covered with rubber vial caps to prevent CO_2 contamination. They were heated to 45-55° C. in a water bath. To this was added 5 ml. of $BaCl_2$-NH_4Cl mixture forcefully from a hypodermic syringe fitted with a needle. The precipitated samples were allowed to remain at 45° C. for ten minutes. They were then centrifuged for a period of ten minutes at about 2,500 r.p.m.; the supernatant fluid was

aspirated; the precipitate was then washed in water and resuspended in 3:1 alcohol-ether, aspirated, and ground to a smooth paste. This paste was transferred by repeated washings with alcohol-ether to the precipitation cups containing weighed aluminun plates and centrifuged. Semi-dry plates so prepared were again centrifuged for an hour. This length of time was sufficient to dry $BaCO_3$ firmly onto the plates. They were further dried in air to constant weights.

F. Counting and Calculation of Results

The method and calculations used in this thesis have been previously described (64).

All samples were combusted and plated in duplicate. The plate weight was maintained in excess of 20 mgs. of $BaCO_3$ per square centimeter. At this weight, because of self absorption phenomena, the observable activity was contributed only by the top 20 mgs. per cm^2 layer of $BaCO_3$ plate. Since the diameter of the plate used in this work was five square centimeters, this layer weighed 100 mgs.

The "infinite thickness" sample on the aluminum plate was placed 2 mm. under a GM tube and counted. The counts were reported in terms of unit time. A standard gas flow counter was used when the radioactivity of the sample was low. The efficiency of the two counters was empirically determined (64).

The definition of terms and the method of calculations used in this thesis are as follows:

I. Activity of top layer = total c.p.m. per plate less background c.p.m.

II. C.p.m./mg. $BaCO_3$ = $\frac{\text{activity of top layer}}{20 \text{ mg./cm}^2 \times \text{area (cm}^2\text{)}}$

$= \frac{\text{activity of top layer}}{100}$

III. Total plate count = c.p.m./mg. $BaCO_3$ x wt. $BaCO_3$ (mg.)

IV. Specific activity of substance studied =

$\frac{\text{Total plate count}}{\text{mg. of substance combusted}}$ = c.p.m./mg. substance

V. C.p.m. of radioactivity incorporated in substance

= c.p.m./mg. substance x mg. substance isolated

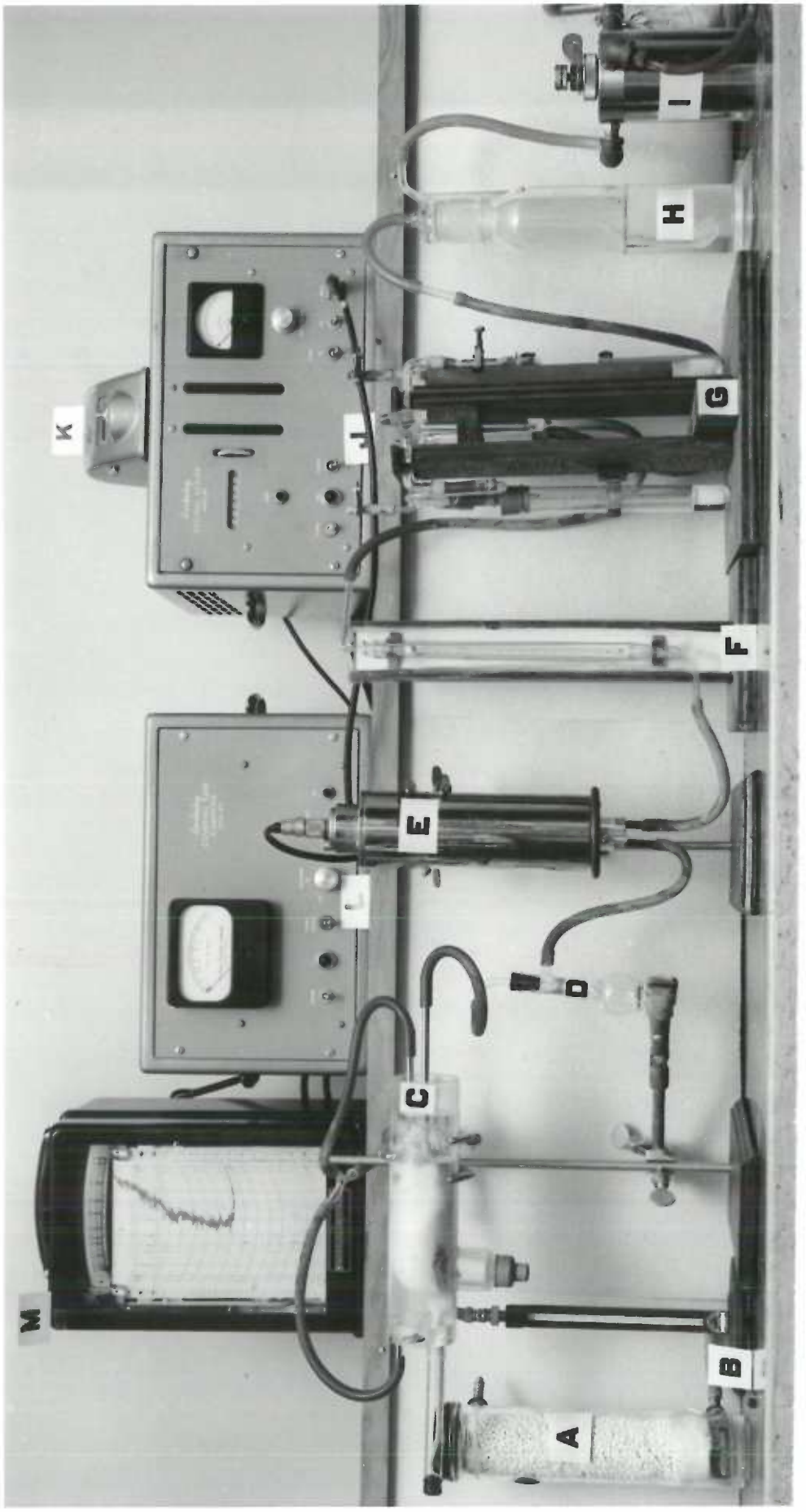
= c.p.m./mg. x total substance

VI. Per cent incorporation into substance

$= \frac{\text{c.p.m./mg. x total substance x 100}}{\text{c.p.m. acetate injected}}$

Figure I.

The metabolism assembly consists of a soda lime tower (A) from which CO_2-free air is drawn through a "C" clamp Flowrator (B) into a polystyrene metabolism chamber (C) through two inlets. Air containing $C^{14}O_2$ is led out of the metabolism chamber (C) through an outlet into a H_2O trap bottle (D) into a specially constructed Geiger-Mueller tube assembly (E). Another flow meter (F) is also connected into the system. Fractional CO_2 absorbers (G) containing 20 ml. each of 1 N NaOH solution are connected into the circuit with two three-way "T" shape stop cocks. These absorbers are used singly and one can be by-passed by the use of water or dilute acid instead of NaOH. The residual CO_2 is collected in another absorber (H) containing 200 ml. 1 N NaOH solution. A Cartesian Manostat (I), a vacuum regulating device, is inserted ahead of a water pump to maintain a constant flow of air through the system. The Berkeley Decimal Scaler (J) registers the total counts. An electric timer (K) records the running time. The Berkeley Counting Rate Computer (L) and the Esterline-Angus recorder (M) indicate and continuously record the relative specific activity of the respired $C^{14}O_2$.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G. Details of a Typical Experiment

1. Injection of animal

An alloxan-diabetic rat was injected intraperitoneally with one ml. of acetate-1-C^{14} with radioactivity of 1×10^6 c.p.m. The injected rat was immediately placed in the metabolism chamber. The rate of air flow through the chamber was adjusted to ten liters per hour.

2. Metabolism

The collection of respiratory CO_2 was begun within a few seconds after the animal was placed in the chamber. After one to six hours, the rat was removed from the metabolism chamber and chloroformed. The lightly anaesthesized rat was quickly killed by decapitation.

3. KOH Digestion of the Tissues

Liver, central nervous system (brain and spinal cord), skin, and the gastrointestinal tract were quickly dissected from the carcass. The liver and the central nervous system tissues were weighed on an analytical balance, transferred into flasks containing 100 ml. of freshly prepared 25% alcoholic KOH solution, and digestion was started. Skin, carcass, and gut were weighed and transferred respectively into 300, 300, and 200 ml. of alcoholic KOH.

After four hours of KOH digestion under reflux, the flasks were disconnected from the assembly, and the contents allowed to cool. Since carcass and gut flasks usually contained solid residues, they were decanted carefully. The solid residues were washed successively with alcohol, water, and petroleum ether; washings were combined with the KOH solutions; and residues were discarded. The flasks containing clear KOH digest were evaporated under air stream on a steam table to one half their original

volume. Enough water was then added to each flask to restore the volume of digest to the original mark.

4. Extraction of Unsaponifiable Fraction

KOH digests of skin, carcass and gut fractions were extracted successively with 400, 300, 200, and 100 ml. of petroleum ether (b.p. 30-60°C.). Similarly, liver and central nervous system were extracted with 300, 200, 100, and 100 ml. of petroleum ether. The pooled petroleum ether extracts of each tissue fraction were washed twice with 100 ml. of 1 N KOH and twice with 50 ml. of water. The washings were combined with the aqueous residues of the respective tissue digests.

Petroleum ether fractions were dried over anhydrous sodium sulphate overnight, filtered, and distilled cautiously to almost dryness. The final trace of solvent was driven off with the aid of an air stream. The cholesterol residues in the flasks were successively extracted five times with 95% alcohol. Each extract was heated to boiling, cooled, and filtered into a volumetric flask. The cholesterol content of the samples was determined as follows: Aliquots, as well as standard cholesterol solutions, in 15 ml. centrifuge tubes, were evaporated to dryness in a water bath, then transferred to a 110° C. oven for an hour. While hot, these samples were dissolved in 2 ml. of glacial acetic acid. These were then cooled. Color was developed with Liebermann-Burchard reagent at 25.0° C., and the density determined with a Model 14 Coleman Spectrophotometer. The accuracy of the method (modified Sperry-Schoenheimer) used in this laboratory was previously reported (65).

5. The Extraction of Saponifiable Fraction

KOH and water washes were combined with the water phase and acidified to Congo red with concentrated HCl, cooled, and extracted four times with

petroleum ether. The volumes of solvent used were the same as the volumes used in the cholesterol extractions. Ether extracts were washed successively three times with 50 ml. of water, dried over anhydrous sodium sulphate, filtered, and distilled. The last trace of petroleum ether was blown off under an air stream. The fatty acid residues left in the flasks were dissolved in hot acetone, cooled, and transferred to tared flasks by filtration in five successive extractions. The acetone in the tared flasks was driven off at 75° C. to complete dryness. The fatty acid residue was transferred to a vacuum dessicator and dried to constant weight.

III. EXPERIMENTAL RESULTS AND DISCUSSIONS

In this thesis diabetic animals showing constant hyperglycemia of more than 400 milligrams of glucose per 100 milligrams of blood were used. Several blood and urine sugar determinations were made prior to the experiments. Table I shows the history of the diabetic animals used. The glucose levels are the values determined at the time of sacrifice. Nonfasting glucose values of the fasted diabetic rats were obtained just prior to fasting. Blood sugar values ranged between 435 milligram per cent to 683 milligram per cent. Urine sugar values represent the amount of glucose excreted in the urine over a 24 hour period. The values of urine glucose have a range of 4.6 grams to 10.5 grams per 24 hours.

Nonfasted diabetic animals were used from one to seven and a-half months after alloxan treatment, and fasted diabetic animals were used from nine to eleven months after alloxan injection.

The weights of the diabetic animals used had a spread of 144 grams to 198 grams at the time of the experiments.

Control rats used in this thesis consisted of eleven adult male rats of the Sprague-Dawley strain of the University of Oregon Medical School colony. Eight of these rats were used one hour after the last feeding, and three were used without exact control over fasting. The average body weight of these control rats was 216 grams. These animals were used as controls in experiments in which a control feeding regime was not used.

Table II lists the weights of the tissues, and the isolated fatty acid and cholesterol fractions of the five tissues of the diabetic animals. Table III shows the corresponding weights of the tissues and the lipid contents of the control animals. The animals listed as pairs (ie., 25, 26) represent the actual pooling of tissues of two animals prior

TABLE I

HISTORY OF ANIMALS PRIOR to $CH_3C^{14}OONa$ INJECTION

Nonfasting Diabetic Animals

Animal No.	Body Weight Gm.	Duration of Diabetes Months	Blood Sugar Mg.%	Urine Sugar Gm./24 hr.
1 AD	155	1	783	4.6
3 AD	186	2.5	474	10.5
4 AD	153	2.5	435	8.4
5 AD	148	4.0	642	7.0
1 DR	144	7.5	550	4.8

18 hr. Fasting Diabetic Animals

Animal No.	Body Weight Gm.	Duration of Diabetes Months	Blood Sugar Mg. %	Fasting Blood Sugar Mg. %	Urine Sugar Gm./24 hr.
3 DR	170	9	520	254	9
4 DR	198	9	440	246	9
5 DR	149	11	441	380	11

TABLE II

TISSUE WEIGHTS AND WEIGHTS of FATTY ACID AND CHOLESTEROL FRACTIONS OF DIABETIC RATS

Unfasted Diabetic Animal		Brain and Spinal cord	Liver	Skin	Carcass	Gut
1 AD	Tissue Wt., gm.	2.11	8.36	15.5	72.0	42.5
	Fatty Acid, mg.	129.3	208.	253.7	1297.7	602.7
	Cholesterol, mg.	51.5	15.0	80.5	121.9	65.0
3 AD	Tissue Wt., gm.	1.91	8.15	23.5	72.5	29.6
	Fatty Acid, mg.	139.0	284.9	2658.	3505.	658.
	Cholesterol, mg.	41.5	10.5	46.5	107.	54.0
4 AD	Tissue Wt., gm.	2.08	7.95	19.5	83.5	32.5
	Fatty Acid, mg.	140.5	284.8	1386.4	4134.	780.
	Cholesterol, mg.	48.5	17.4	71.4	66.9	41.1
5 AD	Tissue Wt., gm.	2.09	8.20	20.0	69.5	42.5
	Fatty Acid, mg.	96.3	254.9	410.6	668.7	624.7
	Cholesterol, mg.	41.5	9.4	105.0	52.0	75.9
1 DR	Tissue Wt., gm.	1.90	7.86	18.8	65.5	26.7
	Fatty Acid, mg.	140.4	259.2	488.0	1913.	610.7
	Cholesterol, mg.	49.5	16.9	94.0	120.6	59.8
18 hr. Fasted Diabetic Animal		Brain and Spinal cord	Liver	Skin	Carcass	Gut
3 DR	Tissue Wt., gm.	2.00	9.69	25.0	92.3	24.3
	Fatty Acid, mg.	109.1	276.2	643.2	1356.	330.4
	Cholesterol, mg.	52.3	27.3	144.	185.	63.
4 DR	Tissue Wt., gm.	2.28	9.33	33.2	110.0	20.0
	Fatty Acid, mg.	161.0	355.6	2174.	2788.	379.6
	Cholesterol, mg.	63.	24.8	105.	185.	36.
5 DR	Tissue Wt., gm.	1.96	8.34	25.5	75.4	21.1
	Fatty Acid, mg.	102.8	268.9	901.1	1724.	200.8
	Cholesterol, mg.	46.4	19.2	107.5	137.5	53.5

TABLE III

TISSUE WEIGHTS and WEIGHTS of FATTY ACID and CHOLESTEROL FRACTIONS of CONTROL RATS

Animal No.		Brain and Spinal Cord	Liver	Skin	Carcass	Gut
	Tissue Wt., gm.	4.40	18.9	63.0	250	83.6
25, 26	Fatty Acid, mg.	228.7	539.	2884.	6115	1658.
	Cholesterol, mg.	60.3	32.5	277.	274	105.
	Tissue Wt., gm.	4.54	16.4	55.3	229	63.5
27, 28	Fatty Acid, mg.	195.7	439.	2460.	5505	1017.
	Cholesterol, mg.	98.3	19.5	225.	281	106.
	Tissue Wt., Gm.	4.18	20.0	70.6	270	82.5
31, 32	Fatty Acid, mg.	157.2	361.	6076.	9936	1868.
	Cholesterol, mg.	45.1	25.5	250.	361	133.
	Tissue Wt., gm.	4.18	20.3	63.5	232	62.0
33, 34	Fatty Acid, Mg.	178.6	367	5529.	9731	1989.
	Cholesterol, mg.	23.2	23.2	234.	300	70.
	Tissue Wt., gm.	1.87	7.9	19.9	134	16.0
N 3	Fatty Acid, mg.	179.3	251.	2646.	6466	577.
	Cholesterol, mg.	39.5	16.2	78.	139	34.6
	Tissue Wt., gm.	1.95	9.9	38.6	139	20.3
N 4	Fatty Acid, mg.	107.8	325.	6077.	10116	1822.
	Cholesterol, mg.	38.3	22.0	126.	137	39.0
	Tissue Wt., gm.	2.28	11.8	43.0	156	26.4
N 6	Fatty Acid, mg.	95.0	339.	4266.	6605	826.
	Cholesterol, mg.	32.2	25.4	187.	173	55.

TABLE IV

MILLIGRAMS FATTY ACIDS RECOVERED PER GRAM TISSUE

	Brain and Spinal Cord	Liver	Skin	Carcass	Gut
Diabetic Unfasted (5)	63.9	32.2	49.4	30.9	19.6
Diabetic 18 hr. fasted (3)	59.9	33.0	42.2	21.0	14.0
Control (11)	48.8	24.9	84.5	38.6	27.5

MILLIGRAMS CHOLESTEROL RECOVERED PER GRAM TISSUE

Diabetic Unfasted (5)	22.9	1.7	4.2	1.3	1.7
Diabetic 18 hr. fasted (3)	25.9	2.6	4.4	1.8	2.3
Control (11)	14.2	1.6	3.9	1.2	1.7

to the alcoholic KOH digestion. The data of these animals have been previously presented (64,67) and were included here because of this writer's participation in these experiments. The data clearly demonstrate the wide variations that exist among the sizes of the lipid compartments of different animals. Since the age of the animals differed at the time of their use, one is tempted to correlate the age with the change in the lipid contents. The data, however, show variation of the tissue weights and the lipid contents and show no trend or smooth progression of these changes with advancing age. Thus, such correlation is difficult to make. In general, tissue weights correlate with the size of the animals, and the lipid weights are related to the corresponding tissue weights; therefore, the lipid weights were expressed as unit weights (milligram/gram) of the tissue to normalize the values. In Table IV the concentrations of lipids are expressed as milligram/gram, and these figures represent the average values of the animals comprising each group.

The weights of the brains and the spinal cords of the animals were relatively constant. Since the lipid content of the central nervous system is high, the brain and the spinal cord fatty acid concentration is higher than that of most other tissues. The relative constancy of the lipid contents of the central nervous system of the animal is an expected finding since these lipids are derived from the essential lipid of the organ. The slight increase of lipid contents in the diabetic rats is probably related to the tissue dehydration in these animals.

The lipid concentration of the liver tissue represents both "essential lipid" and lipid which is being transported to the metabolic mills of the animal body. The role of liver in accumulating the depot lipid prior to utilization of these substances in the metabolic processes in the

tissues is well known. The liver is an active site of the degradation of fatty acids to C_2 and C_4 fragments, and it is an equally active site of the synthesis of new fatty acids from these fragments. In the diabetic state in which the utilization of fat is increased, the increased lipid contents of the liver tissue is an expected finding, but the increase shown is not remarkable.

Normally, the lipid contents of the skin and the carcass represent the major part of the depot lipid of the rat. The wide variations shown in these tissues in the diabetic rats are undoubtedly the reflections of the changes in the depot lipids.

The values of the skin fatty acid concentrations probably represent the amount of subcutaneous fat in the animals. The decreased concentrations of the fatty acids in the skin, the carcass, and the gut of the diabetic animals as compared to the controls are significant findings. Decrease in fatty acid concentrations in the tissues, in part, represents the defect in fatty acid synthesis in the alloxan-diabetic rats. The gut and carcass fatty acid stores are subjected to rapid depletion in the normal fasted animals (18). The similar changes in fatty acid stores in these tissues in the fasted diabetic animals are shown in Tables II and IV.

Cholesterol concentrations of the diabetic and the control groups varied less than the corresponding concentrations of fatty acids in the tissues. The comparison of unfasted control and diabetic rats reveals apparent differences in the concentrations between the two groups. Since the state of hydration of the diabetic animals varied considerably from that of the control rats, the increase may not be real. A state of dehydration was probably present in all severely diabetic animals even though

water was constantly provided. Hotta and Chaikoff (48a) concluded that cholesterol synthesis was grossly elevated in the livers of alloxan diabetic rats. If cholesterol synthesis is grossly elevated in diabetic rats, a greater accumulation of cholesterol in the tissues of chronically diabetic rats should be an expected finding. The absence of accumulation of tissue cholesterol in the chronically diabetic rats, as shown in Table IV, casts doubts upon the conclusions of Hotta and Chaikoff.

The sizes of the rats determine partly the lipid contents of the tissues analyzed. The amount of lipid in the tissue, in turn, influences the specific activities of the isolated lipid. The specific activities of fatty acids and cholesterol fractions were not presented for the following reasons: The weights of the control rats were heavier, tracer doses used in the experiment differed in amount, and the counters used in the isotope assays varied in sensitivity. Per cent of incorporation is calculated from the specific activities of lipids, the total amount of lipid present in the tissue, and the amount of tracer dose injected into the animal, and thus compensates for certain variables. The method used was previously discussed (64).

Tables V and VI contain data on the per cent incorporation of the tracer dose of acetate-1-C^{14} into the lipids of five tissues. The uniformly low per cent of incorporation of label into the lipids of the brain and the spinal cord is due to factors peculiar to this tissue. The "turnover" of brain lipid is rapid in the young animal, but the rate drops to a very low value in the adult. Low "turnover" of the brain lipids does not permit the label to enter appreciably into the lipid fraction in the short period allowed for these experiments. Much of the activity found in these lipids may be contributed by the blood lipids.

TABLE V

C^{14} INCORPORATED INTO FATTY ACID and CHOLESTEROL FRACTIONS of DIABETIC RATS

Unfasted Diabetic Rats

Animal No.		Per Cent Incorporation				
		Brain and Spinal Cord	Liver	Skin	Carcass	Gut
1 AD	Fatty Acids	0.024	0.254	0.118	0.324	0.138
	Cholesterol	0.004	0.384	0.038	0.200	0.343
3 AD	Fatty Acids	0.043	0.477	0.433	0.382	0.202
	Cholesterol	0.007	0.345	0.058	0.214	0.062
4 AD	Fatty Acids	0.035	0.168	0.270	0.459	2.07
	Cholesterol	0.005	0.35	0.061	0.087	0.028
5 AD	Fatty Acids	0.023	0.091	0.179	0.087	2.89
	Cholesterol	0.0001	0.058	0.050	0.020	0.266
1 DR	Fatty Acids	0.033	0.088	0.189	0.32	1.40
	Cholesterol	0.034	0.074	0.106	0.156	0.485

18 hr. Fasted Diabetic Rats

Animal No.		Per Cent Incorporation				
		Brain and Spinal Cord	Liver	Skin	Carcass	Gut
3 DR	Fatty Acids	0.021	0.112	0.214	0.229	1.31
	Cholesterol	0.004	0.137	0.081	0.177	0.275
4 DR	Fatty Acids	0.018	0.260	0.422	0.286	0.898
	Cholesterol	0.006	0.153	0.108	0.210	0.200
5 DR	Fatty Acids	0.053	0.132	0.452	0.326	0.815
	Cholesterol	0.004	0.083	0.171	0.181	0.358

TABLE VI

C^{14} INCORPORATION INTO FATTY ACID and CHOLESTEROL FRACTIONS of CONTROL RATS

Animal No.		Per Cent Incorporation				
		Brain and Spinal Cord	Liver	Skin	Carcass	Gut
25, 26	Fatty Acids	0.008	1.44	0.53	1.90	1.88
	Cholesterol	0.002	0.24	0.03	0.07	0.02
27, 28	Fatty Acids	0.008	0.92	0.32	1.55	0.10
	Cholesterol	0.001	0.11	0.04	0.08	0.21
31, 32	Fatty Acids	0.013	1.61	0.99	1.89	2.53
	Cholesterol	0.004	0.37	0.11	0.34	0.48
33, 34	Fatty Acids	0.017	1.08	0.40	1.85	1.70
	Cholesterol	0.003	0.32	0.07	0.14	0.14
N 3	Fatty Acids	0.017	0.13	0.40	1.42	1.25
	Cholesterol	0.003	0.10	0.10	0.20	0.27
N 4	Fatty Acids	0.02	0.20	0.81	2.15	1.42
	Cholesterol	0.026	0.11	0.21	0.24	0.25
N 6	Fatty Acids	0.016	0.18	0.50	1.82	1.13
	Cholesterol	0.003	0.12	0.19	0.27	0.31

TABLE VII

PER CENT OF RADIOACTIVITY INCORPORATED INTO FATTY ACIDS and CHOLESTEROL

Fatty Acids

Animals	No. of Rats	Brain and Spinal Cord	Liver	Skin	Carcass	Gut
Unfasted Diabetic	5	0.03	0.22	0.24	0.31	1.34
18 hr. fasted Diabetic	3	0.03	0.17	0.36	0.28	1.01
Control	11	0.01	0.79	0.56	1.80	1.43

Cholesterol

Animals	No. of Rats	Brain and Spinal Cord	Liver	Skin	Carcass	Gut
Unfasted Diabetic	5	0.01	0.24	0.06	0.14	0.29
18 hr. fasted Diabetic	3	0.01	0.17	0.12	0.19	0.28
Control	11	0.01	0.20	0.09	0.19	0.24

The complete removal of blood and interstitial fluids from the tissues was not practical. Therefore, the small differences found to exist among the tissues were not significant.

The data shown in Table V exhibit the usual wide variations of per cent of incorporation. These data and the data in Table VI were collected before the trained feeding program was instituted in this laboratory. Since these data were obtained, Van Bruggen et al (68) and Hutchens et al (18) have shown that in normal intact rats lipogenesis is dependent upon simultaneous glycolysis or upon simultaneous "feeding". Some of the variations seen in Tables V and VI may be due to unequal feeding. The diabetic animals lose much of their ingested food as urinary glucose, as shown in Table I. Greatly increased consumption of food and water in the diabetic animals made the feeding time prolonged and increased the residual food in the gut of nonfasted diabetic above that of the normal rats. For these reasons, restrictions on the amount of food and the time of eating were considered inadvisable. Table VII represents average values of per cent incorporation of radioactivity into fatty acids and cholesterol.

The diabetic animals showed a uniformly decreased per cent incorporation of radioactivity into fatty acid fractions of the liver, the skin, and the carcass. Differences in the % incorporations of label into fatty acids among fasted and nonfasted diabetic animals are slight. Defects in carbohydrate metabolism in the diabetic state may diminish glycerol production. That glycerol may be a limiting factor in fatty acid synthesis is suggested by the report (69) of Balmain's group on fatty acid synthesis in the mammary gland.

The findings of high lipogenesis in the gut tissue is not easily explainable. This high lipogenic activity of gut in the intact normal rat

was previously described by this laboratory (68). In the unfasted diabetic animals, the gut tissues are about six times more active than the liver. The decrease of fatty acid synthesis in the fasted diabetic gut is less than in the fasted normal rats. This difference is either due to the increased mass of active gut tissue for lipogenesis or the increased activity of the tissues in the diabetic state. Tables II and III show that the weights of gut tissues are relatively heavier in the diabetic than in the normal. Since other tissues are less active in fatty acid synthesis in the diabetic animals, it is possible that diversion of acetate-1-C^{14} to the gut is responsible for the high synthesis of fatty acids in the gut of the diabetic animals.

The factor which must also be considered at this point is that in this laboratory all injections of the tracer substances were made intraperitoneally. It is possible, then, to expose the gut tissues preferentially to higher concentrations of the tracer substances. The work of Claycomb (64) showed that the metabolism of acetate normally occurs rapidly. The $T\frac{1}{2}$ of the utilization of injected acetate was estimated by Hutchens (70) to be less than 7 minutes. It is possible, therefore, that the per cent incorporation of the tissues may be influenced by the local concentration factor. Hotta and Chaikoff, who obtained high specific activity in the lipid fraction of the liver by a slice technique, subjected the liver tissues to a high tracer dose. Brady and Gurin (28), using a slice technique, found that in the alloxan-diabetic liver the synthesis of fatty acids from acetate was greatly reduced, but the synthesis of cholesterol was only slightly decreased from the normal. In this thesis the per cent of incorporation of label into cholesterol fractions of all tissues of the intact diabetic rats is essentially equal

to the control normal rats, as shown in the data of Table VII. This finding of lipogenesis in the intact alloxan-diabetic rats essentially confirms the reports of Brady and Gurin, but the findings presented here are the first such demonstration in intact animals.

CARBON DIOXIDE

When sodium acetate-C^{14} is injected into an animal it is rapidly mixed with the endogenous acetate of the animal body. A small amount of this acetate is incorporated into lipids, carbohydrates, proteins, and their precursors. The greater portion, however, is rapidly oxidized and eliminated as respiratory CO_2. The amount of carbon dioxide produced in unit time, its specific activity, and the per cent incorporation of label should yield information on the fate of the injected acetate.

According to Gould (66) 86% of injected C^{14}-acetate is eliminated in a rat as CO_2 in four hours. Table VIII shows millimols of carbon dioxide formed, specific activity, and per cent of incorporation of label into the carbon dioxide eliminated by 18 hour fasted diabetic rats and normal rats. The CO_2 collection apparatus described previously on page 22 was developed by our group during the time this thesis work was done. Some of the CO_2 data were obtained with an inferior equipment. These data were, therefore, considered less reliable. Only the data collected from the new equipment are presented in Table VIII, and no attempt is made to compare the CO_2 data from the two groups. The result obtained from the diabetic rats in this laboratory agrees with Gould's work cited above. There appears to be no major defect in the diabetic rat in oxidizing acetate.

TABLE VIII

DATA ON RESPIRATORY CARBON DIOXIDE
OF 18 HOUR FASTED DIABETIC RATS

Dose injected: 1.18×10^6 C.P.M.

Animals	3 DR*	4 DR	5 DR
Total CO_2 mM	40.93	41.6	50.2
CO_2 mM per hr.	10.23	10.4	8.4
Specific activity	5.5×10^3	5.2×10^3	4.5×10^3
% Incorporation	86.9%	79.3%	84.3%

DATA ON RESPIRATORY CARBON DIOXIDE
OF NORMAL UNFASTED RATS

Dose injected: 2.2×10^6 C.P.M.

Animals	N 3†	N 4	N 6
Total CO_2 mM	53.2	54.4	65.0
CO_2 mM per hr.	13.3	13.6	13.0
Specific Activity	1.6×10^4	1.4×10^4	1.5×10^4
% Incorporation	93.5%	92.0%	95.0%

*DR, Diabetic rat with respiratory CO_2 data.
†N, Normal rat with respiratory CO_2 data.

IV. SUMMARY

1. Incorporation of C^{14} of acetate 1-C^{14} into fatty acid and cholesterol fractions of the brain and spinal cord, liver, skin, carcass, and the gut of intact diabetic and normal rats was studied.
2. A defect of fatty acid synthesis in the alloxan-diabetic rat was confirmed.
3. Normal fatty acid formation was noted in the gut of alloxan-diabetic rats.
4. Cholesterol synthesis in the tissues of the alloxan-diabetic rat was not appreciably different from that of the normal rat.
5. Fatty acid stores of all the tissues of alloxan-diabetic rats, except the liver, were reduced. The liver contained slightly more fatty acids than the normal.
6. Cholesterol stores of the alloxan-diabetic rat were essentially equal to those of the normal rat.
7. The alloxan-diabetic rats showed no major defect in oxidation of acetate.

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