THE INFLUENCE OF REFEEDING
UPON LIPOGENESIS
IN THE INTACT FASTED RAT

by

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I. INTRODUCTION

In the course of human history many excellent opportunities for studying the phenomenon of starvation have occurred. Both wars and famines have resulted in the death of millions of humans from this cause. World War II provided conditions from which came invaluable observations on the physiology and biochemistry of chronic malnutrition and starvation.

During starvation many alterations of normal physiologic processes occur, the most readily detectable of which is the change in body weight. Loss of weight begins soon when the food intake is restricted and continues until inanition and death result. The three major organic components of animal tissue, carbohydrate, protein, and fat, are available for use when food is withheld and are utilized by the animal for energy. When the weight loss which results from the use of body elements reaches 20 to 50% of the prefast weight[1], death may result.

Glycogen is a carbohydrate formed from food and stored primarily in liver and muscle cells, but the amount present is small under normal conditions*. As food is withheld this is the first cell constituent to be utilized by the animal. Hershey and Orr[2] have shown the rapid depletion of this substance from the liver and muscle of starved rats, minimal amounts being detected in 48 hours. Glycogen is not completely removed from tissues until terminally, but is maintained at low levels by the

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*The total body glycogen for a normal 200 gram rat might be estimated as follows: the muscle mass = ca. 40% of body weight or 80 gm., 1.5% of which is muscle glycogen (1.2 gm.). The liver weighs ca. 8 gm., 4% of which is liver glycogen (0.3 gm.). This totals only 1.5 gm. glycogen per 200 gm. rat, or less than 0.8% of the body weight.

process of gluconeogenesis. Also, blood glucose is maintained at low normal levels by this mechanism[3]. When the normal glycogen stores have been utilized, energy requirements are met from reserves of fat and from protein.

Protein is not stored in animal tissues, but is a basic component of all cells. As fasting is begun, a negative nitrogen balance occurs which progressively increases. Decreases in the tissue protein content and the plasma albumin concentrations occur as protein is used as a fuel, and it is estimated that in starvation 58% of the body protein metabolized is converted to glucose[4]. Concomitant with tissue protein catabolism is the decreased formation of enzymes and nitrogenous hormones. The body tends, however, to conserve the most essential components, so that the lowering of "metabolic enzyme" levels is accelerated only preterminally, while enzymes secreted into the gastrointestinal tract seem to be decreased at a rate proportional to that of general body protein depletion[3]. The endocrine changes occurring during starvation result principally because of decreased pituitary function. Decreased production of somatotropic, thyrotropic, adrenocorticotropic, and gonadotropic hormones results secondarily in profound alterations in carbohydrate, protein, and fat metabolism.

One of the most important of these secondary changes is the change in thyroid function. In the Minnesota Experiment of Keys and associates[6], the basal metabolic rate (an index of thyroid activity) was shown to decrease at a rate proportional to the rate of weight loss in semi-starved humans. The body temperature also declines somewhat during starvation, slowing to some small degree all chemical reactions in the body.

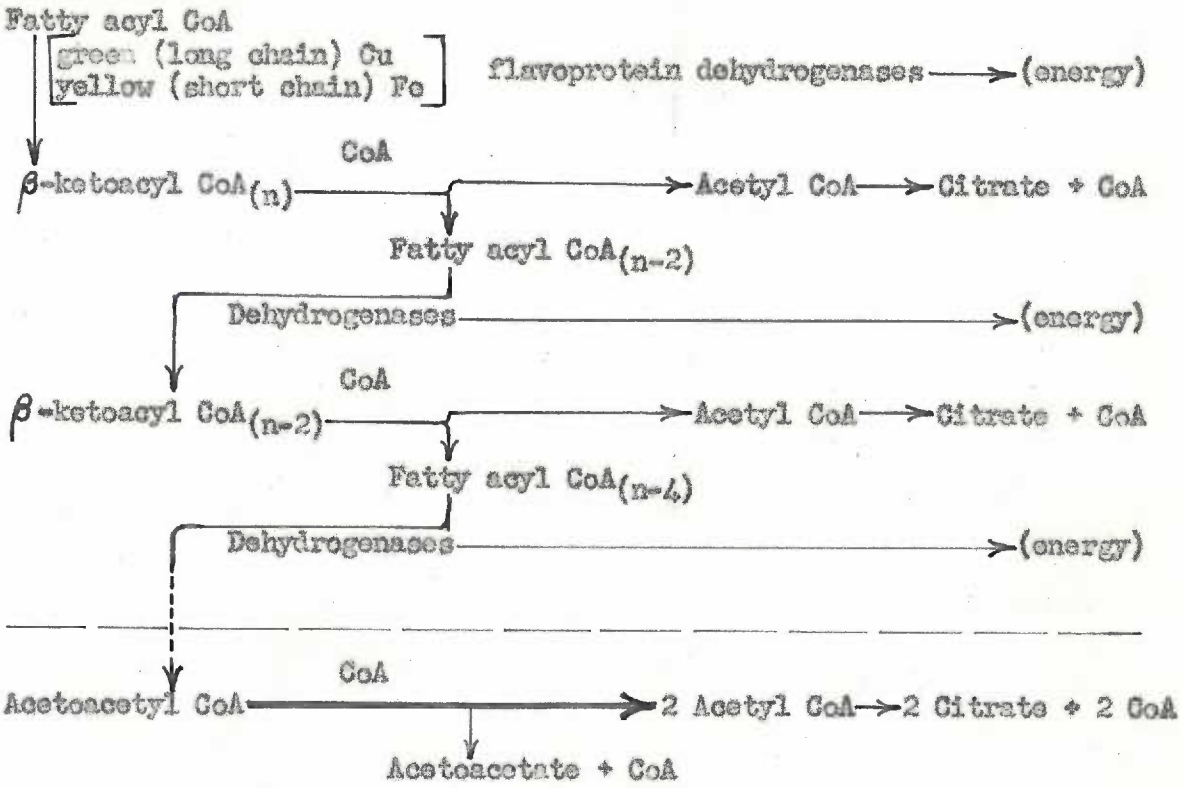
Oxidative reactions are decreased in tissues of starved individuals. One indication of this is the decreased tolerance to administered glucose. This response to glucose may be due to the failure of insulin action, but studies of Kaplan and Greenberg[7] indicate that a low adenosine triphosphate concentration in the liver associated with lowered body phosphorus levels is responsible. The accumulation of lactate and pyruvate following glucose administration to starved humans is also indicative of decreased oxidative processes. One possible explanation for certain of the findings in starvation is the decreased concentration of vitamins, some of which are integral parts of the oxidative enzymes necessary for pyruvate oxidation and other energy yielding reactions of the tricarboxylic acid cycle.

Oxidation of body fat stores contributes the major portion of derivable energy in the fasted state. In Benedict's famous study of a fasted subject[6] the estimated contribution of energy by fat was 85%. The respiratory quotient for this man decreased from 0.84 to 0.72 by the sixth fasting day and remained at this figure (which is characteristic of fat oxidation) for the rest of the 31 day fast.

In the normal breakdown of glucose, energy is derived from a series of reactions known as anaerobic glycolysis. This process begins with the formation of glucose-PO_4, which is subsequently degraded by enzymic action, and ends with the formation of pyruvic acid. This latter compound is enzymatically decarboxylated and the residual C_2 unit is converted to acetyl Coenzyme A. Acetyl-CoA is also formed from amino acid and fatty acid breakdown, and hence becomes a common intermediate in the metabolism of carbohydrate, protein, and fat. The concentration of acetyl Coenzyme A

in the tissues is very low, and the turnover (i.e., rate of formation and rate of breakdown) of this substance is very rapid. Acetyl Coenzyme A combines with oxaloacetate for oxidation to carbon dioxide and water in the tricarboxylic acid cycle. Additional portions of this compound condense to form aceto-acetyl Coenzyme A, and further condensations result in the formation of fatty acids. A small but measurable amount condenses in a still different fashion to form 5 carbon units which then condense in the formation of cholesterol and related steroids. Recent studies of the synthesis of cholesterol by Bloch[9] indicate that the 5 carbon compound is dimethyl acrylic acid and that condensation of this isoprenoid unit results in squalene formation. Indications that squalene is an immediate precursor of cholesterol were found by Diturit[10] and associates. Cholesterol is not known to be degraded by a reversal of this process. It appears to be excreted entirely through the bile. The time at which half the liver cholesterol of rats is replaced has been estimated to be ca. 6 days by Pihl, Bloch, and Anker[11].

The mechanisms involved in fatty acid breakdown and synthesis were recently reviewed in excellent fashion by Green[12]; the following discussion is largely from this reference. In the breakdown of body fat, energy must first be supplied in the formation of fatty acyl Coenzyme A from triglycerides. Following this, beta-dehydrogenation occurs with the formation of beta-ketoacyl-Coenzyme A. The following scheme[13] depicts these reactions:



In these reactions it is seen that with the removal of each acetyl CoA fragment (beta oxidation), one molecule CoA is made available for the next step. Energy is needed only at the start of this process in the formation of fatty acyl CoA, and it is provided by adenosine triphosphate in the presence of CoA. Thus, once started, the reaction is autocatalytic and will run to completion in the presence of the dehydrogenase enzymes. Further oxidation of the acetyl CoA occurs in the tricarboxylic acid cycle. In the last step, where acetoacetyl CoA is being broken down, the reaction proceeds primarily toward the formation of 2 acetyl CoA's, but in all tissues some deacylation occurs and acetoacetic acid is formed. Under normal conditions, all tissues but the liver are capable of reforming acetoacetyl CoA so that little free acetoacetate accumulates. The

acetoacetate formed in the liver is transported to the peripheral tissues where it is reconverted to acetoacetyl CoA and there broken down to acetyl CoA.

In conditions where fat breakdown is occurring at an accelerated rate, i.e., diabetes mellitus and starvation, acetoacetate may accumulate in appreciable quantities because the rate of acetyl CoA formation is increased while the rate of its oxidation is decreased. In such states the quantity of acetoacetate converted to ketone bodies is increased[14]. When carbohydrate is again utilized at a normal rate, oxaloacetate is provided by the addition of CO_2 to pyruvate. Availability of this catalytic C_4 molecule enables more acetyl CoA to be oxidized to CO_2 and H_2O. The equilibrium concerned in acetyl CoA production may be reversed and fatty acids then form.

Lipogenesis, which is the reverse of beta oxidation, is dependent upon three factors, the first of which is the presence of acetyl CoA. Normally this is provided by pyruvate from carbohydrate breakdown. The second requirement is the presence of synthesizing enzymes, and the third is provision of adequate energy to initiate the reaction and carry it to completion. Energy is provided from dehydrogenation reactions in which reduced Coenzyme I (DPNH) is formed. DPNH provides hydrogen for the endergonic hydrogenation of the beta-keto-acyl CoA. Whereas energy (or DPNH) is produced by each step of the beta oxidative breakdown of fatty acids, DPNH must be provided for each step of reduction in the synthesis of fatty acids, and it is normally produced from DPN as a hydrogen acceptor in glycolytic and tricarboxylic acid cycle reactions.

The relationship between glycolysis and lipogenesis has been emphasized by many investigators. Haugaard and Stadie[15] correlated a high liver glycogen content with rapid lipogenesis, and they showed that the conversion of C^{14} glucose to fatty acids is greatest following a high carbohydrate meal. These conditions are not met, however, in the fasted state, and these workers felt that appreciable glycogen deposition is necessary before increased fatty acid synthesis occurs.

That glycogen deposition per se is a prerequisite to lipogenesis was argued by Hirsch, Baruch, and Chaikoff[16], who studied lipid synthesis in lactating mammary gland slices (a tissue in which no glycogen is stored). They found that glucose stimulates the conversion of acetate-1-C^{14} and pyruvate-2-C^{14} to C^{14} labeled fatty acids, suggesting that the process of glycolysis diverts acetyl CoA units from oxidative routes to synthetic pathways. It was found by Baker et al[17] that the low lipogenic ability of diabetic rat liver slices can be enhanced by the addition of insulin and glucose, or by fructose alone. Since fructose is phosphorylated without the help of insulin and since glucose did not stimulate lipogenesis without insulin, it was felt that restoration of the glycolytic process is necessary for lipogenesis in the diabetic rat liver.

Further evidence that glycolysis is stimulatory to lipogenesis was provided by Lyon, Masri, and Chaikoff[18]. Rats were fasted 18 hours and a comparison was made of the lipogenic response to glucose, fat, and a protein hydrolysate. Glucose was found promptly to stimulate lipogenesis, which was not the case with fat or protein. If the animals were fasted following ingestion of much carbohydrate, fat, or protein, the lipogenic response remained high for a longer period of time following glucose

administration than after fat or protein. These investigators postulated from these and previous findings that "high activity in the glycolytic system is required for maintenance of lipogenesis".

The disturbed fat synthesis in fasting is then best rehabilitated by the administration of carbohydrate, as the biochemistry of these substances is so closely interrelated. The old axiom that "fat burns in the flame of carbohydrate" would more aptly read "fat forms in the flame of carbohydrate".

II. STATEMENT OF THE PROBLEM AND THE EXPERIMENTAL DESIGN

The work embodied in this thesis is, in part, a continuation of fasting experiments conducted by Dr. C. K. Claycomb[19], who helped to establish many of the techniques used herein. In his work, the effect of fasting upon lipogenesis in the whole rat was studied, by the use of tracer amounts of acetate-1-C^{14}. The time of maximum incorporation of the labeled carbon into fatty acids, cholesterol, and expired CO_2 was established for certain tissues of the rat. In the fasting experiments of Claycomb, the shortest fast period reported was 24 hours. After metabolism of the injected acetate-1-C^{14} label for a desired time, the rat was quickly dissected into liver, gut, skin, brain and spinal cord, and carcass, so that the time of maximum label incorporation could be determined in these separated tissues.

In this thesis, animals were separated for analysis into liver, gut, skin, and carcass, all dissections taking place four hours after the intraperitoneal injection of 20 μc Na Acetate-1-C^{14}. By varying the nutritional factors which preceded the acetate administration, different levels of incorporation of label into tissue lipids may be expected to occur. The experiments reported here are divided into three groups which cover the three aspects of lipogenesis under investigation, namely: (1) the effect of fasting, (2) the effect of refeeding fasted rats, and (3) the effect of substituting carbohydrate or carbohydrate intermediate compounds for food in previously fasted rats.

A. The Fasted Group

It was first necessary to study the effect of short term fasting on lipogenesis in these four tissue fractions. For purposes of clarity, "fasting" is defined as "the time period elapsing between removal of food from the rat cage and the injection of the labeled acetate". The fasting time ranged from short periods of one hour to longer periods of 240 hours. The influence of the previous meal upon lipogenesis was studied in short fast periods of 1, 3, and 5 hours. Additional groups of rats were fasted 7, 9, 13, and 19 hours.

Another metabolic phase occurred during extensive fasting, for this produced profound alterations in tissue lipid composition as seen in rats fasted 4 days (96 hours), 7 days (168 hours), and 10 days (240 hours). The fasting experiment, then, was a time course study of lipogenesis following withdrawal of all nutriment. A pattern of peak and minimum, and a time of most rapid decline of lipogenesis were established in the four tissue fractions studied.

B. The Refed Fasted Group

At a point during the period of fasting when lipogenesis in the tissues was approaching minimal values, food was given to the animals, to study the recovery of lipogenesis from fasting. Rats were first fasted 13 to 15 hours and oral feeding was permitted for one-half hour in one group of two animals, and for one hour in another group of four rats. Labeled acetate was injected immediately at the end of the feeding periods, and the recovery of lipogenesis was marked and rapid. However, more prolonged fasting made the effect of feeding less pronounced, as was

evidenced by single rats fasted 22 and 166 hours before refeeding and then being injected with acetate.

C. The Fasted Group Refed Carbohydrate Intermediates

That carbohydrate ingestion influences lipogenesis has been pointed out. It was felt desirable to compare the lipogenic response to total diet with the response to the carbohydrate component therein. The administration of compounds intermediate in the metabolic degradation of carbohydrate might be expected to yield results differing from those obtained after the administration of the more complex substances found in the normal diet. For this purpose rats fasted 13-15 hours were either intubated or injected intraperitoneally with one of the following: glucose, glycerol, sodium lactate, lactic acid, pyruvic acid, sodium succinate, and sodium citrate.

The work of this thesis, then, was designed to help elucidate some of the in-vivo factors which affect lipogenesis and to determine the influence of carbohydrate metabolism upon this process.

III. METHODS, MATERIALS, AND TECHNIQUES

A. Animals

The experimental animals of this work were albino rats of the Sprague-Dawley strain. All animals except two (N-7 and N-8) were males, and all were of young adult age, with weight ranges of ca. 200 to 250 gm. Males were used because the fat composition of adult female rats is known to be subject to considerable variation due to the cyclic influence of sex hormones, and because of the different fat distribution in adult females. The two female rats used in this study were of a younger age at which these factors would have only minor importance. All of the animals except three were obtained from the Sprague-Dawley Rat Farm in Madison, Wisconsin. The three others (N-7, N-8 and CF-9) were obtained from the rat colony of the University of Oregon Medical School; these rats being descendants of S-D rats obtained from the above Wisconsin colony.

On arrival the weanling rats, weighing about 50-100 gm. each, were placed in cages in groups of 3 to 5 rats. These cages were placed inside ventilated hoods which were devised in this laboratory[20].

B. Trained Feeding

The rats were placed on ad libitum Purina Laboratory Chow and water until they reached an arbitrary weight of 150 gm., at which time they were marked by removing segments of the ear pinna; and controlled feeding was instituted. Controlled feeding consisted of giving each rat, in two daily feedings, the amount of food it would consume in one hour periods

and yet maintain a normal weight gain. This feeding program was initiated because it was necessary to control accurately the time period during which the animals consumed food, not only for the refeeding experiments, but also to serve as the point of reference from which fasting was to begin. It was determined that our rats consumed ad lib. about 24 gm. per rat per 24 hours while gaining weight normally. Feeding experiments are presented at this time without further reference to verify this observation. Six rats were maintained on the trained feeding regimen of 12 gm. food given twice daily for 35 days. The average daily weight gain was 2.2 gm./day (see below).

Rat No.	Starting Weight	Final Weight	Weight Gain	Weight Gain per Day
1	285	359	74	2.1
2	264	310	46	2.1(fed only 22 days)
3	246	337	62	1.8
4	246	341	84	2.4
5	257	308	80	2.3
6	250	330	91	2.6
			Average	2.2 gm./day

(Average weight gain for rats fed ad libitum from 200 to 300 gm. for Main's group[21] was 2.0 grams body weight per day.)

When the twice daily feeding was begun, ca. 12 gm. of food (2 medium sized checkers) were given at 8:00 a.m. and again at 6:00 p.m. It was noticed that only about one-half of the food was consumed in the first 60-90 minutes, but by 4 hours the remainder had been consumed. After a week of such a program the rats became accustomed to this method of feeding and adjusted to it by eating 80-90% of their food during the first

hour and the remainder during the second hour. After this time they would fast until the next feeding. This regime will be called "trained feeding". Only rats that had been "trained-fed" for a minimum of one week were used for experiments. A daily weight record for each rat was kept during the trained-feeding period.

C. Preparation for Metabolism of Acetate

For the fasting experiments, the rats were weighed and given the morning feeding at 8:00 a.m. One hour later the remaining food was removed from the cage, the animal was reweighed, and fasting began. For the refeeding experiments, food was given at 5:00 p.m. the day preceding an experiment, and any remaining food was removed from the cage at 6:00 p.m. At the following 8:00 a.m. the rat was weighed and another 12 gm. of food were given. One-half hour later (rats CF-19 and CF-22), or one hour later (rats CF-21, -23, -24, and -26), the rats were removed from the cage, weighed, and immediately injected with the radioactive tracer acetate. Rat CF-20 was fasted 22 hours before feeding 1/2 hour, and rat CF-25 was fasted 168 hours before feeding one hour. For the experiments in which carbohydrate intermediates were administered, the same procedure was followed as in the refeeding experiments up until the time the compound was given, i.e., the animals each had a 14 hour fast; each rat was allowed to metabolize the administered chow substitute, and the labeled acetate was administered. Carbohydrate intermediate compounds were given to the animals as shown in Table I.

None of the rats given these compounds orally or intraperitoneally showed untoward symptoms or change in behavior except for rat DF-12 (I.P.

Table I

AMOUNTS OF CARBOHYDRATE INTERMEDIATE COMPOUNDS ADMINISTERED TO RATS

Rat No.	Method of Administration	Substance Given (all in 0.9% NaCl)	Amount (grams)
DF-11	Intubation*	5 ml. 10% Glucose	0.5
DF-1	Intraperitoneally	5 ml. 10% Glucose	0.5
DF-2	Intraperitoneally	5 ml. 10% Glucose	0.5
DF-6	Intubation*	5 ml. 20% Glycerol	1.0
DF-13	Intraperitoneally	5 ml. 8.5% Glycerol	0.4
DF-4	Intubation*	5 ml. 20% Lactic Acid	1.0
DF-9	Intubation*	5 ml. 20% Sodium lactate‡	1.0
DF-12	Intraperitoneally	5 ml. 8.5% Sodium lactate‡	0.4
DF-5	Intubation*	5 ml. 20% Pyruvic acid	1.0
DF-8	Intubation*	5 ml. 10% Sodium succinate	0.5
DF-7	Intubation*	5 ml. 10% Sodium citrate	0.5

* Intubation was effected with the aid of a 5 ml. syringe attached to a small rubber catheter.

‡ Sodium lactate was prepared by titrating 85% (commercial grade) Lactic acid with solid Na_2CO_3 to pH 7.0 with the aid of the Beckman pH meter.

Na. lactate), which began to show signs of listlessness and apathy lasting about one half hour 15 minutes after the injection. This was thought to be due to an intercompartmental fluid shift because of the relative hypertonicity of the intraperitoneal content. The amount of each compound given was a compromise between the amount of carbohydrate in the diet (60% of 10 gm. or 6 gm.) and a maximally tolerable dose of the compound.

D. Preparation of Na Acetate-1-C^{14}

$BaCO_3$ containing 12-14% C^{14} was obtained from Oak Ridge, Tennessee, and from it acetic acid 1-C^{14} was synthetized in this laboratory. Methyl magnesium iodide was reacted with CO_2 evolved by acidification of the standard $BaCO_3$. Reproduceable yields of acetate of 90-95% were obtained by methods previously developed in this laboratory[32]. 0.5 millicurie lots of the carboxyl labeled acetic acid were stored as dried sodium acetate in 40 ml. narrow mouth centrifuge tubes covered with rubber caps. As needed, each such 0.5 mc. lot was dissolved in 25.0 ml. distilled water, and 1.00 ml. of this solution, containing about 20 μc, was used for the intraperitoneal injection. For radio-assay 1.0 ml. was diluted to 200 ml. in a volumetric flask. The same 2 ml. calibrated syringe was used throughout all the rat injections, as well as for the assays of the different batches of acetate. Between injections each batch of acetate was kept frozen in the refrigerator to minimize contamination and loss. In preparation for the injection the solution was warmed to room temperature and shaken before the 1.00 ml. was drawn into the syringe.

E. Metabolism of Acetate-1-C^{14}

Twenty microcuries of Na Acetate-1-C^{14} in 1.00 ml. was delivered intraperitoneally to all rats through a 1-1/2" 21 ga. needle from a 2 ml. syringe, after aspiration to be assured that the tip of the needle was only in the peritoneal space. Following the injection, the rat was immediately placed in a plastic metabolism chamber and was allowed to metabolize the injected acetate for 4.0 hours. As previously shown[23,24], four hours is adequate for the collection of the greater part of the label as CO_2.

F. Metabolism Assembly

Figure 1 shows the metabolism assembly used in many of these experiments. (I) is a constant pressure vacuum regulator, which is connected to a water pump. Control over the flow rate of air through the metabolism assembly is afforded by a thumb screw regulator on top of (I). Room air first enters the assembly through the air inlet at the top of the CO_2 absorber jar (A). The now CO_2-free air then leaves at the bottom of (A) and is next drawn through an air flow rate meter (B). The CO_2-free air stream is divided as it is drawn into the plastic metabolism chamber (C) which confines the injected rat. This chamber is so constructed that the rat's nose extends into a cone shaped piece of plastic, the apex of which contains the main entrance and the exit of the air stream. This allows the rats constant fresh air and permits the immediate withdrawal of expired C^{12} and $C^{14}O_2$. The other division of the air stream is drawn into the rear of the metabolism chamber so that the chamber is constantly being swept free of CO_2. The air containing expired CO_2 is next drawn

Explanation of Figure 1.

A. Soda-lime CO_2 absorber.

B. Air Flow rate meter.

C. Plastic metabolism chamber with rat inside.

D. Sulfuric acid water vapor trap.

E. End window Geiger Müller tube.

F. Air flow rate moniter.

G. CO_2 absorber for small samples.

H. Alkali trap for total CO_2 collection.

I. Constant pressure vacuum regulator, connected to a water pump.

J. Berkeley Decimal Scaler, Model 100.

K. Timer.

L. Berkeley Count Rate Computer, Model 1600.

M. Esterline-Angus milliammeter recorder.

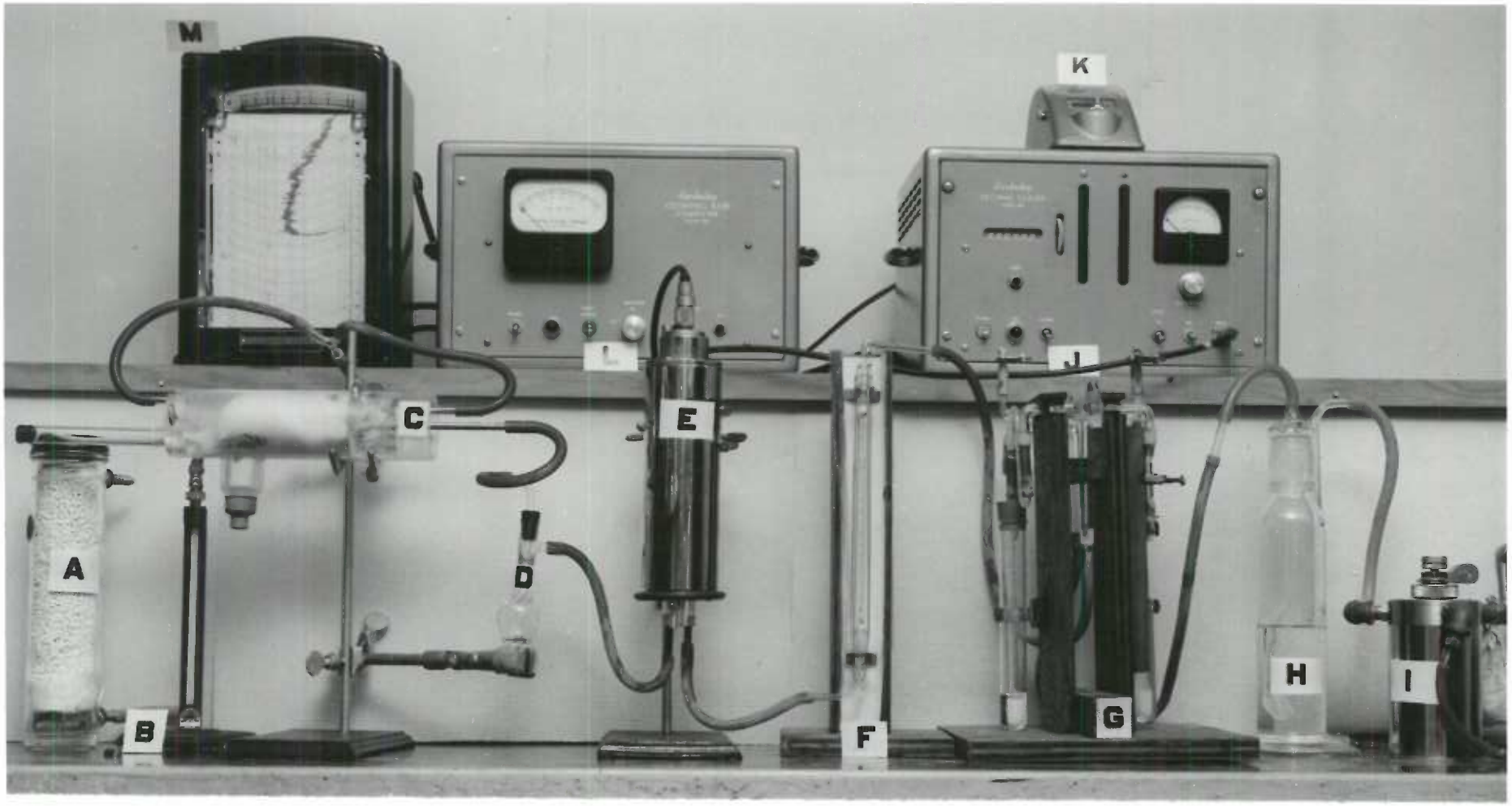


Figure 1. Rat Metabolism Assembly

through a moisture trap (D) containing concentrated sulfuric acid. From there the CO_2-laden air stream enters a lead shielded chamber (E) containing an end window Geiger Müller tube which is used to detect radioactivity in the expired CO_2. Next the air stream is drawn through another air flow rate meter (F), used to monitor the air flow rate. A reading of 14 on (F) indicates that 10 liters of air per hour are being withdrawn from the metabolism chamber. This amount is in excess of the 3 L./hr. normally used by a rat (as previously determined in this laboratory). A similar reading of 14 on air flow rate meter (B) gives assurance that no air leak is occurring in the system. From (F), the air stream is drawn into a serial carbon dioxide sample absorbing device (G), which was not used in these experiments. Here the air is drawn from (F) into (H), which is the total CO_2 absorber. A gas diffuser immersed in 250-300 ml. of 1 N NaOH disperses the air stream into fine bubbles, allowing efficient CO_2 collection by the alkali in (H). The total CO_2 collected during the four hour period is later analyzed for radioactivity and for amount. Impulses from (E) reach the scaler (J) (Berkeley Decimal Scaler Model 100) from which can be read, at any time, the accumulated counts from the radioactive CO_2 which has passed through the GM tube chamber. A timer clock (K) is connected to (J) and used to time the experiments. Impulses from (J) are transmitted to (L), a count rate computer (Berkeley Count Rate Computer, Model 1600), and when coupled with the recording device (M), the count rate (activity/time) is thus automatically recorded. Use of the continuous gas flow counting was made for some of these experiments (this data will not be presented here), but not all, in which cases (E), (J), (L), and (M) were removed from the system. The clock (K) was

connected to a standard electrical outlet, and (D) was connected directly to (F). At the termination of each experiment, when the rat had been removed from the chamber, the system was quickly re-closed and flushed out for an additional 15 minutes to allow the absorption of any remaining $C^{14}O_2$.

C. Termination of an Experiment (Dissection Procedure)

At the end of four hours in the metabolism chamber, each rat was placed immediately in a jar containing chloroform. The rat was then weighed and quickly decapitated with large scissors. The blood was collected in the carcass fraction digestion flask, to which the weighed head was also added. The abdomen was quickly incised and the liver removed *in toto* by blunt dissection and placed in a tared pan for weighing on a Gramatic Balance. When liver glycogen content was being determined, duplicate 0.5 gm. samples were removed from the blotted liver by slicing with a sharp razor blade. These samples were added to tared, stoppered 6" x 1" test tubes containing 2.0 ml. 30% KOH. The gut with its mesentary was removed by blunt dissection and weighed. The skin was stripped from the carcass and each was weighed. Tissues were placed in reflux flasks containing 25% KOH in 95% ethanol. The total time elapsing between removing the rat from the metabolism chamber and placing all the fractions in the digest mixture never exceeded five minutes. The digestion mixture was prepared about 30 minutes before terminating the experiment, and was warmed on a steam bath, so that when the tissues entered the solutions all enzymatic and autolytic activity would quickly cease. The flasks were prepared as follows:

Fraction	Flask size	Mouth, ℑ ground glass	ml. 95% ethanol	grams KOH
Liver	200 ml.	24/40	100	25
Gut	500 ml.	29/42	200	50
Carcass	1000 ml.	45/50	300	75
Skin	1000 ml.	45/50	300	75

Each fraction was digested under reflux for 4 hours to completely saponify the lipids. Complete tissue disruption occurred in the first half hour.

H. Lipid Extraction and Purification

The solutions were next reduced to half volume on a steam bath with the aid of an air stream. The digests were made ready for extraction by first transferring the warm solutions to separatory funnels, and then washing the flasks several times with warm water and warm alcohol. The final alcohol washes restored the volumes to their original size, and the resultant solutions in the separatory funnels were 20% with respect to alcohol. This was found to be optimal in reducing emulsion formation during the extractions. The extractions of carcass and skin fractions were carried out in 2 liter separatory funnels, while the liver and gut fractions were extracted in one liter separatory funnels. Each fraction was extracted four times with low boiling petroleum ether (B.P. 30-60° C.) to remove non-saponifiable lipid. The following amounts of solvent were used.

	Volumes of Petroleum Ether			
	1st ext.	2nd ext.	3rd ext.	4th ext.
Liver	200	100	100	100
Gut	200	200	150	150
Carcass	300	200	150	150
Skin	300	200	150	150

The four extracts of each fraction were combined and washed once with 1 N KOH (50 ml. for carcass and skin, and 25 ml. for liver and gut), and each once again with 50 ml. water. These washings were added to the respective aqueous phases. The petroleum ether extracts were dried over night with Na_2SO_4. The aqueous phases remaining after removal of the non-saponifiable lipid were acidified with concentrated HCl to pH 4-5 (Congo red paper). The fatty acids were removed from each fraction by extracting 4 times with petroleum ether, using the volumes shown above. The combined extracts of each fraction were washed twice with 50 ml. water, and these washings, along with the remaining aqueous phases, were discarded. These fatty acid extracts were also dried over Na_2SO_4.

The dry extracts were next filtered into two liter narrow mouth reflux boiling flasks with ground glass joints, and the sodium sulfate was washed four times with P.E. The petroleum ether was distilled until only a small amount remained. This remainder was removed by placing the flasks on a steam bath and directing fine air streams into them. 10-15 ml. of 95% ethanol were added to each flask containing non-saponifiable lipid and the flask was briefly heated on the steam bath. Each flask was then cooled by cold running tap water, and the condensing alcohol washed down the inner surfaces of the flasks. Non-saponifiable lipid (mainly cholesterol) remained in solution, while some impurities separated and precipitated. The flasks containing fatty acids were treated in a similar manner except that about 25 ml. acetone instead of alcohol were used to dissolve and transfer the fatty acids. The fatty acid extracts were filtered through Whatman No. 42 filter papers into tared 125 ml. Erlenmeyer

flasks with four 10 ml. portions of acetone. The filter papers were washed thoroughly with each washing.

The non-saponifiable fractions were transferred, with four 10 ml. washes of ethanol, to ground glass stoppered volumetric flasks through Whatman No. 42 filter papers. Liver cholesterol fractions were diluted to 50 ml. and the cholesterol fractions of the other three tissues to 100 ml., and set aside for combustion and colorimetric determination of cholesterol content.

The fatty acid fractions were evaporated over hot water, and a fine air stream was directed into the flasks to remove the transferring solvent. The last traces of acetone were removed in from one to two hours, depending on the amount of lipid present, and then the flasks were placed in a vacuum desiccator over night. The following day the flasks were weighed to determine the fatty acid.

I. Lipid Combustion

The technique of wet combustion of non-volatile lipid samples described by Hutchens[25] was used. Apparatus developed in this laboratory for this wet combustion has been described. Measured amounts of lipids were placed in the combustion arm and 5 ml. Van Slyke-Folch Solution* were delivered into the oxidant arm of the combustion flask, which was then attached to one arm of a two-arm vacuum evacuation system. 10 mg. of fatty acid were weighed in small tared porcelain boats which were

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* 167 ml. Conc. H_3PO_4
25 ml. CrO_3 anh.
333 ml. fuming H_2SO_4
20% free SO_3)
heated to 150° C. and cooled.

placed directly into the combustion arm. Samples containing about 76 mg. of cholesterol were pipetted into the combustion arm of other combustion flasks and evaporated to dryness in a hot water bath with an air stream. Combustion flasks containing the sterols then were dried for one hour in a 110° C. oven to remove the last traces of water before combustion.

Six ml. 0.5 N NaOH were placed in absorption flasks and attached to the other arm of the Y tube assembly. The system was evacuated to 15-20 mm. Hg by a water pump and then closed off. The combustion flask was inverted by rotation, allowing the combustion mixture to come in contact with the lipid. When most of the intense bubbling had subsided, the combustion arm was immersed in a 160° wax bath, and an ice bath was placed around the absorption flask. The combustion was complete in 15-20 minutes when the mercury in the manometer arm rose again to its original mark, indicating that no more CO_2 was being evolved from the mixture and that the CO_2 had been completely distilled over into the alkali. The system was removed from the hot wax bath and allowed to cool for about 5 minutes before the vacuum was released. The absorption flasks were removed from the system, and the contents quantitatively transferred to 40 ml. pyrex centrifuge tubes for precipitation of the $CO_3^{=}$ with Ba^{++}. A few drops of phenolphthalein added to the last transfer wash indicated complete transfer of alkali. Duplicate samples were combusted for each lipid fraction of each tissue.

J. Plating Technique

Rubber caps were placed on the centrifuge tubes, which were then heated to 60° C. in a water bath and allowed to cool to 45-50° C. for

the precipitation. 3-5 ml. of 0.25 M $BaCl_2$ in 0.4 N NH_4Cl were added at this temperature because the fine precipitate formed is best for triturating and washing. After cooling, the precipitated solution was layered with a few milliliters of a 1:3 ether-alcohol solution to lower the surface tension and prevent the precipitate from creeping up the sides of the tube. After centrifugation at 3000 RPM, the supernatant fluid was aspirated and the $BaCO_3$ was triturated with a glass rod and washed thoroughly with ca. 30 ml. distilled water added in small portions. Three such water washings were used with centrifugation. A final washing with 30 ml. of the same ether-alcohol solution was used to dehydrate the $BaCO_3$ precipitate. After the final centrifugation, the supernatant was partially aspirated. The small portion of solvent remaining was used to make a paste of the precipitate.

This paste and several ether-alcohol washings of the tube were transferred with a 3 ml. blunt end pipette to plating cups[26]. These consisted of duraluminum cups with drain holes in the bottom. Two round filter papers beneath a tared aluminum planchette were placed in the bottom of a cup and an inner aluminum sleeve was placed inside the cup and held in place with three thumb screws. The inside diameter of the sleeve was such that the cross-sectional area was 5.0 square centimeters, allowing the precipitate to cover only this area of the tared disc. After the $BaCO_3$ had been quantitatively transferred to the plating cups, the cups were placed in the 250 ml. brass carriers of the International centrifuge and centrifuged at 2000 RPM for 5 minutes. The remaining supernatant was carefully aspirated, and the cups were then centrifuged again at 2000 RPM for 30 minutes and dried with the aid of a heating

element inside the centrifuge. The mounted samples were removed from the plating cups after they had cooled to room temperature and were weighed in preparation for counting.

Duplicate determinations of the amount and radioactivity of the expired CO_2 were made in the following manner. The total CO_2 pool, from flask H (figure 1, page 18) was diluted to 500 ml. in a volumetric flask. 5.0 and 10.0 ml. samples were placed in 40 ml. centrifuge tubes and precipitated and plated as described above.

K. Radioassay

Low activity $BaCO_3$ samples derived from the lipid combustions were counted in a gas flow counter. Q gas (0.4% Butane in Helium) flowed through the windowless counting chamber across which was an applied potential differential of 1450-1500 volts. A pre-count Scaler (Nuclear Model 183) was used to count a total of 6400 counts, which is statistically that number needed to produce a counting error of not greater than 3%[27].

Samples of higher activity from the CO_2 pool and from the acetate assay were counted by an end window self-quenching G-M tube (Tracerlab 1.4 mg./cm^2. mica window) housed in a thick lead chamber. The pre-time scaler used was the Nuclear Model 165 at a voltage of 1500.

The background counts and the counts of a standard source of C^{14} were determined each time the counters were used. Background on the gas flow counter varied from 25 to 30 c.p.m., while the background on the end window counter was 24 to 28 c.p.m. The standard C^{14} source counted 1280-1330 c.p.m. on Q gas and with the end window tube it counted 575-600 c.p.m., with a Q gas to end window ratio on the average of 3.60-3.70.

L. Acetate Assay

The sodium acetate-1-C^{14} used for injection was assayed by placing two or four 0.5 ml. (0.05 μc) samples of the 1:200 dilution of a one ml. injection (0.020 millicurie) in the combustion arms of the combustion flasks. Two drops of concentrated NaOH (18 N) were added to each acetate solution. The solutions were evaporated to dryness by directing a fine CO_2-free air stream onto the surface and placing the flasks in the 160° C. wax bath. To maximally dry the acetate, the heating and air stream were continued for 5-6 hours. If the samples contained much absorbed water, it was found to decrease the final yield obtained from the Van Slyke wet combustion. 8-10 cc. of combustion solution were placed in the combustion arm. The same combustion procedure was used as described above, with the exception that the combustion solution was added all at once and the combustion flasks were at the same time plunged into the hot wax bath and left undisturbed for 20 minutes. 1.5 ml. of carrier 0.5 M Na_2CO_3 were added to the centrifuge tubes before precipitating, plating, and counting as above. Triplicate assay counts of each sample were made on the Q gas counter and on the first and second shelves of the end window counter. The result was recorded as the number of counts per minute per milliliter of injectable dose. Reproducible high values were difficult to attain even though the procedure was carefully followed, necessitating as many as 8 combustions to obtain satisfactory results. These values checked with values obtained using the persulfate method[28] of combusting aqueous samples (these combustions were done by Mr. R. J. Emerson in this laboratory). Assay values ranged from 2.3 to 2.7 x 10^6 c./m./ml. (Q gas counts)

and 1.00 to 1.12 x 10^6 c./m./ml. (end window counts) for the five acetate batches used in these experiments.

M. Colorimetric Cholesterol Determination

A modified Schoenheimer-Sperry method of cholesterol determination was used. The determination was done as follows: 0.1-0.4 mg. samples of the alcohol solutions of the non-saponifiable fractions were pipetted into Coleman cuvettes. The alcohol was evaporated with the use of a fine air stream while the cuvettes were immersed in a hot water bath. Samples were analyzed in quadruplicate (duplicate samples at two levels of concentration, and simultaneous standards were always run). All samples were placed for one hour in a 110° C. drying oven to remove the last traces of adsorbed water. 2.0 ml. glacial acetic acid from a 50 ml. burette were added to the hot tubes just as they were removed from the drying oven. After cooling to room temperature, 4.0 ml. acetic anhydride-concentrated sulfuric acid (20:1) reagent were added to each tube, and to a blank containing 2 ml. acetic acid, by rapidly blowing this solution into the sterol solution to promote mixing. Each tube was placed in a dark, 25° C. water bath for 40 minutes before being read in a Coleman junior colorimeter at 640 mu. After the optical densities of the standard cholesterol and the tissue cholesterols had been read, the amount of cholesterol present in each tissue was calculated from the optical densities given by the standard cholesterol (0.25 mg. gave an O.D. at 640 mu of 0.27, and 0.5 mg. gave an O.D. of 0.54.).

N. Quantitative Liver Glycogen Procedure

The method of Good, Kramer and Somogyi[29] for quantitative tissue glycogen determination was slightly modified. Duplicate 0.5 gm. fresh

liver samples were placed in stoppered, tared 6" x 1" test tubes which contained 2 ml. 30% KOH. The liver sample weights were determined by re-weighing the tared tubes. These tubes were placed in a boiling water bath for 30 minutes to allow complete dissolution of the liver tissue. The glycogen was precipitated by adding 1.1 to 1.2 volumes 95% ethanol, and the solutions were again brought to a boil to aid flocculation of the glycogen. Complete precipitation was allowed to occur by standing over night. Next the tubes were centrifuged 15 minutes at 2000 RPM and the supernatant drained. The precipitate was washed once with 5-10 ml. 60-40 alcohol in water, recentrifuged, and the supernatant again drained. The glycogen was next dissolved in 5 ml. 1 N H_2SO_4 and hydrolyzed for 2-1/2 hours in a boiling water bath. After completion of the acid hydrolysis, the glucose solution was neutralized to phenol red (2 drops) by adding 30% KOH (ca. 1.1 ml.) drop-wise. This neutralized solution was transferred quantitatively to 50 or 100 ml. volumetric flasks, the size depending upon the amount of glycogen present which was estimated at the time of precipitation. After dilution to the mark, these solutions were filtered into beakers from which 5 ml. aliquots were taken for the quantitative glucose determination.

The glucose was determined using Somogyi's Reagent for Iodometric Titration[30]. 5 ml. glucose solution, with 5 ml. of this reagent, were placed in 8" x 1" test tubes, covered with large marbles, and heated in a boiling water bath for exactly 15 minutes. Duplicate samples of each liver glycogen sample, duplicate 5 ml. water blanks, and duplicate 5 ml. standard glucose solutions (10 mg. % glucose in distilled water, freshly prepared each time) were analyzed. After the glucose had been oxidized,

the tubes were removed from the boiling water bath and 2 ml. 2.5% $K_2C_2O_4$ were added to form CuC_2O_4 after the addition of acid. 5 ml. 1 N H_2SO_4 were added rapidly to each tube by forceful ejection from a 5 ml. syringe. This allowed the liberation of iodine from the KIO_3 and KI present, part of which reoxidized the cuprous ions formed in the glucose oxidation. The remaining free iodine was titrated with 0.005 N $Na_2S_2O_3$ (prepared each time from stock 0.1 N $Na_2S_2O_3$) to a starch end point. The volume of 0.005 N $Na_2S_2O_3$ was then directly proportional to the amount of free I_2 present and inversely proportional to the amount of glucose originally present. This relationship was used to calculate the amount of glucose in each tube. The results were reported as total mg. glucose in the liver, and the grams glucose per 100 grams of liver.

O. Calculations

The figure "percent incorporation" used in this laboratory is the percent of the injected counts found in the lipids of each tissue and in the expired CO_2. The counts per minute obtained by counting the $BaCO_3$ samples of combusted lipid, or from expired CO_2, were used in calculating the amount of labeled acetate incorporated into the lipids. The total plate count is determined by dividing the c.p.m. (corrected by subtracting the background activity, and corrected to infinite thickness[31] and for coincidence counts[32] when necessary) by 100 mg., (which is infinite thickness for $BaCO_3$ absorption) and multiplying by the plate weight in milligrams. The total plate count is divided by the mg. of lipid combusted to give a figure for the specific activity (c.p.m./mg. lipid or c.p.m./mM CO_2). The specific activity multiplied by the total amount of

lipid gives the total activity in each lipid fraction, and this figure divided by the injected counts (determined from the acetate assay) times 100 gives the % incorporation. The results to follow are given in mg. lipid per gm. tissue, specific activity, and % incorporation.

IV. RESULTS AND DISCUSSION

A. Fasting Experiments

From experiments on the metabolism of acetate-1-C^{14} injected in tracer amounts, we know that approximately 80% of the label is found in the expired carbon dioxide four hours later. The remaining C^{14} is detected at this time in the body lipids, with minute amounts being found in urea, glycogen, and steroids. Following injection and absorption, the labeled acetate is rapidly activated and mixes in the tissues with unlabeled acetate (as acetyl CoA). Within seconds, radioactive CO_2 begins to appear in expired air; and excretion of CO_2 containing the maximum C^{14} concentration occurs in from 7 to 15 minutes following the injection. Maximum acetate-1-C^{14} oxidation has been determined to occur approximately 30 minutes following intraperitoneal injection[33]. The time at which maximum C^{14} activity accumulated in fatty acids and cholesterol was determined by Van Bruggen et al.[34] to be 20 minutes. Following synthesis of labeled lipids by the tissues, appreciable degradation of the labeled compounds did not occur during the four hour experiments.

The radio-activity appearing in the respiratory carbon dioxide is partially indicative of the amount of tricarboxylic acid cycle oxidation occurring during this period. One of the greatest influences upon oxidative processes, other than direct poisoning or anoxia, is the state of nutrition of the animal prior to the time of the CO_2 formation.

Decreased oxidation occurs during fasting due mainly to a lack of dietary foodstuffs, largely carbohydrates, which give the readily combustible materials for the energy needed for sustenance of life. To

supply this energy, first the carbohydrate stores are rapidly depleted. With continued fasting, the body protein and fat stores are mobilized and utilized for the potential energy derivable from them.

1. Body Weight Changes

In Table II are presented the body weight changes which occurred due to fasting the rats. No weight changes were observed during the first 9 hours of fasting, and the first slight weight decrease was observed 13 hours following withdrawal of food. Noticeable decreases, i.e., over 10%, occurred with a fast of only 19 hours. By four days the rats had lost 20% of their body weight. Extending the fast to 7 days produced a 26% loss of body weight; and by the end of a 10 day fast 36%, or a little over one-third, of the body weight had been consumed to supply basic energy requirements during this time period. These values agree with those found in the literature[35,36,37].

2. Lipid Composition for Whole Rats

Although body weight decreases at the rate described above, the depletion of liver glycogen and body fat is much more rapid. Relative fatty acid, cholesterol and liver glycogen concentrations are seen in Table III. The mg. fatty acid or cholesterol isolated from each tissue fraction were totaled and compared to the total tissue weights determined at time of dissection for each fast level. During the fasting phase up to 13 hours, a slight increase in relative fatty acid concentration is seen; this is likely a reflection of the loss of non-lipid components such as carbohydrate stores, feces, and renal and extrarenal fluids. During more prolonged fasting, the fatty acid reserves are depleted faster than

Table II

RAT BODY WEIGHT CHANGES WITH FASTING

Animal	Fast(hrs)	Weight before Fast (grams)	Weight after Fast (grams)	Weight Loss (grams)	% Weight Loss
N-7	1	207	207	0	0
N-8	1	205	205	0	0
N-10	1	240	240	0	0
N-12	1	210	210	0	0
N-13	1	223	223	0	0
N-14	1	220	220	0	0
Average				0	0
CF-7	3	209	209	0	0
CF-10	3	230	230	0	0
Average				0	0
CF-11	5	231	231	0	0
CF-13	5	240	240	0	0
CF-14	5	225	225	0	0
Average				0	0
CF-18	7	221	221	0	0
CF-17	9	238	242	4 (gain)	0
Average				0	0
CF-15	13	246	235	11	4.5%
CF-16	13	230	234	4 (gain)	0
Average				6	2.5%
CF-8	19	209	187	22	10.5%
CF-9	19	212	192	20	9.4
CF-12	20	237	214	23	10.3
Average				21.7	10.1%
CF-1	96	256	202	54	21 %
CF-2	96	250	201	51	20
Average				52.5	20.5%
CF-3	168	283	210	73	26 %
CF-4	168	286	212	74	26
Average				74	26 %
CF-5	240	326	210	116	35.6%
CF-6	240	317	198	129	37.5
Average				122.5	36.5%

Table III

TOTAL TISSUE LIPID COMPOSITION AND LIVER GLYCOGEN CONTENT FOR FASTED RATS

Animal	Fast (hr)	Total mg F.A. per gram Animal	% of A‡	Total mg Chol. per gram Animal	% of B†	Liver Glycogen as gm. Glucose per 100 gm. Liver	% of C‡
N-7	1	81.38		1.50		-	
N-8	1	70.94		2.02		-	
N-10	1	82.80		1.56		3.03	
N-12	1	86.78		1.92		3.40	
N-13	1	93.46		1.90		-	
N-14	1	65.33		1.79		-	
Average		80.12	A= 100%	1.78	B = 100%	3.22	C = 100%
CF-7	3	71.88		2.18		-	
CF-10	3	74.88		2.14		-	
Average		73.38	91%	2.16	121%		
CF-11	5	95.47		2.00		-	
CF-13	5	84.78		1.96		-	
CF-14	5	96.89		2.02		-	
Average		92.38	114%	1.99	112%		
CF-18	7	102.80		2.05		-	
CF-17	9	104.84		1.72		-	
Average		103.82	129%	1.88	106%		
CF-15	13	90.40		1.81		-	
CF-16	13	139.82		1.68		-	
Average		115.11	143%	1.74	98%		
CF-8	19	45.57		2.37		-	
CF-9	19	47.63		2.33		-	
CF-12	20	78.02		2.16		-	
Average		57.07	71%	2.29	129%		
CF-1	96	67.17		2.18		.84	
CF-2	96	44.29		2.23		.97	
Average		55.73	69%	2.20	124%	.90	28%
CF-3	168	98.66		1.63		1.38	
CF-4	168	85.56		2.09		.82	
Average		92.11	114%	1.86	104%	1.10	34%
CF-5	240	48.93		2.34		-	
CF-6	240	11.24		2.58		-	
Average		30.08	37%	2.46	138%		

‡For animals fasted only one hour, fatty acid, cholesterol, and liver glycogen weight was arbitrarily set at 100%. These figures for animals fasted longer periods of time are compared on a percentage basis.

most other body constituents, so a decrease in seen (the 114% figure for the rats fasted 7 days is unexplainable).

Changes in relative cholesterol concentration are less apparent. A decline in blood cholesterol levels has been reported by Bose et al[38], and others in severely starved humans, but they gave no reports of tissue cholesterol levels. The free and esterified cholesterol levels in tissues were found to be unchanged in adult cats fasted for 7 days, according to Gardner and Lauder[39]. In the experiments reported in this thesis, the total amount of tissue cholesterol remained constant. The slight increase (over 20% from 19 hours to 240 hours of fasting) seen in the latter part of starvation, where the amount reported is relative to the body tissue weights, is a reflection of the constancy of total tissue cholesterol levels with decreasing tissue weights.

Liver glycogen values are reported here only for extended starvation and for the one hour time‡ following withdrawal of food. Hershey and Orr[40] (1928) reported a rapid fall in liver glycogen levels in rats fasted 24 hours. The minimal values attained were 0.5 to 1.0 gm. percent, which were about one-quarter the prefast levels. These findings led them to believe that glycogenesis was occurring from protein and fat sources even during the six day fast period. The liver glycogen values for the four and seven fasting day times seen in Table III are in agreement with these findings.

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‡Since the length of each experiment was 4 hours, the liver glycogen could be isolated only after this minimum time. The one hour post prandial rat is designated as such for the purposes of lipid labeling, so the one hour post cibal animals would be 5 hours post cibal with respect to glycogen collection.

3. Fatty Acids[‡]

a. Liver

The incorporation of labeled acetate into liver fatty acids of fasted rats is seen in Table IV. Liver fatty acids showed a slight increase in total weight when animals were fasted 13 hours before isolation of the lipid. Following this, slight decreases were noted during further starvation. The mg. fatty acid per gm. tissue ratio remained fairly constant during the entire fasting time. MacLachlan[41] in 1942 isolated liver fatty acids from starved mice and found that infiltration of fat (a histologic intracellular fat increase) occurred increasingly up to the second day, at which point an increase of 11.5% was found. Liver fat has been found by a number of other workers[42,43,44] to be increased with starvation. Treadwell et al[45], however, found that statistically no increase in liver lipids occurred in rats starved 36 hours. Our results are in accord with this latter observation.

Percent incorporation of labeled acetate into the fatty acids isolated from livers of fasted rats is indicative of the synthesis of these substances. The highest figure seen in these experiments (0.8%) occurred in rats which had been fasted only one hour. As fast periods were extended, the percent incorporation was found to decrease rapidly. Just 5 hours of fasting reduced liver fatty acid label incorporation to one-third of the highest amount, and 13 hours of fasting reduced this incorporation to one-quarter of the highest amount. These findings are not in accordance with those of Lyon, Masri, and Chaikoff[46], who tabulated the

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[‡]The term "lipogenesis" will apply to fatty acid synthesis only, and cholesterol synthesis will be designated by the term "cholesterologenesis".

Table IV

THE INCORPORATION OF LABELED ACETATE INTO LIVER FATTY ACIDS IN FASTED RATS

Animal	Fast (hr)	Gm. F.A.	Mg. F.A. per gm. Liver	% of A*	Specific Activity c.p.m/mg	% of B*	% Incorporation	% of C*
N-7	1	.089	33.98		136.40		1.500	
N-8	1	.278	38.40		81.00		.940	
N-10	1	.318	34.30		32.30		.446	
N-12	1	.334	32.31		63.42		.922	
N-13	1	.338	30.08		38.20		.576	
N-14	1	.277	33.69		40.50		.416	
Average			33.79	A= 100%	65.30	B=100%	.800	C= 100%
CF-7	3	.351	39.04		59.40		.772	
CF-10	3	.301	33.67		52.10		.580	
Average			36.36	107%	55.75	85%	.676	84%
CF-11	5	.289	35.91		23.05		.247	
CF-13	5	.294	27.99		25.79		.281	
CF-14	5	.284	29.54		31.22		.328	
Average			31.15	92%	26.68	41%	.285	36%
CF-18	7	.323	34.09		36.12		.506	
CF-17	9	.338	33.22		9.56		.140	
Average			33.66	99%	22.84	35%	.323	40%
CF-15	13	.358	35.33		9.60		.506	
CF-16	13	.354	31.32		15.48		.238	
Average			33.32	98%	12.54	19%	.194	24%
CF-8	19	.276	34.49		5.70		.058	
CF-9	19	.234	36.19		9.61		.083	
CF-12	20	.220	29.10		4.76		.039	
Average			33.26	98%	6.69	10%	.060	8%
CF-1	96	.279	37.70		5.20		.063	
CF-2	96	.202	29.93		6.82		.060	
Average			33.82	100%	6.01	9%	.062	8%
CF-3	168	.209	34.75		5.32		.050	
CF-4	168	.203	38.72		7.98		.070	
Average			36.74	108%	6.65	10%	.060	8%
CF-5	240	.254	45.93		6.06		.067	
CF-6	240	.157	32.89		3.54		.023	
Average			39.41	116%	4.80	7%	.045	6%

*Using the average figures from the one hour fasted animals as 100%, data for different fast periods are compared on a percentage basis.

incorporation of labeled acetate into the fatty acid of isolated livers of rats fasted 0, 6, 12, and 18 hours. These investigators found maximum lipogenesis still occurring 6 hours post prandially. Minimal values were reached at the 18 hour fast level in their in-vitro experiments. As seen from Table IV, at the 19 hour fast level, lipogenesis was still detectable but minimal, as the 0.06% incorporation figure shows. Any further fasting produced no change in this low level of liver fatty acid label incorporation.

Specific activity data parallels the percent incorporation figures since there was no change in the relative lipid concentration.

b. Gut

In Table V is presented the data for fatty acids of gut tissue. Gut fatty acid concentrations paralleled the findings mentioned for the whole rat, a relative increase being noted with fasting up to 13 hours, which reflects fecal loss rather than decreasing gut fat content. Further fasting, however, did reduce the amount of fatty acids isolated from this tissue.

The incorporation of labeled acetate into the fatty acids of the gut fraction was at maximal levels (5.6% incorporation) during the short period of fasting, when food had been removed only one to three hours prior to injection of the labeled compound. Incorporations fell to one-third these levels when only two more hours of fasting were added. Minimal incorporation values were attained by only 13 hours fasting, and no essential difference occurred by extending the fasting to 10 days. Considerably more label incorporation was occurring in the gut tissue (5.6%) than in

Table V

THE INCORPORATION OF LABELED ACETATE INTO GUT FATTY ACIDS IN FASTED RATS

Animal	Fast (hr)	Gm. F.A.	Mg. F.A. per gm. Gut	% of A*	Specific Activity c.p.m/mg	% of B*	% Incorporation	% of C*
N-7	1	1.751	88.86		78.40		5.700	
N-8	1	1.433	74.10		125.20		7.500	
N-10	1	2.058	62.90		88.20		7.890	
N-12	1	1.214	48.96		162.44		8.576	
N-13	1	1.303	51.29		30.76		1.743	
N-14	1	1.0979	37.99		58.37		2.382	
Average			60.69	A= 100%	90.56	B=100%	5.632	C= 100%
CF-7	3	1.110	40.96		164.40		6.760	
CF-10	3	1.238	42.19		99.20		4.546	
Average			41.53	68%	131.80	145%	5.653	100%
CF-11	5	1.497	80.46		19.69		1.092	
CF-13	5	1.456	42.69		34.74		1.872	
CF-14	5	1.614	62.56		34.04		2.034	
Average			61.90	102%	29.49	32%	1.666	30%
CF-18	7	1.336	57.84		34.34		1.995	
CF-17	9	1.762	88.54		12.14		.930	
Average			73.19	120%	23.24	26%	1.462	26%
CF-15	13	1.317	68.93		15.50		.887	
CF-16	13	2.125	114.22		8.50		.786	
Average			91.58	150%	12.00	13%	.836	15%
CF-8	19	.658	41.94		36.82		.898	
CF-9	19	.556	35.42		47.68		.982	
CF-12	20	1.008	51.97		21.22		.792	
Average			43.11	71%	35.24	39%	.891	16%
CF-1	96	.836	67.38		25.30		.918	
CF-2	96	.670	44.10		35.60		1.037	
Average			55.74	91%	30.45	33%	.978	17%
CF-3	168	1.358	123.43		13.00		.767	
CF-4	168	.643	61.23		7.92		.222	
Average			92.33	151%	10.46	12%	.494	9%
CF-5	240	.476	45.55		46.30		.957	
CF-6	240	.193	14.32		48.56		.408	
Average			29.94	49%	47.43	52%	.682	12%

*Using the average figures from the one hour fasted animals as 100%, data for different fast periods are compared on a percentage basis.

the liver (0.8%) at the one hour fast level, and this difference was still noticed in the rats fasted 10 days when the incorporations had decreased to 0.7% and 0.04%, respectively.

Specific activity data show a similar pattern of decline with fasting, but in all tissues studied where animals were not fasted beyond 19 hours the specific activities were inversely proportional to the relative lipid concentrations. This might be expected when consideration is taken of the lipogenesis occurring in the four hours of the experiments. A tissue with a high lipid content from one animal may have a rate of lipogenesis similar to that of the same tissue of lower lipid content of another animal, under the same nutritional conditions; and the incorporated label will be diluted more in the tissue of higher lipid content resulting in a lower specific activity. Percent incorporation is a figure which stabilizes these differences and is therefore a much more reliable figure for use.

c. Carcass

Table VI shows the changing values in the carcass fatty acids occurring with fasting. The fatty acid concentration of this tissue is similar to the value obtained on the whole rat when fasted. Samuels and Conant[47] in 1944 reported on the fat content of muscle in fasted rats, and showed a slower decline in muscle fat content with fasting than occurred with liver fat of the same animal. Our figures are in agreement with their findings. Percent incorporation figures for carcass fatty acids were high in rats fasted one hour and even higher average values are seen in rats fasted three hours. Rats fasted five hours showed a decrease in

Table VI

INCORPORATION OF LABELED ACETATE INTO CARCASS FATTY ACIDS IN FASTED RATS

Animal	Fast (hr)	Gm. F.A.	Mg. F.A. per gm. Carcass	% of A*	Specific Activity c.p.m/mg	% of B*	% Incorporation	% of C*
N-7	1	9.022	68.33		15.30		5.750	
N-8	1	7.060	54.30		24.70		7.350	
N-10	1	9.481	64.40		11.70		4.810	
N-12	1	8.094	63.23		10.64		3.744	
N-13	1	10.799	80.95		7.96		3.735	
N-14	1	7.888	55.90		4.63		1.358	
Average			64.52	A=100%	12.49	B=100%	4.458	C=100%
CF-7	3	7.449	62.12		21.20		5.830	
CF-10	3	8.782	64.34		15.25		4.958	
Average			63.23	97%	18.22	146%	5.394	121%
CF-11	5	10.009	69.85		6.53		2.420	
CF-13	5	11.110	77.80		6.96		2.862	
CF-14	5	11.291	81.06		9.66		4.038	
Average			76.24	117%	7.72	62%	3.107	70%
CF-18	7	10.317	77.28		8.54		3.828	
CF-17	9	13.849	90.22		2.43		1.463	
Average			83.75	129%	5.48	44%	2.646	59%
CF-15	13	13.394	87.89		1.50		.873	
CF-16	13	16.388	112.79		1.76		1.258	
Average			100.34	155%	1.63	13%	1.066	24%
CF-8	19	4.437	37.66		3.48		.572	
CF-9	19	5.643	43.95		3.92		.819	
CF-12	20	8.705	63.36		2.42		.782	
Average			48.32	74%	3.27	26%	.724	16%
CF-1	96	8.701	61.27		1.68		.638	
CF-2	96	5.312	38.13		3.53		.815	
Average			49.70	77%	2.60	21%	.726	16%
CF-3	168	13.790	93.05		1.66		.995	
CF-4	168	11.161	75.82		1.82		.882	
Average			84.44	130%	1.74	14%	.938	21%
CF-5	240	7.224	48.03		1.74		.548	
CF-6	240	1.336	10.03		10.86		.631	
Average			29.03	45%	6.30	50%	.590	13%

*Using the average figures from the one hour fasted animals as 100%, data for different fast periods are compared on a percentage basis.

percent incorporation of label into fatty acids which was disproportionate to that seen in liver and gut tissue fatty acid incorporations. The decline in label incorporation during early fasting time periods is less precipitous in the carcass fatty acids, being 70% and 60% of the one hour fasted animals when animals were fasted 5, 7, and 9 hours, respectively. Low values were attained in animals fasted 19 hours before acetate-1-C^{14} injection. In the other tissues, lipogenesis was one-third of the maximum at 5 hours. This phenomenon is perhaps explainable by the fact that carcass tissues are mainly composed of muscle, which is an actively metabolising organ for energy and body heat production. The supply of food to this organ is chiefly vascular, and food is buffered over a longer period of time, due to the interposition of visceral organs where digestion occurs. We expect, then, a longer time of utilization of the products of digestion for fatty acid synthesis by this organ following a meal. The energy transferred from tricarboxylic acid cycle operation to fatty acid synthesis is perhaps contributory during this phase of metabolism.

d. Skin

The skin is an organ containing large storage depots of fat, and its function is that of protection and heat regulation. Though lipids are being transported to and deposited in this tissue, active lipogenesis is seen to occur in the skin as shown in Table VII. The pattern of tracer acetate incorporation is similar to that seen in the carcass tissue where high levels of incorporation occur in one hour post prandial rats, while rats fasted for increasing time periods show decreasing incorporation of

Table VII

THE INCORPORATION OF LABELED ACETATE INTO SKIN FATTY ACIDS IN FASTED RATS

Animal	Fast (hr)	Gm. F.A.	Mg. F.A. per gm. Skin	% of A*	S.A. c.p.m/mg	% of B*	% Incorporation	% of C*
N-7	1	4.644	144.20		4.30		.830	
N-8	1	4.488	144.90		3.80		.710	
N-10	1	6.921	184.50		5.60		1.660	
N-12	1	7.766	207.10		4.36		2.945	
N-13	1	6.961	184.68		3.80		1.152	
N-14	1	4.908	126.82		3.14		.572	
Average			165.37	A= 100%	4.17	B=100%	1.312	C= 100%
CF-7	3	4.662	142.12		8.46		1.460	
CF-10	3	5.465	151.79		4.30		.871	
Average			146.96	88%	6.38	153%	1.166	89%
CF-11	5	8.826	191.86		1.51		.494	
CF-13	5	6.274	163.82		2.48		.576	
CF-14	5	7.333	197.66		3.16		.856	
Average			184.45	111%	2.38	57%	.642	49%
CF-16	7	8.266	268.36		3.10		1.114	
CF-17	9	7.789	181.98		2.75		.932	
Average			225.17	135%	2.92	70%	1.023	78%
CF-15	13	4.693	126.85		1.89		.386	
CF-16	13	11.671	270.15		1.71		.866	
Average			198.50	119%	1.80	43%	.626	48%
CF-8	19	2.531	79.34		2.70		.253	
CF-9	19	2.112	73.33		2.78		.218	
CF-12	20	5.827	154.96		1.56		.336	
Average			102.54	62%	2.35	56%	.269	21%
CF-1	96	3.310	98.51		1.45		.209	
CF-2	96	2.256	76.99		2.00		.196	
Average			87.75	53%	1.72	41%	.202	15%
CF-3	168	4.624	123.98		.84		.167	
CF-4	168	5.439	132.66		1.11		.262	
Average			128.32	77%	.98	24%	.214	16%
CF-5	240	2.019	53.27		2.18		.192	
CF-6	240	.432	11.68		8.54		.160	
Average			32.48	19%	5.36	129%	.176	13%

*Using the average figures from the one hour fasted animals as 100%, data for different fast periods are compared on a percentage basis.

C^{14} acetate into fatty acids. The decrease seen is unlike that of liver and gut tissues which showed a precipitous decrease at the five hour fast level, but is like that of the carcass, which showed the delayed decline in percent incorporation up to the 13 hour fast level. In the skin fatty acids of rats fasted 13 hours, the percent incorporation was half the value obtained in one hour post cibal animals, and minimal levels of incorporation were encountered in animals fasted 19 to 240 hours.

If fat transport was occurring to depots in the skin in animals absorbing the standard meal for 3 to 9 hours before the tracer acetate injection, a relatively high amount of unlabeled fatty acids might be expected to be found in skin, and by simple dilution of the labeled fatty acids formed during the experiment on these animals, a low specific activity but high percent incorporation would be expected to result. The findings for specific activity and percent incorporation, however, do not agree with such reasoning, but lend further support to observations of other workers, e.g. Wick and Drury[48], that extrahepatic lipid synthesis is highly active. During the period of severe fasting, i.e., 7 to 10 days, the skin shows the greatest decrease in fatty acid content of any of the tissues studied. Tuorkischer and Wertheimer[49] have shown that lipogenesis occurs in fat depots of fasted rats. They depleted skin fat depots of rats by fasting, and the subsequent glycogen deposited in this tissue by a high carbohydrate diet was found to be converted in-vitro to fatty acids. Mirski[50] has also demonstrated the conversion of glycogen to fatty acids in-vitro in adipose tissue from rats starved to remove the fat content, then fed high CHO to deposit glycogen in the adipose tissue. He noted a rise in fatty acid concentration with decreasing glycogen levels in

incubated tissues. Concurrently a rise in R. Q. values from 0.7 to 1.0 was seen and is consistent with conversion of adipose tissue components to compounds of low O_2 content, namely fat.

Though fat was being removed by transport, direct oxidation in situ, or both, four times as much labeled acetate was still being incorporated into fatty acids of the skin of rats fasted 10 days than into liver fatty acids of the same animals. Skin contained from 10 to 15 times as much fatty acid as did liver, but incorporated only four times as much acetate C^{14}.

In general, the overall pattern of the percent incorporation of fatty acid of the four tissues was similar; the magnitudes, however, differed. The gut tissue was seen to be the most active, with about 5% of the acetate dose being incorporated into fatty acids in one hour post prandial animals. The carcass was the next most active lipogenic tissue, with percent incorporation figures for these same animals closely approximating the figures for gut. The tissue having the next lowest percent incorporation figures for fatty acid was the skin, which had values around 1%. The liver always had the lowest fatty acid percent incorporation figures (less than 1%).

4. Cholesterol

The percent of injected acetate dose found in tissue cholesterols of fasted animals was very small, never greater than 0.5% in any tissue of any animal, but the specific activities of the cholesterols were several times higher in most tissues than the fatty acid specific activities.

a. Liver

Data for liver cholesterol is presented in Table VIII. The milligrams of cholesterol per gram of liver were seen to increase as animals were fasted past 19 hours. Fasting up to this time produced no change in cholesterol concentration. A 60% fall in percent incorporation was noted in rats fasted 5 hours before the labeled acetate injection compared to rats fasted only one hour. Five hours post cibal is the time when the circulation to the liver is being enriched by absorption products of the previous meal, one of the constituents of which is cholesterol. As the amount of liver cholesterol is not increased in the 5 hour fasted rat and since both specific activity and percent incorporation are decreased, it is then assumed that the influx of preformed unlabeled cholesterol influenced the amount of new (labeled) cholesterol being formed. As this influence is increasingly less manifest at 7, 9, and 13 hours post cibal, a rise in percent incorporation for cholesterol is seen in the liver of these animals. After a fast period of 19 hours, however, the liver appears to be synthesizing even less cholesterol, and further fasting gives low but detectable levels of incorporation. Specific activity data are in line with the percent incorporation figures.

b. Gut

The cholesterol isolated from the gut tissue of fasted rats was seen to increase relative to the amount of tissue, as seen in Table IX. This increase is most noticeable in gut fractions of rats which had been fasted more extensively than 13 hours, where the mg. cholesterol per gm. tissue ratio was 1.5 to 2 times greater than that seen for animals fasted

Table VIII

THE INCORPORATION OF LABELED ACETATE INTO LIVER CHOLESTEROL IN FASTED RATS

Animal	Fast (hr)	Mg. Chol.	Mg. Chol. per gm. Liver	% of A*	S.A. c.p.m/mg	% of B*	% Incorporation	% of C*
N-7	1	15.30	1.94		130.50		.063	
N-8	1	15.95	2.20		233.80		.160	
N-10	1	36.20	2.80		125.50		.143	
N-12	1	17.46	1.69		240.00		.182	
N-13	1	21.15	1.88		133.80		.129	
N-14	1	19.65	2.39		264.80		.193	
Average			2.15	A= 100%	188.07	B= 100%	.147	C= 100%
CF-7	3	30.66	3.41		106.80		.121	
CF-10	3	22.14	2.48		109.80		.090	
Average			2.94	137%	108.30	57%	.106	72%
CF-11	5	19.73	2.45		60.71		.044	
CF-13	5	21.00	2.00		85.78		.066	
CF-14	5	19.47	2.03		92.04		.066	
Average			2.16	100%	79.51	42%	.059	40%
CF-18	7	20.28	2.14		104.20		.092	
CF-17	9	25.04	2.46		126.60		.138	
Average			2.30	107%	115.40	61%	.115	78%
CF-15	13	25.08	2.47		133.80		.146	
CF-16	13	25.05	2.22		181.70		.198	
Average			2.34	109%	157.75	84%	.172	117%
CF-8	19	26.97	3.37		61.80		.062	
CF-9	19	15.00	2.32		32.32		.018	
CF-12	20	20.80	2.75		59.58		.046	
Average			2.81	131%	51.23	27%	.042	29%
CF-1	96	24.60	3.32		7.95		.008	
CF-2	96	23.27	3.45		22.70		.023	
Average			3.38	157%	15.32	8%	.016	11%
CF-3	168	17.12	2.84		35.50		.026	
CF-4	168	24.49	4.67		12.66		.014	
Average			3.76	175%	24.08	13%	.020	14%
CF-5	240	21.39	3.87		14.72		.014	
CF-6	240	14.34	3.01		12.91		.008	
Average			3.44	160%	13.82	7%	.011	7%

*Using the average figures from the one hour fasted animals as 100%, data for different fast periods are compared on a percentage basis.

Table IX

THE INCORPORATION OF LABELED ACETATE INTO GUT CHOLESTEROL IN FASTED RATS

Animal	Fast (hr)	Mg. Chol.	Mg.Chol. per gm. Gut	% of A*	S.A. c.p.m/mg	% of B*	% Incorporation	% of C*
N-7	1	35.00	1.77		106.10		.160	
N-8	1	38.10	1.96		143.20		.230	
N-10	1	62.20	1.90		126.80		.343	
N-12	1	47.58	1.92		222.55		.460	
N-13	1	49.22	1.94		105.10		.224	
N-14	1	48.05	1.66		378.30		.676	
Average			1.86	A= 100%	180.35	B=100%	.349	C= 100%
CF-7	3	56.60	2.09		183.80		.385	
CF-10	3	53.58	1.82		158.70		.315	
Average			1.96	105%	171.25	94%	.350	100%
CF-11	5	43.72	2.35		88.70		.144	
CF-13	5	55.40	1.62		76.80		.158	
CF-14	5	51.65	2.00		109.40		.210	
Average			1.99	107%	91.63	50%	.171	49%
CF-18	7	51.45	2.23		106.70		.238	
CF-17	9	43.88	2.20		121.05		.231	
Average			2.22	119%	113.88	63%	.234	67%
CF-15	13	46.95	2.46		133.40		.272	
CF-16	13	44.18	2.38		132.25		.254	
Average			2.42	130%	132.82	73%	.263	75%
CF-8	19	51.22	3.26		138.50		.262	
CF-9	19	43.68	2.78		173.10		.280	
CF-12	20	54.12	2.79		122.90		.246	
Average			2.94	158%	144.83	80%	.263	75%
CF-1	96	47.50	3.83		8.18		.017	
CF-2	96	54.95	3.62		106.50		.254	
Average			3.72	200%	57.34	32%	.136	39%
CF-3	168	36.50	3.32		113.50		.180	
CF-4	168	37.90	3.61		133.01		.219	
Average			3.46	186%	123.26	68%	.200	57%
CF-5	240	31.02	3.10		192.46		.260	
CF-6	240	37.72	2.79		66.30		.108	
Average			2.94	158%	129.38	71%	.184	53%

*Using the average figures from the one hour fasted animals as 100%, data for different fast periods are compared on a percentage basis.

one hour. The fatty acids from these same tissues decreased as was shown above, so even though the fat was "dissolving" from these starved tissues, the cholesterol content remained fairly constant. Since the gut fraction represents the contents, as well as tissue, a change in cholesterol content might be expected when the bulk of feces had been expelled, but as cholesterol synthesis proceeds normally without the presence of food in the G.I. tract, and as cholesterol is excreted by and is constantly recycled through the bile, no sudden change in gut cholesterol occurred.

The pattern of labeled acetate incorporation into the gut cholesterol fraction of fasted animals is different from that into the fatty acid fraction, and also from liver cholesterol percent incorporation, in the latter periods of fasting. The same decreased percent incorporation into liver cholesterol of the rats fasted five hours is seen in gut cholesterol percent incorporation, and the same mechanism (increased preformed dietary cholesterol) is believed to operate in the gut tissue of these animals. Similarly, following the utilization of this dietary unlabeled cholesterol, the percent incorporation again is greater in animals fasted 7 to 9 hours and longer. Even the rats starved 7 and 10 days showed remarkably high levels of label incorporation (greater than 50% of that for the one hour fasted animals in this tissue) indicating that cholesterologenesis is far less influenced by fasting than is fatty acid synthesis. This is reasonable when one considers that the amounts of cholesterol in tissues is small and that the function of cholesterol, though largely unknown, seems entirely different from that of the fatty acids, but is probably more closely related to cell structure.

Table X

THE INCORPORATION OF LABELED ACETATE INTO CARCASS CHOLESTEROL IN FASTED RATS

Animal	Fast (hr)	Mg. Chol.	Mg.Chol. per gm.Carcass	% of A*	S.A. cpm./mg.	% of B*	% Incorporation	% of C*
N-7	1	154.50	1.25		18.20		.117	
N-8	1	212.35	1.62		28.50		.252	
N-10	1	168.60	1.14		26.10		.206	
N-12	1	195.06	1.52		29.50		.250	
N-13	1	190.50	1.43		23.40		.194	
N-14	1	177.50	1.26		28.46		.188	
Average			1.37	A= 100%	26.03	B= 100%	.201	C= 100%
CF-7	3	219.56	1.83		28.30		.230	
CF-10	3	228.55	1.67		15.48		.131	
Average			1.75	128%	21.89	84%	.180	90%
CF-11	5	208.19	1.45		12.78		.098	
CF-13	5	216.22	1.51		14.00		.112	
CF-14	5	213.15	1.53		18.55		.146	
Average			1.50	109%	15.11	58%	.119	59%
CF-18	7	169.12	1.42		22.70		.186	
CF-17	9	182.20	1.19		28.80		.228	
Average			1.30	95%	25.75	99%	.207	103%
CF-15	13	197.80	1.30		25.32		.218	
CF-16	13	172.40	1.19		32.12		.242	
Average			1.24	91%	28.72	110%	.230	114%
CF-8	19	225.75	1.92		18.58		.156	
CF-9	19	231.62	1.80		14.30		.122	
CF-12	20	218.18	1.59		20.87		.169	
Average			1.77	129%	17.92	69%	.149	74%
CF-1	96	207.40	1.46		15.05		.136	
CF-2	96	200.70	1.44		18.04		.157	
Average			1.45	106%	16.54	64%	.146	73%
CF-3	168	193.20	1.30		17.76		.150	
CF-4	168	210.95	1.43		17.34		.159	
Average			1.36	99%	17.55	67%	.154	77%
CF-5	240	278.75	1.85		14.55		.176	
CF-6	240	268.00	2.01		16.20		.188	
Average			1.93	141%	15.38	59%	.182	91%

*Using the average figures from the one hour fasted animals as 100%, data for different fast periods are compared on a percentage basis.

Table XI

THE INCORPORATION OF LABELED ACETATE INTO SKIN CHOLESTEROL IN FASTED RATS

Animal	Fast (hr)	Mg. Chol.	Mg.Chol.per gm. Skin	% of A*	S.A. c.p.m/mg	% of B*	% Incorporation	% of C*
N-7	1	85.50	2.65		14.10		.050	
N-8	1	115.20	3.70		15.90		.080	
N-10	1	97.40	2.60		22.50		.095	
N-12	1	125.68	3.35		19.31		.106	
N-13	1	134.80	3.57		17.51		.102	
N-14	1	143.42	3.71		17.68		.094	
Average			3.26	A= 100%	17.83	B= 100%	.088	C= 100%
CF-7	3	105.56	3.22		30.49		.119	
CF-10	3	146.50	4.07		10.77		.058	
Average			3.64	112%	20.62	115%	.088	100%
CF-11	5	160.17	3.48		12.00		.071	
CF-13	5	150.44	3.93		10.33		.058	
CF-14	5	144.62	3.90		14.08		.076	
Average			3.77	116%	12.14	68%	.068	77%
CF-18	7	142.25	4.62		15.05		.093	
CF-17	9	137.20	3.21		31.46		.188	
Average			3.92	120%	23.26	130%	.140	159%
CF-15	13	124.80	3.37		23.22		.126	
CF-16	13	124.40	2.88		35.79		.194	
Average			3.12	96%	29.50	165%	.160	182%
CF-8	19	106.80	3.35		20.14		.080	
CF-9	19	127.62	4.43		17.01		.080	
CF-12	20	143.19	3.81		21.63		.115	
Average			3.86	118%	19.59	110%	.092	105%
CF-1	96	146.80	4.37		10.73		.068	
CF-2	96	145.30	4.96		8.19		.052	
Average			4.66	143%	9.46	53%	.060	68%
CF-3	168	82.70	2.22		15.08		.054	
CF-4	168	153.50	3.74		14.09		.094	
Average			2.98	91%	14.58	82%	.074	84%
CF-5	240	145.81	3.85		11.37		.072	
CF-6	240	166.25	4.50		8.10		.059	
Average			4.18	128%	9.74	55%	.066	75%

*Using the average figures from the one hour fasted animals as 100%, data for different fast periods are compared on a percentage basis.

The gut was the tissue in which the highest percent incorporation occurred (0.35% for one hour post prandial rats), and was followed next in rank by the carcass with average values of 0.2% for the same animals. The liver incorporated even less label (0.15%), while the skin was least active (0.09%) in using C^{14} acetate for cholesterol synthesis.

5. CO_2 Data

Data for the expired carbon dioxide in rats subjected to fasting are presented in Table XII. The amount of CO_2 excreted by rats fasted short lengths of time, i.e., up to five hours, was quite constant. As the fasting time was lengthened, less CO_2 was produced relative to the whole rat weight, but the decrease seen was slight, being 15% of the value for the one hour post prandial rat in animals fasted 13 hours. Rats fasted 7 and 10 days excreted much less CO_2. With less fat and protein to be oxidized to CO_2 in these severely starved rats it is expected that the amount of CO_2 would be less.

Early in fasting, the CO_2 of the rats showed fairly constant specific activities. These data for rats extensively fasted (7 to 10 days) were found to be 1.5 times the one hour post prandial animals.

With the decreased CO_2 production and concomitant rise in specific activities seen in animals fasted a long time, the percent incorporation into CO_2 tended to remain fairly stable at any fasting level, but there is a rather wide variation in these percent incorporation figures. That the amount of label incorporated into CO_2 is unchanged with fasting indicates that the tricarboxylic acid cycle oxidation is functional, even after the withdrawal of all nutriment for 10 days. It would seem that the formation of acetyl Coenzyme A would therefore be unimpaired.

Table XII

THE INCORPORATION OF LABELED ACETATE INTO RESPIRATORY CARBON DIOXIDE IN FASTED RATS

Animal	Fast (hr)	mM CO_2	mM CO_2 per 100 gm. rat	% of A*	S.A. c.p.m/mM		% of B*	% Incorporation	% of C*
N-7	1	49.66	23.99		1.45	10^4		76.60	
N-8	1	48.86	23.83		1.56	"		81.40	
N-10	1	58.95	24.56		1.18	"		70.60	
N-12	1	50.48	24.04		1.42	"		71.86	
N-13	1	55.40	24.84		1.49	"		82.67	
N-14	1	60.32	27.42		0.92	"		49.47	
Average			24.78	A= 100%	1.34	"	B=100%	72.10	C= 100%
CF-7	3	50.97	24.39		1.44	10^4		63.00	
CF-10	3	59.19	25.73		1.08	"		55.30	
Average			25.06	100%	1.26	"	94%	59.15	82%
CF-11	5	65.06	26.18		1.13	10^4		63.40	
CF-13	5	53.47	22.28		1.43	"		65.80	
CF-14	5	55.42	24.63		1.72	"		82.00	
Average			25.02	100%	1.43	"	107%	70.40	97%
CF-16	7	46.06	20.84		1.64	10^4		75.60	
CF-17	9	57.22	23.64		0.97	"		55.70	
Average			22.24	89%	1.31	"	98%	65.65	91%
CF-15	13	59.44	25.29		1.50	10^4		89.60	
CF-16	13	51.08	21.83		1.46	"		74.40	
Average			23.56	94%	1.48	"	110%	82.00	113%
CF-8	19	40.15	21.47		1.67	10^4		57.80	
CF-9	19	41.36	21.54		2.11	"		75.30	
CF-12	20	45.10	21.07		2.07	"		80.50	
Average			21.36	85%	1.95	"	146%	71.20	98%
CF-1	96	47.50	23.51		1.79	10^4		85.95	
CF-2	96	-	-		-			-	
Average		47.50	23.51	94%	1.79	"	134%	85.95	119%
CF-3	168	39.64	18.88		2.00	10^4		80.00	
CF-4	168	-	-		-			-	
Average			18.88	75%	2.00	"	149%	80.00	110%
CF-5	240	34.06	16.22		2.06	10^4		71.11	
CF-6	240	-	-		-			-	
Average			16.22	65%	2.06	"	154%	71.11	98%

*Using the average figures from the one hour fasted animals as 100%, data for different fast periods are compared on a percentage basis.

Condensation of acetyl Co A units for fatty acid formation was not maintained to a similar degree, for within a few hours after eating, lipogenesis of some tissues was quite low. This finding might indicate that, even though energy from tricarboxylic acid cycle oxidation was available for fat synthesis, some other limiting factors were present. Besides the amount of energy needed for lipogenesis, which could be readily supplied by TCA cycle oxidation, C_2 fragments are needed. These are ordinarily supplied from carbohydrate sources, but in severely fasted rats the source is from catabolized fat, protein and, in some part, from carbohydrates formed anew from fats and proteins. That carbohydrate ingestion is a stimulant to the lipogenic process is well documented[51]. The degradation of carbohydrate not only may provide the two carbon units for fatty acid synthesis, but adds additional energy to the system by its passage through the Embden-Meyerhof scheme of carbohydrate breakdown before reaching the tricarboxylic acid cycle.

B. Refeeding Experiments

The contribution of ingested food to the restoration of lipogenic processes is a large one. The food given rats fasted for about three hours was Purina Laboratory Chow, composed of 60% CHO, 5% Fat, and 23% protein, with added essential vitamins and minerals. This proved to be a potent stimulator of fatty acid synthesis, as will be shown. The 13 hour fast level was selected for the period of fasting because it corresponds approximately to the amount of time elapsing between feedings in the trained feeding procedure. The rats which had been fed one-half hour or one hour should, therefore, correspond well to the animals which were one

hour post prandial. The recoverability of lipogenic processes from more extensive fasting was tested in single animals fasted 22 hours and 168 hours before refeeding was instituted.

1. Tissue Lipid Composition

Data for the lipid composition in the refed animals are shown in Table XIII. The amount of food consumed during refeeding was estimated to be 5-8 grams from the animal weights before and after feeding. The food consumed was consistent from rat to rat, although animals fed for one-half hour consumed less food than those fed one full hour.

The total milligrams fatty acid per gram combined rat tissue weights for rats fed one-half or one hour shows a slight increase above a similar figure for the one hour post prandial animals. This finding is unexpected, because of similar animal weights and because only one hour of fasting occurred in the latter group, during which time the only weight losses occurring would be due to feces and water loss. In comparison to animals fasted 13 hours, refeeding produces a fall in fatty acid concentration for the total animals refed one-half or one hour. This is explained by the fact that the lipid content of 13 hour fasted animals was higher, due to non-lipid losses, than that of the one hour post prandial animals. The fatty acid concentration in the bodies of rats fasted more extensively before refeeding was on a level similar to that of the one hour post prandial animals, but was less than that seen for the animals refed one-half or one hour.

The mg. cholesterol per gm. combined tissue weights for the refed animals were identical with those values seen in animals not refed. The

Table XIII

BODY WEIGHTS AND TOTAL TISSUE LIPID COMPOSITION
FOR REFED FASTED RATS

The percentage figures in this table are derived by comparing the average values listed below with values in Table III

Animal	Fast (hrs)	Body Weight after refeeding (grams)	Total Fatty Acid per gram animal mg.	%	Total Cholesterol per gram animal mg.	%
CF-19	13	211	94.16		1.84	
CF-22	13	226	91.58		1.87	
	Average of rats refed 1/2 hour		92.87		1.86	
	% of 1 hr. fast			116%		104%
	% of 13 hr. fast (not refed)			81%		107%
CF-21	13	249	80.68		1.93	
CF-23	13	236	108.17		1.55	
CF-24	13	236	114.71		1.71	
CF-26	13	198	82.17		1.93	
	Average of rats refed one hour		96.43		1.78	
	% of 1 hr. fast			120%		100%
	% of 13 hr. fast (not refed)			84%		102%
CF-20 (refed 1/2 hr.)	22	205	74.32		2.07	
	% of 1 hr. fast			93%		116%
	% of 19 hr. fast			130%		90%
	% of 13 hr. fast (refed 1/2 hr)			80%		111%
CF-25 (refed 1 hr.)	168	190	79.24		1.77	
	% of 1 hr. fast			99%		99%
	% of 168 hr. fast			86%		95%
	% of 13 hr. fast (not refed)			69%		102%
	% of 13 hr. fast (refed 1 hr.)			82%		99%

animals fasted 168 hours before refeeding for one hour likewise showed cholesterol concentrations equal to those of the latter group.

2. Fatty Acids

a. Liver

In Table XIV are shown the liver fatty acid data for the refed group. The incorporation of labeled acetate into the liver fatty acids of fasted animals refed one half hour was nearly three times as great as that into this compartment of animals fasted 13 hours but not given food. In comparison to the one hour post prandial animals the amount of label incorporated was two-thirds. Even greater increases in percent incorporation were seen as the refeeding time was extended to one hour, thereby allowing more time for absorption of the products of digestion. For the rat fasted 22 hours before refeeding, the percent incorporation was only one-half that observed in one hour post prandial animals, but was six to seven times greater than the value for liver fatty acid percent incorporation in animals fasted 19 hours. The liver of rats fasted 19 hours may be impaired in ability to form fatty acids, but the decreases seen might not be as evident if the refeeding time were extended. In the refed rat fasted 168 hours, however, percent incorporation figures for liver fatty acids remained low even after an hour of refeeding. This low level of incorporation (0.1%) is slightly increased (1.7 times) over the incorporation into liver fatty acid of the 168 hour fasted animals, but is only 17% of the radioactivity incorporated into liver fatty acid of animals refed one hour following a 13 hour fast. The liver is certainly impaired by extensive fasting in its ability to form fatty acids even after

Table XIV

THE INCORPORATION OF LABELED ACETATE INTO LIVER FATTY ACIDS IN REFED FASTED RATS

The percentage figures in this table are derived by comparing the average values listed below with values in Table IV.

Animal	Fast (hrs)	Grams F. A.	mg. F.A. per gm Liver		Specific Activity		Percent Incorporation	
CF-19	13	.2771	30.52		40.70		0.490	
CF-22	13	.3213	39.39		42.18		0.590	
Average of rats refed 1/2 hr.			34.96		41.44		0.540	
	% of 1 hr. fast			103%		63%		68%
	% of 13 hr. fast (not refed)			105%		330%		278%
CF-21	13	.3944	36.12		58.48		1.003	
CF-23	13	.3063	32.69		38.00		0.506	
CF-24	13	.2667	33.96		39.54		0.458	
CF-26	13	.2547	29.66		43.02		0.475	
Average of rats refed 1 hr.			33.11		44.76		0.610	
	% of 1 hr. fast			98%		69%		76%
	% of 13 hr. fast (not refed)			99%		356%		314%
CF-20 (refed 1/2 hr.)	22	.3112	31.98		30.26		0.410	
	% of 1 hr. fast			95%		46%		51%
	% of 19 hr. fast (not refed)			96%		452%		683%
	% of 13 hr. fast (refed 1/2 hr.)			91%		73%		76%
CF-25 (refed 1 hr.)	168	.827	33.60		12.95		0.102	
	% of 1 hr. fast			99%		20%		13%
	% of 168 hr. fast			91%		195%		170%
	% of 13 hr. fast (not refed)			101%		103%		53%
	% of 13 hr. fast (refed 1 hr.)			101%		29%		17%

refeeding. Just how the liver is injured by fasting is unknown, but sluggish responses such as this would imply liver damage, or certainly decreased metabolic activity. Decreased capacity for the synthesis of enzymes necessary in metabolic processes would be expected in the fasting liver, and this may be important in relation to lipogenesis.

It has been reported[52] that the livers of rats fasted seven days had a 40% decrease in liver protein, whereas the total body protein loss was only 8%. This might well explain a decreased synthesis of enzymes. Carbohydrate tolerance is also decreased during extensive fasting[53] and glucosuria with a high blood sugar is often seen. Other evidence of liver damage with fasting was suggested by Malmros[54], who found a positive urobilin reaction in the urine of fasted animals. If liver damage occurred with fasting, then a decreased percent incorporation of acetate into lipids would not be an unexpected finding. However, it is not suggested from this work that damage has occurred, only that impaired function has resulted from fasting.

b. Gut

The responses in the gut tissue following refeeding are tabulated in Table XV. In this tissue, immediate increases in acetate-1-C^{14} incorporation were noted in the fasted animals refed one-half hour, being equal to the one hour post prandial animals and seven times greater than 13 hour fasted animals. The animals refed one hour incorporated less acetate into the gut fatty acids than did animals refed only one-half hour, but the figures seen are not significantly different because of the wide variability of incorporation for fatty acid of this tissue of one

Table XV

THE INCORPORATION OF LABELED ACETATE INTO GUT FATTY ACIDS IN REFED FASTED RATS

The percentage figures in this table are derived by comparing the average values listed below with values in Table V.

Animal	Fast (hrs)	Grams F. A.	Mg. F.A. per gm Gut	Specific Activity	Percent Incorporation
CF-19	13	1.3623	52.20	97.39	5.770
CF-22	13	1.4706	55.29	93.30	5.965
Average of rats refed 1/2 hr.			53.74	95.34	5.868
	% of 1 hr. fast		89%	105%	104%
	% of 13 hr. fast (not refed		59%	79%	702%
CF-21	13	1.6939	47.85	78.34	5.770
CF-23	13	2.2741	77.09	27.00	2.669
CF-24	13	1.5929	59.22	51.60	3.573
CF-26	13	1.7844	60.28	36.31	2.815
Average of rats refed 1 hr.			61.11	48.31	3.707
	% of 1 hr. fast		101%	53%	66%
	% of 13 hr. fast (not refed)		67%	403%	443%
CF-20 (refed 1/2 hr.)	22	1.4912	63.19	71.40	4.628
	% of 1 hr. fast		104%	79%	82%
	% of 19 hr. fast (not refed)		147%	203%	519%
	% of 13 hr. fast (refed 1/2 hr.)		118%	75%	79%
CF-25 (refed 1 hr.)	168	0.7184	41.29	35.56	1.110
	% of 1 hr. fast		68%	39%	20%
	% of 168 hr. fast		45%	340%	225%
	% of 13 hr. fast (not refed)		45%	296%	133%
	% of 13 hr. fast (refed 1 hr.)		68%	74%	30%

hour post prandial rats. The animal fasted 22 hours before refeeding likewise showed high lipogenic activity in the gut. The gut tissue of the animal fasted 168 hours and then refed one hour, however, showed a poor response to the refeeding, as did the liver of this same animal; the percent incorporation figure for the gut was one-fifth that of the one hour post prandial animals. That lipogenesis increased somewhat because of refeeding is seen by comparing the percent incorporation in this tissue (1.11%) to that of the seven day fasted rat which received no refeeding (0.49%).

c. Carcass

Table XVI shows the data for carcass fatty acids from fasted, refed rats. Feeding for one-half hour or one hour brings a rapid increase in and restores to high values the amounts of C^{14} incorporated into fatty acids by the carcass of rats fasted 13 or 22 hours. As with the liver and gut fatty acids of the animal fasted 7 days before refeeding, the carcass showed a decreased capability of utilizing tracer acetate for fat synthesis, for in this tissue no increase was found upon refeeding.

d. Skin

The data for skin fatty acids of refed fasted animals are presented in Table XVII. Here, as with the carcass fatty acids, increases in percent incorporation to "normal" levels are seen by rats fasted 13 and 22 hours before refeeding one-half or one hour. The skin fatty acids of the rat subjected to 7 days fasting before being refed showed a lessened lipogenic response to food, as was noted for the other tissues of this animal.

Table XVI

THE INCORPORATION OF LABELED ACETATE INTO CARCASS FATTY ACIDS IN REFED FASTED RATS

The percentage figures in this table are derived by comparing the average values listed below with values in Table VI.

Animal	Fast (hrs)	Grams F. A.	Mg. F. A. per gm Carcass		Specific Activity		Percent Incorporation	
CF-19	13	10.3345	79.68		13.70		6.154	
CF-22	13	10.2772	77.92		15.22		6.798	
Average of rats refed 1/2 hr.			78.80		14.46		6.476	
	% of 1 hr. fast			122%		116%		145%
	% of 13 hr. fast (not refed)			79%		887%		894%
CF-21	13	10.0242	70.44		13.85		6.034	
CF-23	13	13.4545	92.28		8.02		4.690	
CF-24	13	12.4119	89.68		17.70		9.554	
CF-26	13	8.3049	82.88		6.22		2.240	
Average of rats refed 1 hr.			83.82		11.45		5.630	
	% of 1 hr. fast			130%		92%		126%
	% of 13 hr. fast (not refed)			84%		702%		528%
CF-20 (refed 1/2 hr.)	22	7.4709	59.62		13.28		4.312	
	% of 1 hr. fast			92%		106%		97%
	% of 19 hr. fast (not refed)			123%		406%		596%
	% of 13 hr. fast (refed 1/2 hr.)			76%		92%		65%
CF-25 (refed 1 hr.)	168	8.5708	69.06		2.66		0.989	
	% of 1 hr. fast			107%		21%		22%
	% of 168 hr. fast			82%		153%		105%
	% of 13 hr. fast (not refed)			69%		163%		93%
	% of 13 hr. fast (refed 1 hr)			82%		23%		18%

Table XVII

THE INCORPORATION OF LABELED ACETATE INTO SKIN FATTY ACIDS IN REFED FASTED RATS

The percentage figures in this table are derived by comparing the average values listed below with values in Table VII.

Animal	Fast (hrs)	mg. F. A.	Mg. F. A. per gm Skin		Specific Activity		Percent Incorporation	
CF-19	13	6.8299	196.26		6.22		1.848	
CF-22	13	6.6371	176.52		3.48		1.004	
Average of rats refed 1/2 hr.			186.39		4.85		1.426	
	% of 1 hr. fast			113%		116%		109%
	% of 13 hr. fast (not refed)			94%		269%		228%
CF-21	13	6.0993	164.40		4.72		1.250	
CF-23	13	8.1049	210.52		3.84		1.354	
CF-24	13	10.9351	234.66		3.88		1.844	
CF-26	13	4.6840	171.60		5.94		1.200	
Average of rats refed 1 hr.			195.30		4.60		1.412	
	% of 1 hr. fast			118%		110%		106%
	% of 13 hr. fast (not refed)			98%		256%		226%
CF-20 (refed 1/2 hr.	22	5.1754	144.56		4.84		1.088	
	% of 1 hr. fast			87%		116%		83%
	% of 19 hr. fast (not refed)			141%		206%		404%
	% of 13 hr. fast (refed 1/2 hr.)			78%		100%		76%
CF-25 (refed 1 hr.)	168	4.7472	146.07		1.52		0.312	
	% of 1 hr. fast			88%		36%		24%
	% of 168 hr. fast			114%		155%		146%
	% of 13 hr. fast (not refed-)			74%		84%		50%
	% of 13 hr. fast (refed 1 hr.)			75%		33%		22%

3. Considerations Regarding Prolonged Fasting

All the tissues of the 7 day fasted rat (CF-25) failed to show rises in percent incorporation of label into fatty acids following refeeding. Several reasons might be advanced to provide an explanation for this finding. Previous mention was made to possible injury to tissue cells which would impair metabolic function by such factors as membrane permeability with fluid imbalance and electrolyte shifts toward acidity, thus reducing enzymatic action. Decreased enzyme synthesis and concentration may be a factor. Another point to consider is the time involved in the digestion and absorption of food in the G.I. tract. If insufficient time elapsed between the eating of the food and the tracer acetate injection, then the absorbed products of digestion would enter the tissues after the labeled acetate had been acted upon, and the amount of label found in the fatty acids would be diluted by later formed fatty acid. The specific activities of the fatty acids of this animal were quite low in comparison to those of the one hour post prandial animal, but were somewhat higher than values for the fatty acids of the tissues of the non-refed 168 hour fasted animal, indicating that label incorporation was influenced to a slight degree by digestion products.

The G.I. tract in severely starved subjects has been shown[55] to undergo atrophic changes, and it was also shown that the digestive enzymes are reduced in amount due to generalized nitrogen depletion. Whether or not the ingested food was being digested as rapidly in the 168 hour fasted animal which was refed as in the animal fasted for a shorter time is also an important factor in considering whether or not absorption occurred in time for metabolites to influence oxidative processes in the tissues. The

amount of carbon dioxide produced by this refed 168 hour fasted animal was low, indicating that very little of the ingested food had been oxidized (See Table XXII.) This leads to the consideration that if the ingested food were assimilated and utilized, the energy produced and the carbon compounds present would be used for more important functions, such as reconstruction of nitrogenous cellular elements[56], heat production, and glycogen production, rather than fat synthesis and storage. Fat synthesis and storage would not be expected to be the most immediate readjustment mechanism in tissue rehabilitation. Again, the decreased CO_2 production from this animal shows that energy production had certainly not increased, and the percent incorporation and specific activity of labeled acetate into the expired CO_2 remained the same as in the non-fed 168 hour fasted animal. The activity of the adrenal cortical hormones, the thyroid hormone, and anterior pituitary hormones would show effects on the amount of CO_2 produced. The hypophysis has been shown[57] to decrease in weight and to have decreased hormone output in fasting. The secondary influences through the hypophyseal hormones upon the thyroid and adrenal glands produce decreasing hormone output by these glands which in turn would show low circulating hormone levels with fasting. Low thyroid hormone levels at the time metabolites are entering the system could explain to some degree the failure of metabolic activity to increase spontaneously.

It is felt that the main reason for the delayed metabolic response of this animal was slowed metabolism in general, and that if given time enough the food utilized could eventually restore metabolic activity to a higher level.

The stimulation of lipogenesis by refeeding is graphically illustrated in Figure 2. As was mentioned previously, refeeding provides a means of rapidly stimulating lipogenesis in tissues of rats subjected to periods of short fasting. That so large a stimulus to fat synthesis is provided by only one-half hour of refeeding is remarkable when consideration is taken of the time between initiation of eating and the final derivation of energy from the metabolic fuels contained in food. The liver appears to be the organ having the greatest lability in responding to the stimulus of refeeding.

4. Cholesterol

The incorporation of carboxyl-labeled acetate into the cholesterol found in the liver, gut, carcass, and skin of fasted refed animals is shown in Tables XVIII, XIX, XX, and XXI, respectively. Since the four tissues studied showed a similar cholesterologenic response to refeedings, the responses of the tissues will not be discussed singly.

The cholesterol concentration of each tissue of animals fasted 13 or 19 hours before refeeding was equal to that of the one hour post prandial animals. This was true also for the animal fasted 168 hours before refeeding. The percent of labeled acetate incorporated into the cholesterol of each tissue of animals fasted 13 hours and refed one-half hour was found to be 60 to 80% of that incorporated into the tissue cholesterols of one hour post cibal animals. Even less (50-60%) acetate was incorporated into the liver, carcass, and skin of these animals as compared to those 13 hour fasted animals which were not refed; while the percent incorporation figure for the gut cholesterol was 87% that in the 13 hour

Explanation of Figure 2.

Each curve represents the percent of acetate-1-C^{14} incorporated into tissue fatty acids under conditions of fasting and refeeding. Refeeding is represented by the area marked by diagonal lines in each graph: point A., animals refed ½ hour; point B., animals refed 1 hour.

The dotted line on each ordinate represents the previous 13 hour fast level; and the fasting time course of lipogenesis is shown following refeeding. The vertical lines represent the range of percent incorporation figures for each group of animals.

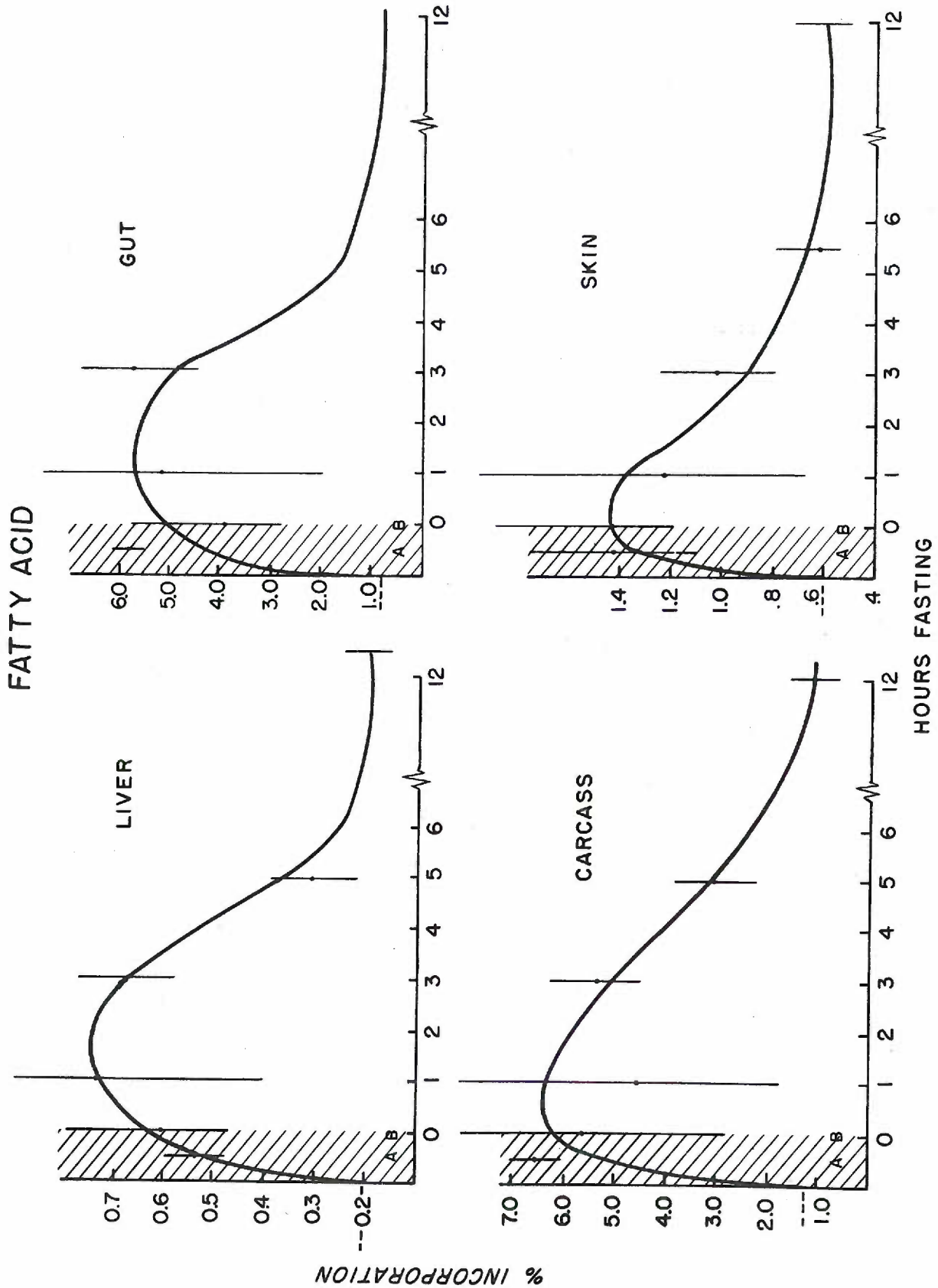
FATTY ACID
GUT
LIVER
SKIN
CARCASS
% INCORPORATION
HOURS FASTING
A
B

Figure 2.

Table XVIII

THE INCORPORATION OF LABELED ACETATE INTO LIVER CHOLESTEROL IN REFED FASTED RATS

The percentage figures in this table are derived by comparing the average values listed below with values in Table VIII.

Animal	Fast (hrs)	mg Chol.	mg. Chol. per gm Liver		Specific Activity		Percent Incorporation	
CF-19	13	18.51	2.04		91.80		.074	
CF-22	13	19.81	2.43		122.60		.106	
Average of rats refed 1/2 hr.			2.24		107.20		.090	
	% of 1 hr. fast			104%		57%		61%
	% of 13 hr. fast (not refed)			96%		68%		52%
CF-21	13	17.49	1.60		172.10		.130	
CF-23	13	19.85	2.12		133.26		.115	
CF-24	13	18.86	2.40		124.80		.102	
CF-26	13	16.50	1.92		226.30		.159	
Average of rats refed 1 hr.			2.01		164.12		.126	
	% of 1 hr. fast			93%		87%		86%
	% of 13 hr. fast (not refed)			86%		104%		73%
CF-20 (refed 1/2 hr.)	22	21.75	2.24		71.64		.078	
	% of 1 hr. fast			104%		38%		53%
	% of 19 hr. fast (not refed)			80%		140%		186%
	% of 13 hr. fast (refed 1/2 hr.)			100%		67%		87%
CF-25 (refed 1 hr.)	168	10.19	1.87		69.40		.030	
	% of 1 hr. fast			87%		37%		20%
	% of 168 hr. fast			50%		288%		150%
	% of 13 hr. fast (not refed)			80%		44%		17%
	% of 13 hr. fast (refed 1 hr.)			93%		42%		24%

Table XIX

THE INCORPORATION OF LABELED ACETATE INTO GUT CHOLESTEROL IN REFED FASTED RATS

The percentage figures in this table are derived by comparing the average values listed below with values in Table IX.

Animal	Fast (hrs)	mg. Chol.	mg. Chol. per gm Gut		Specific Activity		Percent Incorporation	
CF-19	13	47.82	1.83		99.89		.208	
CF-22	13	49.15	1.85		115.87		.248	
Average of rats refed 1/2 hr.			1.84		107.88		.228	
	% of 1 hr. fast			99%		60%		65%
	% of 13 hr. fast (not refed)			76%		81%		87%
CF-21	13	50.60	1.43		94.98		.209	
CF-23	13	48.05	1.63		83.36		.174	
CF-24	13	45.02	1.67		77.20		.151	
CF-26	13	49.15	1.66		240.65		.515	
Average of rats refed 1 hr.			1.60		124.05		.262	
	% of 1 hr. fast			86%		69%		75%
	% of 13 hr. fast (not refed)			66%		93%		100%
CF-20 (refed 1/2 hr.)	22	47.00	1.99		102.33		.209	
	% of 1 hr. fast			107%		57%		60%
	% of 19 hr. fast (not refed)			68%		71%		79%
	% of 13 hr. fast (refed 1/2 hr.)			108%		95%		92%
CF-25 (refed 1 hr.)	168	34.98	2.01		103.47		.158	
	% of 1 hr. fast			108%		57%		45%
	% of 168 hr. fast			58%		84%		79%
	% of 13 hr. fast (not refed)			83%		78%		60%
	% of 13 hr. fast (refed 1 hr.)			126%		83%		60%

Table XX

THE INCORPORATION OF LABELED ACETATE INTO CARCASS CHOLESTEROL IN REFED FASTED RATS

The percentage figures in this table are derived by comparing the average values listed below with values in Table X.

Animal	Fast (hrs)	mg. Chol.	mg. Chol. per gm Carcass		Specific Activity		Percent Incorporation	
CF-19	13	178.75	1.38		17.43		.135	
CF-22	13	198.12	1.50		18.22		.157	
Average of rats refed 1/2 hr.			1.44		17.82		.146	
	% of 1 hr. fast			105%		68%		73%
	% of 13 hr. fast (not refed)			116%		62%		63%
CF-21	13	239.60	1.68		15.56		.162	
CF-23	13	171.62	1.18		26.07		.194	
CF-24	13	148.83	1.08		22.36		.145	
CF-26	13	191.50	1.63		26.63		.222	
Average of rats refed 1 hr.			1.39		22.66		.181	
	% of 1 hr. fast			101%		87%		90%
	% of 13 hr. fast (not refed)			112%		79%		79%
CF-20 (refed 1/2 hr.)	22	197.25	1.57		17.22		.148	
	% of 1 hr. fast			115%		66%		74%
	% of 19 hr. fast (not refed)			89%		96%		99%
	% of 13 hr. fast (refed 1/2 hr.)			109%		97%		101%
CF-25 (refed 1 hrs.)	168	165.83	1.34		14.86		.107	
	% of 1 hr. fast			98%		57%		53%
	% of 168 hr. fast			99%		85%		69%
	% of 13 hr. fast (not refed)			108%		52%		47%
	% of 13 hr. fast (refed 1 hr.)			96%		66%		59%

Table XXI

THE INCORPORATION OF LABELED ACETATE INTO SKIN CHOLESTEROL IN REFED FASTED RATS

The percentage figures in this table are derived by comparing the average values listed below with values in Table XI.

Animal	Fast (hrs)	mg. Chol.	mg. Chol. per gm. Skin		Specific Activity		Percent Incorporation	
CF-19	13	122.25	3.51		15.60		.083	
CF-22	13	115.25	3.07		13.84		.070	
Average of rats refed 1/2 hr.			3.29		14.72		.076	
	% of 1 hr. fast			101%		83%		86%
	% of 13 hr. fast (not refed)			105%		50%		48%
CF-21	13	128.20	3.46		12.44		.069	
CF-23	13	107.08	2.78		26.40		.123	
CF-24	13	162.19	3.48		14.78		.104	
CF-26	13	96.50	3.53		26.53		.111	
Average of rats refed 1 hr.			3.31		20.04		.102	
	% of 1 hr. fast			102%		112%		116%
	% of 13 hr. fast (not refed)			106%		68%		64%
CF-20 (refed 1/2 hr.)	22	136.25	3.81		18.76		.111	
	% of 1 hr. fast			117%		105%		126%
	% of 19 hr. fast (not refed)			99%		96%		121%
	% of 13 hr. fast (refed 1/2 hr.)			116%		127%		146%
CF-25 (refed 1 hr.)	168	106.25	3.27		14.30		.066	
	% of 1 hr. fast			100%		80%		75%
	% of 168 hr. fast			110%		98%		89%
	% of 13 hr. fast (not refed)			105%		48%		41%
	% of 13 hr. fast (refed 1 hr.)			99%		71%		65%

post cibal animals. Higher percent incorporation figures were obtained for the tissue cholesterols of animals fasted 13 hours and refed for one hour than for the tissue cholesterols of animals refed only one-half hour but were less than the corresponding figures for the one hour post cibal animals.

The curves for the percent of labeled acetate incorporated into the cholesterol of the four tissues are shown in Figure 3. In the "basal state", i.e., 13 hours post prandial, C^{14} incorporation into cholesterols is high. When animals in the basal are fed one-half hour, this labeled acetate incorporation into the cholesterol of the tissues decreases. Cholesterol synthesis in the tissues of 5 hour post prandial animals reaches the lowest levels. As the influence of preformed dietary cholesterol diminishes, percent incorporation values rise again for the cholesterol of tissues of 13 hour post prandial animals. These changes are most noticeable in the gut tissue, which received the preformed dietary cholesterol, and is least noticeable in the skin, in which the least amount of label incorporation is occurring. The cholesterol of tissues of rats fasted 22 and 168 hours prior to refeeding showed less incorporation of C^{14}-labeled acetate than that of animals fasted 13 hours prior to refeeding. The liver cholesterol of the 168 hour fasted animal which was refed showed a percent incorporation figure greater than that seen for the 168 hour post prandial animals, but this response was not seen in the cholesterol of the gut, carcass, and skin fractions. The factors influencing the lack of response of fatty acid formation to the meal given this animal could well explain the impaired cholesterologenesis seen here.

Explanation of Figure 3.

Each curve represents the percent of acetate-1-C^{14} incorporated into tissue cholesterol under conditions of fasting and refeeding. Refeeding is represented by the area marked by diagonal lines in each graph: point A., animals refed ½ hour; point B., animals refed 1 hour.

The dotted line on each ordinate represents the previous 13 hour fast level; and the fasting time course of cholesterologenesis is shown following refeeding. The vertical lines represent the range of percent incorporation figures for each group of animals.

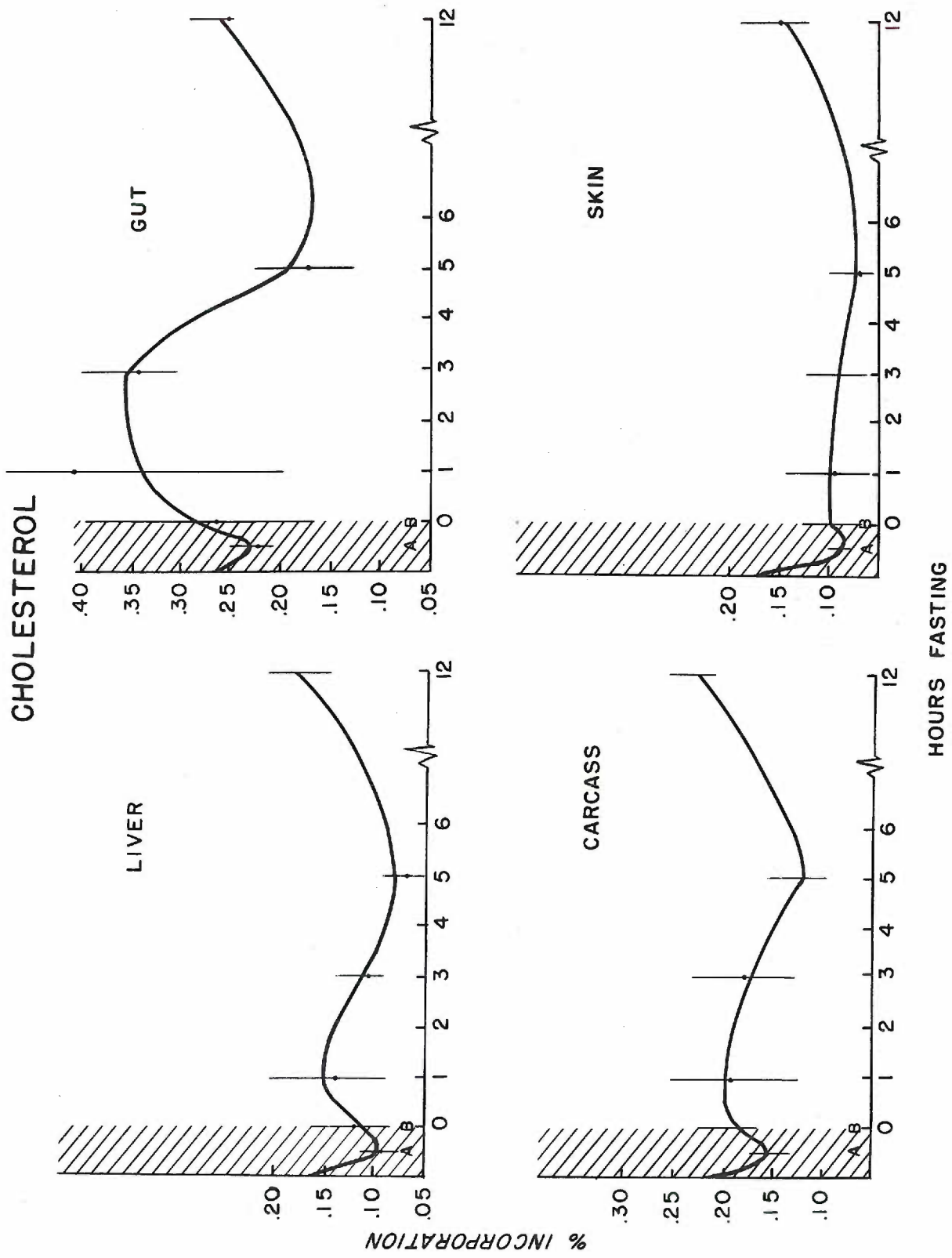
CHOLESTEROL
GUT
LIVER
SKIN
CARCASS
% INCORPORATION
HOURS FASTING
A
B

Figure 3.

5. CO_2 Data

Table XXII shows the carbon dioxide data for the refed group of fasted animals. The significance of the amount of and percent incorporation of labeled acetate into CO_2 of the animal fasted 168 hours prior to refeeding was noted earlier. The amount of CO_2 isolated from animals fasted 13 and 22 hours before refeeding was elevated over fasted animals that were not refed, which is an indication that extra sources of carbon (i.e., the meal) were being utilized. Slight decreases in the percent of labeled acetate incorporated into CO_2 of the 13 hour post cibal refed animals paralleled the increased labeling of fatty acids seen. Increased radioactivity in the CO_2 expired from the animal fasted 22 hours before refeeding is proportionate to the smaller amount of radioactivity in tissue fatty acids of this animal.

C. Refeeding with Carbohydrate Intermediate Compounds

That ingested food is a potent stimulator of the process of lipogenesis has been shown by others. The portion of the diet contributing most to this stimulation is carbohydrate[56,59]. The glycolytic breakdown of glucose has been implicated as the main source of energy for lipogenesis. Other metabolic fuels, i.e., protein and fat, have been shown not to stimulate lipogenesis of isolated livers of fasted rats[60].

A number of compounds naturally found in metabolic systems was given to rats, following which the in-vivo incorporation of labeled acetate into tissue fatty acids was measured. A base of comparison was established for the rats by fasting them nine to sixteen hours before the metabolites were administered, so as best to compare the lipogenic stimulatory ability of these compounds to that shown above by chow ingestion.

Table XXII

THE INCORPORATION OF LABELED ACETATE INTO RESPIRATORY CARBON DIOXIDE IN REFED FASTED RATS

Animal	Fast (hrs)	mM CO_2	mM CO_2 per 100 grams rat		Specific Activity (cts/min/mM)10^4		Percent Incorporation	
CF-19	13	65.82	31.19		1.19(10^4)		78.50	
CF-22	13	55.15	24.40		1.06		57.96	
Average of rats refed 1/2 hr.			27.80		1.12		68.23	
	% of 1 hr. fast*			112%		84%		95%
	% of 13 hr.fast (not refed)*			118%		76%		83%
CF-21	13	62.84	25.24		0.96		60.65	
CF-23	13	57.88	24.53		1.06		61.40	
CF-24	13	54.59	23.13		1.11		60.68	
CF-26	13	54.89	27.72		0.90		49.80	
Average of rats refed 1 hr.			25.16		1.01		58.12	
	% of 1 hr. fast*			102%		75%		81%
	% of 13 hr. fast (not refed)*			107%		68%		71%
CF-20 (refed 1/2 hr.)	22	58.70	28.63		1.38		81.20	
	% of 1 hr. fast*			116%		103%		113%
	% of 19 hr. fast (not refed)‡			134%		71%		114%
	% of 13 hr. fast (refed 1/2 hr.)			102%		123%		119%
CF-25 (refed 1 hrs.)	168	35.52	18.69		1.92		67.93	
	% of 1 hr. fast‡			75%		143%		94%
	% of 168 hr. fast*			99%		96%		85%
	% of 13 hr. fast (not refed)‡			79%		130%		83%
	% of 13 hr. fast (refed 1 hr.)			74%		190%		117%

‡The comparison is to data in Table XII.

In Table XXIII is shown the fatty acid, cholesterol, and liver glycogen concentrations of tissues of rats given carbohydrate intermediate compounds. As expected, the milligrams fatty acid per gram total animal of any of the rats in this group did not exceed comparable values for the 13 hour fasted, refed animals, and also the cholesterol concentrations were approximately the same for these two groups. The liver glycogen concentrations of animals given glucose or glycerol were equal to those of the one hour post prandial animals, while the animal given sodium citrate had a liver glycogen 70% of that average value. Animals given lactate, pyruvate, and succinate had lower liver glycogen concentrations than those of the refed animals.

1. Fatty Acid Data

In Tables XXIV, XXV, XXVI, and XXVII are tabulated the data for the fatty acids isolated from the four tissues of animals given carbohydrate intermediate compounds. Consistently less percent incorporation was noted in the skin fatty acids in comparison to the fatty acids of the other tissues of these animals. The formation of fatty acids in the skin was promptly stimulated by refeeding, but the low responses seen by giving these substances were undoubtedly influenced by concentration factors since the gut and liver are both interposed between the substrate and metabolic portions of the skin.

a. Glucose Administration

When glucose was given as a metabolite in place of chow to 14 hour fasted animals, the percentage injected labeled acetate incorporated into the tissue fatty acids was found to be only 1/2 to 1/3 the amount incorporated when chow was fed, so that in all tissues but skin only a

Table XXIII

BODY WEIGHTS, TOTAL TISSUE LIPID COMPOSITION, AND LIVER GLYCOGEN CONTENT IN FASTED RATS REFED CARBOHYDRATE INTERMEDIATE COMPOUNDS

Animal	Grams Body Wt.	Total mg. F.A. per gram Animal		Total mg. Chol. per gram Animal		Liver Glycogen as gms. Glucose per 100 grams Liver	
DF-1 (glucose)	219	57.80		1.93		-	
DF-2 (glucose)	221	52.97		1.96		-	
DF-11(glucose)	202	61.52		2.02		3.70	
Average		57.43		1.97		3.70	
% of 13 hr fast (not refed)*			50%		113%		
							115%***
% of 13 hr fast (refed ½ hr)**			62%		106%		
DF-6 (glycerol)	228	99.42		1.62		2.74	
DF-13(glycerol)	265	84.28		1.34		3.21	
Average		91.85		1.48		2.98	
% of 13 hr fast (not refed)*			80%		85%		
							93%***
% of 13 hr fast (refed ½ hr)**			99%		80%		
DF-4 (lactate)	234	81.06		1.62		0.08	
DF-9 (lactate)	207	59.93		2.15		1.80	
DF-12(lactate)	237	57.08		1.40		2.01	
Average		66.02		1.72		1.30	
% of 13 hr fast (not refed)*			57%		99%		
							40%***
% of 13 hr fast (refed ½ hr)**			71%		92%		
DF-5 (pyruvate)	225	82.15		1.67		0.05	
% of 13 hr fast (not refed)*			71%		96%		
							2%***
% of 13 hr fast (refed ½ hr)**			88%		90%		
DF-8 (succinate)	206	74.02		1.80		0.82	
% of 13 hr fast (not refed)*			64%		103%		
							25%***
% of 13 hr fast (refed ½ hr)**			80%		97%		
DF-7 (citrate)	215	83.49		1.64		2.26	
% of 13 hr fast (not refed)*			73%		94%		
							70%
% of 13 hr fast (refed ½ hr)**			90%		88%		

* Comparison is to data in Table III
** Comparison is to data in Table XIII
*** Comparison is to the 1 hr. fasted liver glycogen values in Table III

Table XXIV

THE INCORPORATION OF LABELED ACETATE INTO LIVER FATTY ACIDS OF FASTED RATS REFED CARBOHYDRATE INTERMEDIATE COMPOUNDS

Animal	Grams F. A.	mg F.A. per Gm. Liver	Specific Activity (cts/min/mg)	Percent Incorporation
DF-1 (glucose)	.2792	37.27	31.66	.328
DF-2 (glucose)	.2193	30.98	34.60	.282
DF-11 (glucose)	.3142	38.18	19.61	.225
Average		35.48	28.62	.278
% of 13 hr fast (not refed)*		106%	228%	143%
% of 13 hr fast (refed ½ hr)**		101%	69%	51%
DF-6 (glycerol)	.2721	36.40	12.76	.129
DF-13 (glycerol)	.3964	42.67	14.97	.216
Average		39.54	13.86	.172
% of 13 hr fast (not refed)*		119%	111%	89%
% of 13 hr fast (refed ½ hr)**		113%	33%	32%
DF-4 (lactate)	.3118	39.25	28.40	.277
DF-9 (lactate)	.3379	41.88	15.10	.186
DF-12 (lactate)	.3073	37.66	19.04	.214
Average		39.60	20.85	.226
% of 13 hr fast (not refed)*		119%	166%	116%
% of 13 hr fast (refed ½ hr)**		113%	50%	42%
DF-5 (pyruvate)	.2465	33.48	64.56	.592
% of 13 hr fast (not refed)*		100%	515%	305%
% of 13 hr fast (refed ½ hr)**		96%	156%	110%
DF-8 (succinate)	.3415	43.11	17.66	.224
% of 13 hr fast (not refed)*		129%	141%	115%
% of 13 hr fast (refed ½ hr)**		123%	43%	41%
DF-7 (citrate)	.3035	41.27	8.60	.097
% of 13 hr fast (not refed)*		124%	69%	50%
% of 13 hr fast (refed ½ hr)**		118%	21%	18%

* The comparison is to data in Table IV
** The comparison is to data in Table XIV

Table XXV

THE INCORPORATION OF LABELED ACETATE INTO GUT FATTY ACIDS OF FASTED RATS REFED CARBOHYDRATE INTERMEDIATE COMPOUNDS

Animal	Grams F. A.	mg F.A. per gm. Gut	Specific Activity (cts/min/mg)	Percent Incorporation
DF-1 (glucose)	.6799	43.03	104.98	2.654
DF-2 (glucose)	.9639	30.41	37.70	1.350
DF-11 (glucose)	.7388	44.37	79.13	2.134
Average		39.27	73.94	2.146
% of 13 hr fast (not refed)*		43%	616%	245%
% of 13 hr fast (refed ½ hr)**		73%	78%	35%
DF-6 (glycerol)	1.6454	79.11	14.55	.890
DF-13 (glycerol)	1.4092	70.46	22.89	1.177
Average		74.78	18.72	1.034
% of 13 hr fast (not refed)*		82%	156%	124%
% of 13 hr fast (refed ½ hr)**		139%	20%	18%
DF-4 (lactate)	1.5572	48.66	42.72	2.473
DF-9 (lactate)	.6730	21.57	82.84	2.034
DF-12 (lactate)	.8374	42.08	48.04	1.468
Average		37.44	57.87	1.992
% of 13 hr fast (not refed)*		41%	482%	238%
% of 13 hr fast (refed ½ hr)**		70%	61%	34%
DF-5 (pyruvate)	1.4585	42.52	51.03	21766
% of 13 hr fast (not refed)*		46%	425%	331%
% of 13 hr fast (refed ½ hr)**		79%	54%	47%
DF-8 (succinate)	1.0035	49.68	27.56	1.028
% of 13 hr fast (not refed)*		54%	230%	123%
% of 13 hr fast (refed ½ hr)**		92%	29%	18%
DF-7 (citrate)	.8133	45.18	26.60	.804
% of 13 hr fast (not refed)*		49%	222%	96%
% of 13 hr fast (refed ½ hr)**		84%	28%	14%

*The comparison is to data in Table V
**The comparison is to data in Table XV

Table XXVI

THE INCORPORATION OF LABELED ACETATE INTO CARCASS FATTY ACIDS OF FASTED RATS REFED CARBOHYDRATE INTERMEDIATE COMPOUNDS

Animal	Grams F. A.	mg F.A. per gm. Carc.		Specific Activity (cts/min/mg)		Percent Incorporation	
DF-1 (glucose)	7.2047	50.31		12.45		3.334	
DF-2 (glucose)	6.2192	45.73		9.86		2.280	
DF-11 (glucose)	6.0531	48.82		7.54		1.666	
Average		48.29		9.95		2.427	
% of 13 hr fast (not refed)*			48%		610%		228%
% of 13 hr fast (refed ½ hr)**			61%		69%		37%
DF-6 (glycerol)	12.4443	82.41		2.02		.936	
DF-13 (glycerol)	12.0891	72.35		3.32		1.465	
Average		77.38		2.67		1.200	
% of 13 hr fast (not refed)*			77%		164%		113%
% of 13 hr fast (refed ½ hr)**			98%		18%		19%
DF-4 (lactate)	11.2635	76.26		3.43		1.436	
DF-9 (lactate)	6.1331	52.29		3.85		.862	
DF-12 (lactate)	7.9014	50.44		8.24		2.375	
Average		59.66		5.17		1.558	
% of 13 hr fast (not refed)*			59%		317%		146%
% of 13 hr fast (refed ½ hr)**			76%		36%		24%
DF-5 (pyruvate)	10.5520	73.28		4.17		1.636	
% of 13 hr fast (not refed)*			73%		256%		153%
% of 13 hr fast (refed ½ hr)**			93%		29%		25%
DF-8 (succinate)	8.35.26	65.46		3.32		1.031	
% of 13 hr fast (not refed)*			65%		204%		97%
% of 13 hr fast (refed ½ hr)**			83%		23%		16%
DF-7 (citrate)	9.2181	68.95		1.98		.679	
% of 13 hr fast (not refed)*			69%		121%		64%
% of 13 hr fast (refed ½ hr)**			88%		14%		10%

* The Comparison is to data in Table VI
** The comparison is to data in Table XVI

Table XXVII

THE INCORPORATION OF LABELED ACETATE INTO SKIN FATTY ACIDS OF FASTED RATS REFED CARBOHYDRATE INTERMEDIATE COMPOUNDS

Animal	Grams F. A.	mg F.A. per gm.Skin		Specific Activity (cts/min/mg)		Percent Incorporation	
DF-1 (glucose)	3.7608	94.49		4.76		.665	
DF-2 (glucose)	3.5781	98.03		2.88		.384	
DF-11 (glucose)	4.1200	122.62		2.48		.378	
Average		105.05		3.37		.476	
% of 13 hr fast (not refed)*			53%		187%		76%
% of 13 hr fast (refed ½ hr)**			56%		69%		33%
DF-6 (glycerol)	7.2480	186.32		1.24		.334	
DF-13 (glycerol)	6.5011	142.57		1.25		.296	
Average		164.44		1.24		.315	
% of 13 hr fast (not refed)*			83%		69%		50%
% of 13 hr fast (refed ½ hr)**			88%		26%		22%
DF-4 (lactate)	5.0813	134.78		2.63		.497	
DF-9 (lactate)	4.3191	124.47		1.54		.244	
DF-12 (lactate)	3.6172	97.37		2.50		.331	
Average		118.87		2.22		.357	
% of 13 hr fast (not refed)*			60%		123%		57%
% of 13 hr fast (refed ½ hr)**			64%		46%		25%
DF-5 (pyruvate)	6.0054	161.00		2.38		.530	
% of 13 hr fast (not refed)*			81%		132%		85%
% of 13 hr fast (refed ½ hr)**			86%		49%		37%
DF-8 (succinate)	4.3973	126.72		2.13		.348	
% of 13 hr fast (not refed)*			64%		118%		56%
% of 13 hr fast (refed ½ hr)**			68%		44%		24%
DF-7 (citrate)	6.1330	160.55		1.69		.385	
% of 13 hr fast (not refed)*			81%		94%		62%
% of 13 hr fast (refed ½ hr)**			86%		35%		27%

* The comparison is to data in Table VII
** The comparison is to data in Table XVII

1.5 to 2 fold increase in label incorporation was noted above the values found for control animals fasted 13 hours. It is shown that while tissue lipogenesis has been stimulated to a considerable degree by the administration of 500 mg. glucose, the increases seen in the tissues were not comparable to those seen when chow was given. It is interesting to speculate about the quantitative nature of the stimulating effects of chow and of glucose. The animals refed chow might be assumed to have had available some 3000 mg. of carbohydrate (5 gm. chow x 60% CHO). The liver of the rat given glucose contained some 300 mg. glycogen (determined as glucose). This is 200 mg. more carbohydrate than found in the livers of fasted rats. If this 200 mg. are assumed to have come from the 500 mg. glucose fed, then only 300 mg. of glucose would have remained available for glycolytic reaction. The 300 mg. of glucose then produced an effect upon liver lipogenesis half that seen in the rat which theoretically had 3000 mg. of carbohydrate available. Larger doses of glucose might be expected to produce a marked increase in fatty acid synthesis, since it has been estimated[61] that 10 times as much glucose is used to form fatty acid as is used to synthesize glycogen.

b. Glycerol administration

Glycerol was given to animals in twice the dose (1 gram) used when glucose was given. The percent of C^{14} acetate incorporated into the fatty acids of liver, gut, and carcass of animals given glycerol was not materially different from that seen for these tissues of 13 hour fasted rats, and the skin percent incorporation was half that of the fasted group. These percent incorporation figures were only one-fifth to

one-third those of animals which were fasted 13 hours and refed chow for one half hour. The liver glycogen levels of the glycerol treated animals were high, which indicated that much of the absorbed glycerol had been deposited as liver glycogen. The absence of effect upon lipogenesis raises the question as to whether or not the glycerol given these animals was absorbed, but the high liver glycogen values indicate that absorption had occurred. Kortes[62] mentions that exogenous glycerol might be phosphorylated with greater difficulty than the glycerol formed from glucose catabolism. Spritas[63] studied acetate incorporation into fatty acid when glycerol was added to liver slices of starved rats, and found little incorporation of acetate into these liver fatty acids. Hirsch, Baruch and Chaikoff[64] studied the in-vitro incorporation of acetate-1-C^{14} into fatty acids of lactating rat mammary tissue as influenced by various substrates. Glycerol was found to cause low levels of acetate C^{14} incorporation into fatty acids and CO_2 in this tissue which, incidentally, does not form glycogen. Other studies (Tsang[65] et al) indicate that glycerol when given to liver slices of fasted rats is utilized primarily (71%) for glycogen and glucose synthesis and that only small amounts appear in CO_2 (5%), lactate (3%), and fatty acids (1%). The findings of a high liver glycogen level with low levels of acetate incorporation into tissue fatty acids in our in-vivo experiments cited above certainly corroborate these observations.

c. Lactate Administration

The percent incorporation of acetate-1-C^{14} into tissue fatty acids of fasted rats given lactate is shown to be increased over similar

values seen for fasted animals. The percent incorporations found were one to two times greater in the liver, gut and carcass fractions, but only half as great for the skin. The liver glycogen values for the rats given lactate were only 40% of the figure seen in the one hour post prandial animals. The lipogenic response given by lactate compared to chow is about 1/4 to 1/3, showing lactate and glucose have similar quantitative lipogenic abilities.

In the work of Boxer and Stetten[66], when lactate or glucose was incubated with liver slices of fasted rats, the amount of liver glycogen formed from lactate was four times that of the fasted controls, and the glycogen formed from glucose was 17 times greater than the control values. That liver glycogen is formed from lactate has been demonstrated by many investigators. This synthesis is considered an indirect means of restoring blood glucose during acute stresses[67]. Besides contributing to the formation of glycogen, lactate is known to be converted to fatty acids. It has been found that 40% of fed labeled lactate can be incorporated into body fat[68]. Other in-vitro work[69] has shown that labeled lactate is incorporated into liver fatty acids.

The entrance of lactate into the metabolic chain involved in carbohydrate breakdown occurs at a point midway between glycogen or glucose and the final end product of the chain, CO_2. Once in the system, lactate may traverse opposite pathways; that is, on the one hand, by reverse glycolysis to glycogen, a process requiring energy, and on the other hand, continuation down through the course of normal events leading to acetyl-CoA formation and eventual fatty acid synthesis, and/or oxidation to CO_2,

which provides energy. That the rate of reverse glycolysis is rapid at a time when fat synthesis is being stimulated is interesting in view of Chaikoff's concept[70] that glycolysis is the prerequisite for lipogenesis.

d. Pyruvate administration

One gram pyruvic acid was given orally to a rat fasted 15 hours. The stimulus afforded to the lipogenesis of liver, gut and skin fatty acids by pyruvate was greater than that given by glucose, glycerol or lactate. In comparison to the animals which were refed chow, the liver fatty acids incorporated slightly more label, while the other tissue fatty acids incorporated only 1/4 to 1/2 as much label, as did chow fed controls. The liver contained no detectable glycogen after the pyruvic acid had been administered, which indicated that little reversal of the glycolytic processes had occurred in this animal. In the previously mentioned work of Teng[65] and his associates, pyruvate-2-C^{14} was used as a substrate in studying its metabolism by liver slices of fasted rats. In their system, only 9% of the pyruvate was converted to liver glycogen, while 14% was converted to glucose, 16% to CO_2, 16% to lactate, and 1% to lipid. Generally speaking, they found pyruvate to be converted at equal rates to CO_2, glucose, and lactate. Pyruvate from carbohydrate sources is known to be converted into fatty acids[68]. Fat synthesis in lactating mammary glands of rats was studied in-vitro by Hirsch, Baruch and Chaikoff[64]. Pyruvate-2-C^{14} as a substrate in their investigation was found to be converted largely to CO_2 and fatty acids and in amounts nearly equal to those found when the substrate was glucose U-C^{14}*. This

*Glucose uniformly labeled with C^{14}

great a stimulus to fatty acid formation was not seen by these workers in liver slice studies.

Whereas both lactate and pyruvate have been shown to increase the amount of labeled acetate incorporated into tissue fatty acids, liver glycogen formation was demonstrated only by the administration of lactate. The pyruvic acid given to our animal was converted to CO_2 and fatty acids, and no liver glycogen was formed; but there is not any indication as to whether or not any of the pyruvate given was converted to lactate and/or glucose as Teng's findings might lead one to suppose. If this were the case, then pyruvate might act as a brake to the process of glycolysis by mass effect.

e. Succinate administration

Sodium succinate was given in place of food to a single rat to determine its lipogenic stimulatory capacity. The tracer acetate dose given one-half hour later was found to be minimally incorporated into the tissue fatty acids of this rat. The fatty acid percent incorporation values were very similar to those of rats fasted 13 hours, and the liver glycogen content was found to be low. This latter finding is somewhat unexpected, if the fate of succinate is considered. Succinate entering the tricarboxylic acid cycle would be expected to form oxalo-acetic acid, some of which would form pyruvic acid and, by reverse glycolysis, form glycogen. Part of the pyruvate formed could re-enter the tricarboxylic acid cycle for oxidation. That this latter mechanism was probably operating in this animal will be mentioned in the later discussion of CO_2 data. The absorbed succinate was undoubtedly partially oxidized in the

tricarboxylic acid cycle, but the energy derived therefrom apparently was not utilized for fatty acid synthesis.

f. Citrate administration

Sodium citrate was another tricarboxylic acid cycle intermediate compound used in place of food in a single 15 hour post cibal rat to test its ability to stimulate lipogenesis. As with succinate, this compound failed to increase above the 13 hour fast level the percent incorporation of acetate-1-C^{14} into tissue fatty acids. The liver glycogen content was 70% that seen in the livers of the one hour post prandial rats, which indicated that some reverse glycolysis and glycogen formation had taken place.

It might be expected that citrate administration would tend to "flood" the tricarboxylic acid cycle and thereby depress the acetylation of oxaloacetate. Labeled acetate added to such a system might then be diverted through other pathways (i.e., fat synthesis). This concept is inadequate for, as mentioned above, C^{14} label was not found in the lipids.

2. CO_2 Data

The amounts of carbon dioxide excreted by the animals receiving carbohydrate intermediate substances remained unchanged in comparison to 13 hour fasted animals, whether refed or not, and the amount of radioactivity seen in the CO_2 of these animals was greater in each case than when food had been given. The specific activities seen in Table XXVIII are 1.5 to 2 times greater than corresponding values of the refed group. CO_2 percent incorporation figures of the animals which received no lipogenic

Table XXVIII

THE INCORPORATION OF LABELED ACETATE INTO RESPIRATORY CARBON DIOXIDE OF FASTED RATS REFED CARBOHYDRATE INTERMEDIATE COMPOUNDS

Animal	mM CO_2	mM CO_2 per 100 gms Rat		Specific Activity (cts/min/mM)		Percent Incorporation	
DF-1 (glucose)	58.61	26.76		1.39	10^4	72.72	
DF-2 (glucose)	48.84	22.10		1.68	"	73.12	
DF-11 (glucose)	43.26	21.42		1.97	"	72.81	
Average		22.09		1.68	"	72.88	
% of 13 hr fast (not refed)*			94%		114%		89%
% of 13 hr fast (refed ½ hr)**			79%		150%		107%
DF-6 (glycerol)	45.30	19.87		2.08	10^4	84.02	
DF-13 (glycerol)	51.20	19.32		1.57	"	68.80	
Average		19.60		1.82	"	76.41	
% of 13 hr fast (not refed)*			83%		123%		93%
% of 13 hr fast (refed ½ hr)**			70%		162%		112%
DF-4 (lactate)	33.92	14.50		2.09	10^4	63.26	
DF-9 (lactate)	39.36	19.01		2.08	"	69.86	
DF-12 (lactate)	55.33	23.35		1.51	"	71.40	
Average		18.95		1.89	"	68.17	
% of 13 hr fast (not refed)*			80%		128%		83%
% of 13 hr fast (refed ½ hr)**			68%		169%		100%
DF-5 (pyruvate)	31.62	14.05		2.46	10^4	69.56	
% of 13 hr fast (not refed)*			60%		166%		85%
% of 13 hr fast (refed ½ hr)**			51%		220%		102%
DF-8 (succinate)	47.46	23.04		1.86	10^4	78.64	
% of 13 hr fast (not refed)*			98%		126%		96%
% of 13 hr fast (refed ½ hr)**			83%		166%		115%
DF-7 (citrate)	44.90	20.88		2.01	10^4	80.56	
% of 13 hr fast (not refed)*			89%		136%		98%
% of 13 hr fast (refed ½ hr)**			75%		179%		118%

* The comparison is to data in Table XII
** The comparison is to data in Table XXII

stimulation (those receiving glycerol, succinate, and citrate) were slightly increased (10%) above those animals refed or given glucose, lactate or pyruvate. In general, an inverse relationship between percent incorporation into fatty acid and percent incorporation into CO_2 was found, which correlates with findings of others[64].

3. Cholesterol Data

The percent incorporation figures for the tissue cholesterols are presented in Tables XXIX, XXX, XXXI, and XXXII. The cholesterologenic response to the administration of carbohydrate intermediate compounds was greater than the lipogenic response in fatty acids seen above. The percent incorporation of labeled acetate into tissue cholesterol was equal to or greater than that seen in 13 hour post prandial animals. Exception to this is seen in the case of the glycerol and citrate treated animals, and also in the skin cholesterol values of all the animals in this group. Since refeeding of chow (containing 0.12% cholesterol) was seen to cause a decrease in cholesterologenesis as measured by acetate-1-C^{14} incorporation, the increases seen after the administration of these pure compounds are of great interest. It is not known if net cholesterol synthesis occurred when these compounds were given, but the cholesterol concentration, particularly in the livers of these animals, at least, was equal to or slightly greater than that seen in 13 hour fasted or chow fed animals. The effect of preformed cholesterol in inhibiting cholesterologenesis is thus obviously not seen in this group.

In conditions in which there is altered carbohydrate metabolism, such as diabetes mellitus, there is often a rise in blood ketone bodies.

Table XXIX

THE INCORPORATION OF LABELED ACETATE INTO LIVER CHOLESTEROL OF FASTED RATS REFED CARBOHYDRATE INTERMEDIATE COMPOUNDS

Animal	mg Chol.	mg Chol per Gm Liver	Specific Activity (cts/min/mg)	Percent Incorporation
DF-1 (glucose)	16.28	2.17	302.45	.183
DF-2 (glucose)	18.19	2.57	363.08	.246
DF-11 (glucose)	22.31	2.71	139.56	.114
Average		2.48	268.36	.181
% of 13 hr fast (not refed)*		106%	170%	105%
% of 13 hr fast (refed ½ hr)**		111%	250%	201%
DF-6 (glycerol)	17.74	2.37	84.16	.156
DF-13 (glycerol)	23.53	2.53	78.76	.068
Average		2.45	81.46	.062
% of 13 hr fast (not refed)*		105%	52%	36%
% lf 13 hr fast (refed ½ hr)**		109%	76%	69%
DF-4 (lactate)	20.94	2.64	558.65	.435
DF-9 (lactate)	23.71	2.94	179.46	.155
DF-12 (lactate)	22.07	2.70	264.78	.213
Average		2.76	334.30	.268
% of 13 hr fast (not refed)*		118%	212%	156%
% lf 13 hr fast (refed ½ hr)**		123%	312%	298%
DF-5 (pyruvate)	19.46	2.64	461.94	.334
% of 13 hr fast (not refed)*		113%	293%	194%
% of 13 hr fast (refed ½ hr)**		118%	431%	371%
DF-8 (succinate)	21.58	2.72	295.34	.237
% of 13 hr fast (not refed)*		116%	187%	137%
% of 13 hr fast (refed ½ hr)**		121%	276%	263%
DF-7 (citrate)	21.66	2.95	123.00	.099
% of 13 hr fast (not refed)*		126%	78%	58%
% of 13 hr fast (refed ½ hr)**		132%	115%	110%

* The comparison is to data in Table VIII
** The comparison is to data in Table XVIII

Table XXX

THE INCORPORATION OF LABELED ACETATE INTO GUT CHOLESTEROL OF FASTED RATS REFED CARBOHYDRATE INTERMEDIATE COMPOUNDS

Animal	mg Chol.	mg Chol per Gm. Gut	Specific Activity (cts/min/mg)	Percent Incorporation
DF-1 (glucose)	37.65	2.38	155.80	.218
DF-2 (glucose)	56.15	1.77	119.38	.249
DF-11 (glucose)	45.12	2.71	177.30	.284
Average		2.29	150.83	.250
% of 13 hr fast (not refed)*		95%	114%	95%
% of 13 hr fast (refed ½ hr)**		124%	140%	110%
DF-6 (glycerol)	45.38	2.18	121.14	.204
DF-13 (glycerol)	42.50	2.12	95.88	.149
Average		2.15	108.51	.176
% of 13 hr fast (not refed)*		89%	82%	67%
% of 13 hr fast (refed ½ hr)**		117%	101%	77%
DF-4 (lactate)	50.52	1.58	225.28	.423
DF-9 (lactate)	49.05	1.57	196.29	.352
DF-12 (lactate)	44.80	2.25	153.10	.250
Average		1.80	191.56	.342
% of 13 hr fast (not refed)*		74%	144%	130%
% of 13 hr fast (refed ½ hr)**		98%	178%	150%
DF-5 (pyruvate)	47.28	1.38	172.09	.302
% of 13 hr.fast (not refed)*		57%	130%	115%
% of 13 hr fast (refed ½ hr)**		75%	160%	132%
DF-8 (succinate)	43.85	2.17	188.74	.308
% of 13 hr fast (not refed)*		90%	142%	117%
% of 13 hr fast (refed ½ hr)**		118%	175%	135%
DF-7 (citrate)	38.05	2.11	135.84	.192
% of 13 hr fast (not refed)*		87%	102%	73%
% of 13 hr fast (refed ¼ hr)**		115%	126%	84%

* The comparison is to data in Table IX
** The comparison is to data in Table XIX

Table XXXI

THE INCORPORATION OF LABELED ACETATE INTO CARCASS CHOLESTEROL OF FASTED RATS REFED CARBOHYDRATE INTERMEDIATE COMPOUNDS

Animal	mg Chol.	mg Chol per gm carcass	Specific Activity (cts/min/mg)	Percent Incorporation
DF-1 (glucose)	209.38	1.46	39.00	.303
DF-2 (glucose)	208.12	1.53	47.06	.364
DF-11 (glucose)	190.75	1.54	25.00	.174
Average		1.51	37.02	.280
% of 13 hr fast (not refed)*		122%	129%	122%
% of 13 hr fast (refed ½ hr)**		105%	208%	192%
DF-6 (glycerol)	160.88	1.07	24.20	.144
DF-13 (glycerol)	135.75	.81	21.46	.106
Average		.94	22.83	.125
% of 13 hr fast (not refed)*		76%	79%	54%
% of 13 hr fast (refed ½ hr)**		65%	128%	86%
DF-4 (lactate)	173.12	1.17	40.39	.260
DF-9 (lactate)	191.50	1.63	27.37	.192
DF-12 (lactate)	143.62	.92	41.24	.216
Average		1.24	36.33	.223
% of 13 hr fast (not refed)*		100%	126%	97%
% of 13 hr fast (refed ½ hr)**		86%	204%	153%
DF-5 (pyruvate)	158.00	1.10	34.86	.204
% of 13 hr fast (not refed)*		89%	121%	89%
% of 13 hr fast (refed ½ hr)**		76%	196%	140%
DF-8 (succinate)	205.33	1.61	45.00	.344
% of 13 hr fast (not refed)*		130%	157%	150%
% of 13 hr fast (refed ½ hr)**		112%	253%	236%
DF-7 (citrate)	164.62	1.23	25.14	.154
% of 13 hr fast (not refed)*		99%	88%	67%
% of 13 hr fast (refed ½ hr)**		85%	141%	105%

*The comparison is to data in Table X
**The comparison is to data in Table XX

Table XXXII

THE INCORPORATION OF LABELED ACETATE INTO SKIN CHOLESTEROL OF FASTED RATS REFED CARBOHYDRATE INTERMEDIATE COMPOUNDS

Animal	mg Chol.	mg Chol per gm Skin	Specific Activity (cts/min/mg)	Percent Incorporation
DF-1 (glucose)	134.42	3.38	22.70	.114
DF-2 (glucose)	132.31	3.62	24.46	.120
DF-11 (glucose)	109.56	3.26	16.90	.072
Average		3.42	21.35	.102
% of 13 hr fast (not refed)*		110%	72%	64%
% of 13 hr fast (refed ½ hr)**		104%	145%	134%
DF-6 (glycerol)	128.90	3.31	17.68	.085
DF-13 (glycerol)	122.00	2.68	19.78	.088
Average		3.00	18.73	.086
% of 13 hr fast (not refed)*		96%	63%	54%
% of 13 hr fast (refed ½ hr)**		91%	127%	113%
DF-4 (lactate)	122.83	3.26	31.18	.142
DF-9 (lactate)	146.05	4.21	13.76	.074
DF-12 (lactate)	100.06	2.69	26.95	.098
Average		3.39	23.96	.105
% of 13 hr fast (not refed)*		109%	81%	66%
% of 13 hr fast (refed ½ hr)**		103%	163%	138%
DF-5 (pyruvate)	148.40	3.98	16.57	.092
% of 13 hr fast (not refed)*		128%	56%	58%
% of 13 hr fast (refed ½ hr)**		121%	113%	121%
DF-8 (succinate)	72.44	2.09	42.14	.114
% of 13 hr fast (not refed)*		67%	143%	71%
% of 13 hr fast (refed ½ hr)**		64%	285%	150%
DF-7 (citrate)	98.50	2.58	25.62	.094
% of 13 hr fast (not refed)*		83%	87%	59%
% of 13 hr fast (refed ½ hr)**		78%	174%	124%

* The comparison is to data in Table XI
** The comparison is to data in Table XXI

Excess beta oxidation of fat in this disease[71] yields larger than normal amounts of acetic acid and acetoacetic acid which are cholesterol precursors, and a hypercholesterolemic state often develops. A transient hypercholesterolemia is also seen early in acute fasting[72]. In the animals presented here, the time period of fasting (before administration of chow substitutes) is insufficient for excess beta oxidation of fat to occur, so that ketone body formation would not grossly increase. Giving carbohydrate intermediates would tend to accelerate the disappearance of cholesterol precursors by increasing Citryl-CoA formation. An accelerated rate of tricarboxylic acid cycle operation through increased acetate and oxaloacetate coupling might, however, yield additional sources of energy for cholesterol synthesis. As this laboratory has previously shown[73], chronic diabetic rats have normal cholesterol concentrations. A possible explanation for many of these findings is that the turnover rate of cholesterol is increased in conditions of fasting, and that high incorporations of C_2 label might occur without tissue cholesterol concentration being changed.

The lack of effect noted when glycerol was given may be explained by the metabolic inertness of this substance as indicated by decreased conversion of it to fatty acids mentioned previously.

The low percent incorporation of acetate-1-C^{14} into the skin cholesterols of these animals parallels the incorporation into skin fatty acids. As mentioned earlier, the intermediate anatomical position of the gut and liver with respect to the vascular supply of the skin results in lowered amounts of substrate available for metabolism by the skin.

While gut is the tissue receiving the highest fatty acid stimulation by the above compounds, the liver is the organ most highly stimulated to form cholesterol. The liver is known to form large amounts of cholesterol, and it is possible that the metabolites given to these animals were largely acted upon in this organ. It must be pointed out, however, that the decreasing order of absolute label incorporation into tissue fatty acids is similar for this group of animals to that seen for the refed and the fasted groups, i.e., gut > carcass > skin > liver. This order is slightly changed for the incorporation of acetate into tissue cholesterols, i.e., gut > carcass > liver > skin, in the three groups studied.

V. SUMMARY STATEMENTS

1. Rats fasted from one hour to 10 days showed a total body weight reduction of about one-third at the end of the experiment. Reduction in tissue fat was greater than this, while tissue cholesterol levels remained constant during the starvation.

2. Four hours after the intraperitoneal injection of 20 c. acetate-1-C^{14}, the label was distributed 80% in expired CO_2, 10% in tissue fatty acids, and 2% in tissue cholesterols, but the amounts of the incorporation products varied under the nutritional conditions of the experiments. In the four tissues isolated, the order of magnitude of label incorporation into fatty acids was as follows: gut > carcass > skin > liver. This order was maintained for cholesterol incorporations except that skin was less active than liver.

3. Percent incorporation was high in the tissue fatty acids of one hour post prandial rats. One-half to one-third of this maximum was found for rats fasted 5 hours.

4. Lipogenesis was found to be minimal in the tissues of 19 hour fasted rats. The percent incorporation into tissue fatty acids of these rats was 1/10 that of one hour post prandial animals.

5. Extensive fasting produced no further decrease in lipogenesis beyond the 19 hour level. Although lipogenesis was minimal, this process could still be demonstrated by acetate incorporation, indicating that, while fat was being excessively catabolized, a dynamic state existed between synthesis and breakdown.

6. The incorporation of acetate into cholesterol during the absorptive phase of food utilization was 60% that of animals one hour post prandial, which indicated that preformed dietary cholesterol depressed cholesterologenesis.

7. The tissue cholesterol concentration was unchanged under the influence of severe fasting, but labeled acetate incorporation was depressed.

8. The amount of carbon dioxide excreted remained constant during the short fasting periods, as did CO_2 labeling, but the physiologic alterations occurring after 7 days without food resulted in a lowered CO_2 output while the $C^{14}O_2$ concentration was elevated. This was interpreted as due to a decreased activity of oxidative processes.

9. Food was given basal state animals and subsequent administration of tracer acetate demonstrated the prompt labeling of fatty acids of the tissues. That refeeding only one-half hour could result in maximal lipogenic stimulation was surprising because, although glycolysis was occurring, preformed dietary fat was entering the system at the same time, and lipogenesis is evidently not influenced by the presence of fat in a diet containing carbohydrate. Excretory CO_2 was increased in amount but was less radioactive (S.A. was lowered) after refeeding.

10. After extensive fasting, lipogenesis was seen not to be stimulated by refeeding. Finding decreased amounts of CO_2, which were more radioactive, led to the conclusion that the ingested food had not been utilized, because tissue oxidative processes are lower in the fasted state, and nitrogenous components such as digestive enzymes and the anterior pituitary, thyroid, and pancreatic hormones were thought to be decreased.

Impairment of the entire lipogenic mechanism appeared to be involved.

11. Depressed cholesterologenesis was noted when food was given to mildly or severely fasted animals.

12. Glycerol, succinate, or citrate as chow substitutes in 15 hour fasted rats produced no lipogenic stimulation. Liver glycogen was formed from glycerol and citrate, and at the same time increased radioactivity was noted in the excreted carbon dioxide.

13. Glucose, lactate, and pyruvate stimulated lipogenesis when given separately to 15 hour fasted rats. The lipogenic stimulation was only 1/3 to 1/2 that seen by feeding chow, but the amount of substrate was only 1/10 that equivalent of food. Since liver glycogen formed from these compounds and since no increased CO_2 labeling occurred from these animals, glycolysis was probably not operating as a lipogenic stimulus; or, conversely, lipogenesis increased at the same time reverse glycolysis was occurring.

14. Lipogenic stimulation from pyruvate was the highest, while lactate stimulated lipogenesis slightly less than did glucose.

15. Increased cholesterologenesis was noted when glucose, lactate, pyruvate, or succinate was given in place of chow to 15 hour fasted rats, and no effect was noted when glycerol or citrate was administered. The relationship of these substances to cholesterol precursors was discussed.

BIBLIOGRAPHY

1. Keys, A., Brozek, J., Henschel, A., Mickelsen, O., and Taylor, H.L., The Biology of Human Starvation. The University of Minnesota Press, Minneapolis, vol. I, 763 pp., p. 105, 1950.

2. Hershey, J.M., and Orr, M.D., The Removal of Glycogen from Living Muscle. Tr. Roy. Soc. Canada, Sect. V, vol. 22, pp. 151-157, 1928.

3. Keys, A., Brozek, J., Henschel, A., Mickelsen, O., and Taylor, H.L., The Biology of Human Starvation. The University of Minnesota Press, Minneapolis, vol. I, 763 pp., p. 540, 1950.

4. West, E.S., and Todd, W.R., Textbook of Biochemistry. The Macmillan Co., New York, ed. 2, 1356 pp., p. 946, 1955.

5. Cuthbertson, D.P., Discussion: The Physiology and Treatment of Starvation. Proc. Roy. Soc. Med. (London), vol. 38, pp. 390-393, 1945.

6. Keys, A., Brozek, J., Henschel, A., Mickelsen, O., and Taylor, H.L., The Biology of Human Starvation. The University of Minnesota Press, Minneapolis, vol. I, 763 pp., p. 328, 1950.

7. Kaplan, N.O., and Greenberg, D.M., Studies with Radioactive Phosphorus of the Changes in the Acid-soluble Phosphates in the Liver, Coincident to Alterations in Carbohydrate Metabolism. III. The Effect of Fasting and of High Fat, High Carbohydrate, and High Protein Diets. J. Biol. Chem., vol. 156, pp. 543-551, 1944.

8. Benedict, F.G., A Study of Prolonged Fasting. Carnegie Inst. Washington Publ. No. 203, 416 pp., 1915.

9. Bloch, K., Biosynthesis of Cholesterol. A paper presented before: Symposium: Cholesterol Metabolism: Anabolic and Catabolic Mechanisms., at the meeting of the Am. Soc. of Biol. Chem., San Francisco, Cal., April 11-14, 1955.

10. Diturit, F., Gobey, F.P., Warms, J.V.B., and Gurin, S., Terpenoid Intermediates in Biosynthesis of Cholesterol. Fed. Proc., vol. 14, No. 1, Part 1, p. 203, March 1955.

11. Pihl, A., Bloch, K., and Anker, H.S., The Rates of Synthesis of Fatty Acids and Cholesterol in the Adult Rat Studied with the Aid of Labeled Acetic Acid. J. Biol. Chem., vol. 183, pp. 441-450, 1950.

12. Green, D.E., Oxidation and Synthesis of Fatty Acids in Soluble Enzyme Systems of Animal Tissues. Clinical Chemistry, vol. 1, pp. 53-67, 1955.

13. Green, D.E., Oxidation and Synthesis of Fatty Acids in Soluble Enzyme Systems of Animal Tissues. Clinical Chemistry, vol. 1, pp., 53-67, p. 59, 1955.

14. Green, D.E., Fatty Acid Oxidation in Soluble Systems of Animal Tissues. Biological Reviews, vol. 29, pp. 330-366, 1954.

15. Haugaard, E.S., and Stadie, W.C., Relation Between Glycogen Content and Synthesis of Fatty Acids by Rat Liver. J. Biol. Chem., vol. 199, pp. 741-744, 1952.

16. Hirsch, P.F., Baruch, H., and Chaikoff, I.L., The Relation of Glucose Oxidation to Lipogenesis in Mammary Tissue. J. Biol. Chem., vol. 210, pp. 785-797, 1954.

17. Baker, N., Chaikoff, I.L., and Schusdek, A., Effect of Fructose on Lipogenesis from Lactate and Acetate in Diabetic Liver. J. Biol. Chem., vol. 194, pp. 435-443, 1952.

18. Lyon, I., Masri, M.S., and Chaikoff, I.L., Fasting and Hepatic Lipogenesis from C^{14}-Acetate. J. Biol. Chem., vol. 196, pp. 25-32, 1952.

19. Claycomb, C.K., Lipogenesis from C^{14} Acetate. A thesis presented to the University of Oregon Medical School, 101 pp., 1951.

20. Van Bruggen, J.T., Cage-Hood Assembly for Small Animals. Nucleonics, vol. 10, no. 3, pp. 64-66, 1952.

21. Main, R.J., The Care of a Small Rat Colony. C.V. Mosby Co., St. Louis, Mo., 101 pp., p. 46, 1939.

22. Van Bruggen, J.T., Claycomb, C.K., and Hutchens, T.T., Techniques in the Use of C^{14} as a Tracer. III. Semi-micro Synthesis of C^{14}-Labeled Acetic Acid., Nucleonics, vol. 7, no. 3, pp. 45-48, 1950.

23. Gould, G., Sinex, M.F., Rosenberg, I.N., Solomon, A.K., and Hastings, A.B., Excretion of Radioactive Carbon Dioxide by Rats after Administration of Isotopic Bicarbonate, Acetate, and Succinate. J. Biol. Chem., vol. 177, pp. 295-301, 1949.

24. Claycomb, C.K., Lipogenesis from C^{14} Acetate. A thesis presented to the University of Oregon Medical School, 101 pp., p. 89, 1951.

25. Claycomb, C.K., Hutchens, T.T., and Van Bruggen, J.T., Techniques in the Use of C^{14} as a Tracer. I. Apparatus and Techniques for Wet Combustion of Non-Volatile Samples. Nucleonics, vol. 7, no. 3, pp. 38-41, 1950.

26. Hutchens, T.T., Claycomb, C.K., Cathey, W.J., and Van Bruggen, J.T., Techniques in the Use of C^{14} as a Tracer. II. Preparation of $BaCO_3$ Plates by Centrifugation. Nucleonics, vol. 7. no. 3, pp. 41-44, 1950.

27. _____, Manual of Operation and Service Instructions - Model 183 Scaler., Nuclear Instrument and Chem. Corp., Chicago, Ill., 21 pp., p. 8, August 10, 1953.

28. Calvin, M., Heidelberger, C., Reid, J.C., Tolbert, B.M., and Yankwich, P.F., Isotopic Carbon. John Wiley & Sons, Inc., New York, 376 pp., pp. 94-96, 1949.

29. Good, C.A., Kramer, H., and Somogyi, M., The Determination of Glycogen. J. Biol. Chem., vol. 100, pp. 485-491, 1933.

30. Somogyi, M., Notes on Sugar Determination., J. Biol. Chem., vol. 195, pp. 19-23, 1952.

31. Calvin, M., Heidelberger, C., Reid, J.C., Tolbert, B.M., and Yankwich, P.F., Isotopic Carbon. John Wiley & Sons, Inc., New York, 376 pp., p. 317, 1949.

32. Calvin, M., Heidelberger, C., Reid, J.C., Tolbert, B.M., and Yankwich, P.F., Isotopic Carbon. John Wiley & Sons, Inc., New York, 376 pp., pp. 292-302, 1949.

33. Claycomb, C.K., Lipogenesis from C^{14} Acetate., A thesis presented to the University of Oregon Medical School, 101 pp., p. 82, 1951.

34. Van Bruggen, J.T., Hutchens, T.T., Claybomb, C.K., and West, E.S., Time Course of Lipide Labeling in the Intact Mouse and Rat. J. Biol. Chem., vol. 200, pp. 31-37, 1953.

35. Claycomb, C.K., Lipogenesis from C^{14} Acetate., A thesis presented to the University of Oregon Medical School, 101 pp., p. 59, 1951.

36. Dible, J.H., Fat Mobilization in Starvation. J. Path. Bact., vol. 35, pp. 451-466, 1932.

37. Keys, A., Brozek, J., Henschel, A., Mickelsen, O., and Taylor, H.L., The Biology of Human Starvation., The University of Minnesota Press, Minneapolis, vol. I, 763 pp., pp. 167-171, 1950.

38. Bose, J.P., De, U.N., and Mukerjee, P., A Preliminary Study of the Biochemical Changes in Starvation Cases. Indian J. Med. Res., vol. 34, pp. 143-150, 1946.

39. Gardner, J., and Lauder, P., On the Cholesterol Content of the Tissues of Cats under Various Dietetic Conditions and During Inanition., Biochem. J., vol. 7, pp. 576-587, 1913.

40. Hershey, J.M., and Orr, M.D., The Removal of Glycogen from Living Muscle., Tr. Roy. Soc, Canada, Sect. V, vol. 22, pp. 151-157, 1928.

41. MacLachlan, P.L., Hodge, H.C., Bloor, W.R., Welch, E.A., Truax, F.L., and Taylor, J.D., Lipids of the Fasting Mouse. II. The Fat to Water Relation in the Liver and the Fractionation of Liver Phospholipids., J. Biol. Chem., vol. 143, pp. 473-490, 1942.

42. Dible, J.H., Fat Mobilization in Starvation., J. Path, Bact., vol. 35, pp. 451-466, 1932.

43. Hodge, H.C., MacLachlan, P.L., Bloor, W.R., Stoneburg, C.A., Oleson, M.C., and Whitehead, R., Lipids of the Fasting Mouse. V. The Relation between Carcass Lipids and Liver Lipids., J. Biol. Chem., vol. 139, pp. 897-915, 1941.

44. West, E.S., and Todd, W.R., Textbook of Biochemistry., The Macmillan Co., New York, ed. 2, 1356 pp., p. 891, 1955.

45. Treadwell, C.R., Tidwell, H.C., and Grafa, B.C.,Jr., Liver Glycogen and Lipids in Fasted and Glucose-fed Rats., J. Biol. Chem., vol. 149, pp. 209-215, 1943.

46. Lyon, I., Masri, M.S., and Chaikoff, I.L., Fasting and Hepatic Lipogenesis from C^{14}-Acetate., J. Biol. Chem., Vol. 196, pp. 25-32, 1952.

47. Samuels, L.T., and Conant, R.F., The Adrenals and the Mobilization of Stored Fat Formed from Diets Containing Different Fats., J. Biol. Chem., vol.152, pp. 173-179, 1944.

48. Wick, A.N., and Drury, D.R., Metabolism of Acetate by the Extrahepatic Tissues., J. Biol. Chem., vol. 199, pp. 127-131, 1952.

49. Tuerkischer, E., and Wertheimer, E., Glycogen and Adipose Tissue. J. Physiol., vol. 100, pp. 385-409, 1942.

50. Miraki, A., Metabolism of Adipose Tissue in vitro., Biochem. J., vol. 36, pp. 232-241, 1942.

51. Lyon, I., Masri, M.S., and Chaikoff, I.L., Fasting and Hepatic Lipogenesis from C^{14}-Acetate., J. Biol. Chem., vol. 196, pp. 25-32, 1952.

and

Baker, N., Chaikoff, I.L., and Schusdek, A., Effect of Fructose on Lipogenesis from Lactate and Acetate in Diabetic Liver., J. Biol. Chem., vol. 194, pp. 435-443, 1952.

and

Hirsch, P.F., Baruch, H., and Chaikoff, I.L., The Relationship of Glucose Oxidation to Lipogenesis in Mammary Tissue., J. Biol. Chem., vol. 210, pp. 785-797, 1954.

and

Masoro, E.J., Chaikoff, I.L., Chernick, S.S., and Felts, J.M., Previous Nutritional State and Glucose Conversion to Fatty Acids in Liver Slices., J. Biol. Chem., vol. 185, pp. 845-856, 1950.

52. West, E.S., and Todd, W.R., Textbook of Biochemistry., The Macmillan Co., New York, ed. 2. 1356 pp., p. 1056, 1955.

53. Keys, A., Brozek, J., Henschel, A., Mickelsen, O., and Taylor, H.L., The Biology of Human Starvation. The University of Minnesota Press, Minneapolis, vol. I, 763 pp., pp. 545-549, 1950.

54. Malmros, H., A Study of Glycosuria with Special Reference to the Interpretation of the Incidental Finding of a Positive Reduction Test., Acta Med. Scand., Suppl. 27, 309 pp., 1928.

55. Keys, A., Brozek, J., Henschel, A., Mickelsen, O., and Taylor, H.L., The Biology of Human Starvation. The University of Minnesota Press, Minneapolis, vol. I, pp. 1-763, p. 599, 1950.

56. Keys, A., Brozek, J., Henschel, A., Mickelsen, O., and Taylor, H.L., The Biology of Human Starvation., The University of Minnesota Press, Minneapolis, vol. I, 763 pp., p. 389, 1950.

57. Keys, A., Brozek, J., Henschel, A., Mickelsen, O., and Taylor, H.L., The Biology of Human Starvation., The University of Minnesota Press, Minneapolis, vol. I, 763 pp., pp. 214-215, 1950.

58. Masoro, E.J., Chaikoff, I.L., Chernick, S.S., and Felts, J.M., Previous Nutritional State and Glucose Conversion to Fatty Acids in Liver Slices., J. Biol. Chem., vol. 185, pp. 845-856, 1950.

59. Medes, G., Thomas, A., and Weinhouse, S., Nutritional Factors in Fatty Acid Synthesis by Tissue Slices in vitro., J. Biol. Chem., vol. 197, pp. 181-191, 1952.

60. Lyon, I., Masri, M.S., and Chaikoff, I.L., Fasting and Hepatic Lipogenesis from C^{14}-Acetate., J. Biol. Chem., vol. 196, pp. 25-32, 1952.

61. Boxer, G.E., and Stetten, DeW., Jr., Studies in Carbohydrate Metabolism. II. The Glycogenic Response to Glucose and Lactate in the Previously Fasted Rat., J. Biol. Chem., vol. 155, pp. 237-242, 1944.

62. Kerkes, S., Discussion following a paper presented by Chernick, S.S., The Effect of Insulin on Hepatic Utilization of Glucose., Major Metabolic Fuels., Brookhaven Symposia in Biology No. 5, Brookhaven National Laboratory, New York, 234 pp., p. 64, 1952.

63. Sprites, M.A., Discussion following a paper presented by Chernick, S.S., The Effect of Insulin on Hepatic Utilization of Glucose., Major Metabolic Fuels., Brookhaven Symposia in Biology No. 5, Brookhaven National Laboratory, New York, 234 pp., p. 63, 1952.

64. Hirsch, P.F., Baruch, H., and Chaikoff, I.L., The Relationship of Glucose Oxidation to Lipogenesis in Mammary Tissue., J. Biol. Chem., vol. 210, pp. 785-797, 1954.

65. Teng, C.T., Karnovsky, M.L., Landau, B.R., Hastings, A.B., and Nesbett, F.B., Metabolism of C^{14}-Labeled Glycerol and Pyruvate by Liver in vitro. J. Biol. Chem., vol. 202, pp. 705-716, 1953.

66. Boxer, G.E., and Stetten, DeW.,Jr., Studies in Carbohydrate Metabolism. II. The Glycogenic Response to Glucose and Lactate in the Previously Fasted Rat., J. Biol. Chem., vol. 155, pp. 237-242, 1944.

67. Soskin, S., The Production, Disposal and Regulation of the Blood Glucose., Major Metabolic Fuels., Brookhaven Symposia in Biology No. 5, Brookhaven National Laboratory, New York, 234 pp., p. 5, 1952.

68. West, E.S., and Todd, W.R., Textbook of Biochemistry. The Macmillan Co., New York, ed. 2, 1356 pp., p. 919, 1955.

69. Baker, N., Chaikoff, I.L., and Schusdek, A., Effect of Fructose on Lipogenesis from Lactate and Acetate in Diabetic Liver., J. Biol. Chem., vol. 194, pp. 435-443, 1952.

70. Lyon, I., Masri, M.S., and Chaikoff, I.L., Fasting and Hepatic Lipogenesis from C^{14}-Acetate., J. Biol. Chem., vol. 196, pp. 25-32, 1952.

71. Brady, R.O., and Gurin, S., Biosynthesis of Labeled Fatty Acids and Cholesterol in Experimental Diabetes., J. Biol. Chem., vol. 187, pp. 589-595, 1950.

72. Keys, A., Brozek, J., Henschel, A., Mickelsen, O., and Taylor, H.L., The Biology of Human Starvation. The University of Minnesota Press, Minneapolis, vol. I, 763 pp., p. 543, 1950.

73. Van Bruggen, J.T., Yamada, P., Hutchens, T.T., and West, E.S., Lipogenesis of the Intact Alloxan-Diabetic Rat. J. Biol. Chem., vol. 209, pp. 635-640, 1954.