STUDIES ON LIPID SYNTHESIS
AND TURNOVER
IN THE DIABETIC RAT

by

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INTRODUCTION

One of the most extensively studied of all metabolic diseases is diabetes mellitus. Although the etiology of this disease is not known, it is believed to be due to one or more of the following factors: excessive action of the anterior pituitary, overstimulation of the adrenal cortex, an inadequate action of insulin resulting from a decreased number of beta cells in the pancreas, a decreased production of insulin by the beta cells, an inactivation of insulin in the body, an excess insulin excretion, or a lack of response of the cells to insulin[1].

From a clinical standpoint, diabetes is characterized by an initial diabetic syndrome of hyperglycemia and glucosuria, followed by a lipid syndrome of hyperlipemia, ketonemia, and hypercholesteremia. It is the concept of many physicians that, regardless of age, every diabetic is a potential atherosclerotic patient.

While experimental diabetes does not simulate the human disease in every aspect, it has served as a tool to aid in the search for biochemical defects in the human disease. From a biochemical standpoint, diabetes is thought to involve a lesion in the initial stages of glucose utilization. Some investigators[2,3] attribute the decreased glucose utilization to an interference of transfer of glucose through the cell membrane, whereas others[4,5,6] present evidence to indicate a lesion at the stage of the phosphorylation of glucose.

Glucose is phosphorylated as follows:

$$\text{glucose} + \text{ATP} \xrightarrow{\text{hexokinase}} \text{glucose-6-}PO_4 + \text{ADP}$$

In the liver, there exists an active phosphatase enzyme which removes the phosphate grouping from glucose-6-PO_4 to reform free glucose.

$$\text{glucose-6-}PO_4 \xrightarrow{\text{glucose-6-phosphatase}} \text{glucose} + PO_4$$

Thus a lesion may involve an interference in the hexokinase phosphorylation or an increased activity of glucose-6-phosphatase[7]; in either case less than normal amounts of glucose-6-PO_4 become available for metabolism.

As a result of this carbohydrate lesion, fatty acid synthesis from acetyl coenzyme A is depressed, and fat is mobilized to the liver to be catabolized at a rapid rate, resulting in the formation of greater amounts of ketone bodies. The administration of insulin can usually correct the disturbed carbohydrate and fat metabolism and also overcome the ketosis if adequate amounts of carbohydrate are present.

The action of insulin has been extensively studied. A review of this subject by Krahl[8] cites the following actions of insulin. The immediate effects of insulin are: a stimulation of glucose uptake and glycogen synthesis in muscle, stimulation of peptide synthesis in the muscle of the fasting rat when glucose is added to the extracellular medium, stimulation of fatty acid synthesis in liver slices from fed normal rats when glucose is added to the extracellular medium, and stimulation of phosphate turnover in muscle and liver. The delayed effects of insulin are concerned with the repairing of tissues of diabetic animals. They are: a restoration of optimal glucose uptake in muscle and liver, induction of maximal peptide

synthesis in muscle and liver, restoration of optimal fatty acid synthesis from acetate or pyruvate in the liver, and suppression of glucose-6-phosphatase activity in the liver.

The investigation reported in this thesis is concerned with the synthesis and turnover of lipids in the diabetic rat. It is believed that this study provides an increased understanding of the disturbance of fat metabolism associated with diabetes.

While it is mostly agreed that a depressed fatty acid synthesis exists in the diabetic, the subject of cholesterogenesis in the diabetic is controversial. Complete studies on the turnover of both fatty acids and cholesterol in the diabetic have not been previously published.

By using acetate-1-C^{14} as a tracer and radioassaying the isolated lipids, we have gathered data related to lipid synthesis. The rate of disappearance of labeled cholesterol and fatty acids after their initial synthesis from radioacetate provided information on the turnover of lipids.

Total carbon dioxide output and specific activities of the $C^{14}O_2$ expired were measured to determine the ability of the diabetic to oxidize acetate and to ascertain the magnitude of acetate activation by the animal. A comparison was also made in the present studies between the alloxan diabetic and the pancreatectomized rat, since Peterson, Beatty, Bocek, and West[9] and Thorogood and Zimmerman[10] found certain metabolic differences to exist between these two preparations.

The following section is devoted to a historical review of some important phases of lipid metabolism to aid in an appreciation of the sig-

nificance of this study. Experimental diabetes is first discussed, followed by a discussion of the three aspects of acetate metabolism, namely, oxidation, fatty acid synthesis, and cholesterol synthesis, and concluded by a dissertation on the turnover of lipids.

I. HISTORICAL BACKGROUND

EXPERIMENTAL DIABETES

Experimental diabetes may be produced by the administration of repeated large doses of anterior pituitary extracts, by pancreatectomy, and by the administration of alloxan. The last two methods are described in detail below.

Pancreatectomy

The relation of the pancreas to diabetes was established in 1890 by Von Mering and Minkowski who produced diabetes mellitus in dogs by total pancreatectomy.

While total pancreatectomy can be performed in some species of animals, it poses a surgical problem in the rat due to the diffuse distribution of the pancreatic tissue in the mesentery.

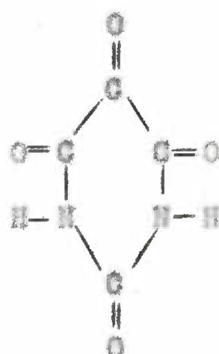
The onset of diabetes is believed to be dependent upon the amount of pancreas removed[11]. With the removal of 90% of the pancreas, the onset of diabetes is six months; with 95% pancreatectomy, it is from one to three months; and with a pancreatectomy of over 95%, it requires only a few days for the development of diabetes. Scow[12] recently reported the successful removal of 99.5% of the pancreas of rats. Within 18 hours these animals became hyperglycemic, had glucosuria and fatty livers, and intracellular fat was found in the renal tubules. These animals were not able to live more than 48 hours without the administration of insulin.

The time of onset of diabetes can also be modified by nutritional factors. High fat diets and high caloric intake accelerate, whereas a protein diet delays, the onset of diabetes. A sex difference has been found in the incidence of diabetes in subtotally pancreatectomized rats[13], for the incidence of diabetes in males was found to be 90% and in females, 10%.

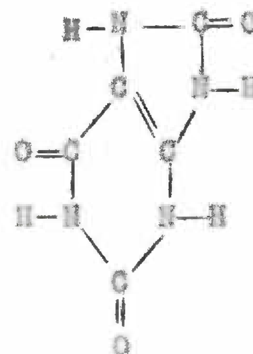
Alloxan Diabetes

In 1943 Dunn, Sheehan and McLetchie[14] demonstrated histologically that, in rabbits, alloxan is capable of producing a selective necrosis of the islets of Langerhans, damaging the beta cells without injuring the alpha cells or the acinar cells of the pancreas. Dunn[15] later produced permanent diabetes in rats with a subcutaneous injection of alloxan. Following these experiments, other investigators have found alloxan to be capable of producing permanent diabetes in the monkey, pigeon, turtle, and other species of animals.

Alloxan is the ureide of mesoxalic acid and has the following chemical structure, which is very similar to that of uric acid.



Alloxan



Uric Acid

Alloxan is a very reactive compound and can act both as a strong reducing and a strong oxidizing agent; it dissolves readily in water and is usually administered in aqueous solution.

Since alloxan is also a general tissue poison, it is important to regulate the dosage given to an animal for the production of diabetes. Large doses can produce necrosis of the renal tubules and frequent toxic deaths, while smaller doses produce only transitory hyperglycemia and glucosuria, which do not result in permanent diabetes. While the dosage is critical, the route of administration, the fasting period prior to injection, and the sex of the animal all may be contributing factors to the development of the disease.

Since alloxan is rapidly destroyed in the body, it is more effective given intravenously than orally or by means of other parenteral routes; however, the intravenous method is not a common route of administration. We have found intramuscular injections to be satisfactory.

Kass[16] has found that a fasting period of 48 to 60 hours before subcutaneous injection of alloxan makes the animal more susceptible to alloxan. On the other hand, Sturtevant[17] attributes the high mortality rates of alloxan diabetic rats to the trauma caused by this fasting period. His method of production of diabetes involves a postprandial injection of alloxan. Beach et al.[18] demonstrated that female albino rats more frequently develop severe diabetes than do males when given alloxan subcutaneously.

The mechanism by which alloxan injures the beta cells is still unexplained. The response of animals to alloxan has been observed to be a triphasic reaction of an immediate hyperglycemia lasting for an hour after

injection, severe hypoglycemia lasting approximately 24 hours, then finally the development of chronic hyperglycemia. Many mechanisms have been proposed to explain this phenomenon. Bailey and Bailey[19] postulated that the hypoglycemia may be due to the destruction of the islets and liberation of an excess amount of insulin. According to Houssay[20], the initial hyperglycemia is attributed to the direct action of alloxan on the liver, since an intact liver was found to be essential for this response. The hypoglycemia may be due to extrapancreatic influences, probably a decreased production of glucose by the liver, while the final hyperglycemia may be due mainly to the destruction of the beta cells of the islets. Bradshaw _et al_.[21] recently reported a significant decrease of the initial hyperglycemia and the intermediate hypoglycemia in the Wistar rat which was given alloxan after partial pancreatectomy, implying that the pancreas is involved in the immediate effects of alloxan.

Alloxan provides a valuable aid in the study of the pathology and physiology of diabetes, but it is important to bear in mind the similarities and differences between alloxan diabetes and the human disease. Both are similar in the following respects[22]:

1. hyperglycemia and glucosuria
2. insulin deficiency
3. condition modified by diet and insulin
4. complications such as cataract may occur
5. hyperlipemia occurs in severe cases.

The differences between the two conditions are:

1. ketosis -- not an ordinary feature of alloxan diabetes

2. alteration of beta cells -- alloxan diabetes results in few beta cells, while a lesion of beta cells is not always demonstrated in the human disease.

Although both alloxan administration and pancreatectomy can produce permanent diabetes in rats, it is interesting to note that there exist some metabolic differences between these two preparations[9,10]. Alloxan diabetics have a more severe glycosuria and a greater insulin requirement. They live much longer without insulin and maintain excellent health for a long period of time as compared to the pancreatectomized animals. They do not develop as severe a ketosis and fail to go into diabetic coma. With alloxan animals, females are more susceptible to diabetes[18], whereas in pancreatectomized animals, males have a higher incidence of the disease[13].

One of the objectives of this thesis is to uncover any differences in lipid metabolism that may exist in these two diabetic preparations.

ACETATE METABOLISM

One of the most important metabolites in intermediary metabolism is the acetate molecule, particularly as it exists in its activated state, acetyl coenzyme A. Acetyl-CoA may be derived from carbohydrate, fat, or protein metabolism. Three of the important fates of this molecule are:

1. oxidation via the tricarboxylic acid cycle to carbon dioxide,
2. utilization for fatty acid synthesis, and
3. utilization for cholesterol synthesis.

A discussion of each of these metabolic pathways follows.

Acetate Oxidation

Acetate oxidation may be studied by administering labeled acetate to an animal and analyzing the rate of excretion of $C^{14}O_2$. Using this method of study, Gould[23] found that the intact animal oxidizes a considerable amount of the administered acetate to carbon dioxide at a very rapid rate. Similar findings have been demonstrated in the eviscerated animal[24], as well as in *in vitro* systems with diaphragm[25], heart[26], and liver[5].

The ability of the diabetic animal to oxidize acetate is of current interest. Since there is evidence of a decreased utilization of carbohydrate, a decreased synthesis of fatty acids, and an increased catabolism of fats, the diabetic has been postulated to have a greater than normal abundance of acetyl coenzyme A, the chief fates of which could be oxidation or ketone body formation. Many *in vitro* studies on the oxidation of acetate in the diabetic are reported in the literature. Chernick and Chaikoff[5] found normal rates of oxidation of acetate in the diabetic rat liver slices and propose an integrity of the tricarboxylic acid cycle enzyme systems in the diabetic. On the other hand, Villee and Hastings[25] suggested an interference in the condensation of acetate with oxalacetate in the tricarboxylic acid cycle from their finding of a decreased conversion of labeled acetate to labeled carbon dioxide in the diaphragm muscle of the diabetic rat.

Studies on the oxidation of acetate in the intact diabetic animal are limited. Harper[27] found a normal rate of acetate utilization in the controlled depancreatized dog and a one-third decreased utilization in the uncontrolled animal. In a study using one alloxan diabetic animal,

Tolbert[28] observed a small reduction of $C^{14}O_2$ output and concluded that no large difference exists between the normal and diabetic in their ability to oxidize acetate-2-C^{14} to $C^{14}O_2$.

Synthesis of Fatty Acids

During the last twenty years, there has been great progress in the field of fat metabolism due largely to the enlightening research of many workers. Prominent among these are Leloir and Munoz[29], Lehninger[30,31,32], Lipmann[33], Lynen[34], Ochoa[35], and Green[36]. Because of this work the metabolic steps and enzyme systems involved in the synthesis and oxidation of fatty acids are now seemingly well established. The stepwise metabolism of a four carbon chain acid is given below to illustrate present concepts. (Page 12.) The systematic names of the enzymes as agreed upon by a committee of participants at the Second International Conference of Biochemical Problems in Lipid Metabolism held in Belgium are also given[37].

Current interest in the field of fat metabolism concerns the intracellular sites of oxidation and synthesis and the cofactors involved in synthesis.

While fatty acid oxidation is known to occur in the mitochondria[38], it has recently been proposed that the synthesis process occurs at a separate intracellular site. By separating liver cells into mitochondria, microsome, and soluble supernatant fractions, Langdon[39] found fatty acid synthesis from acetate to be most efficient in the soluble cytoplasmic fraction and concluded that fatty acid synthesis occurs exclusively in the extramitochondrial portion of the liver cell.

REACTION CATALYZED	COMPOUND	ENZYME SYSTEMS	
		General Name	Specific Name
	BUTYRIC ACID		
ACTIVATION	↓ ATP CoA ↑	THIOKINASE	ACETIC THIOKINASE
	BUTYRYL CoA		
OXIDATION-REDUCTION	↓ ± 2 H (TPN) ↑	ACYL DEHYDROGENASE	BUTYRYL DEHYDROGENASE
	CROTONYL CoA		
HYDRATION-DEHYDRATION	↓ ± H_2O ↑	ENOYL HYDRASE	CROTONYL HYDRASE
	β-OH BUTYRYL CoA		
OXIDATION-REDUCTION	↓ ± 2 H (DPN) ↑	β-OH ACYL DEHYDROGENASE	β-OH BUTYRYL DEHYDROGENASE
	ACETOACETYL CoA		
CONDENSATION-CLEAVAGE	↓ ± CoA ↑	THIOLASE	ACETOACETYL THIOLASE
	2 ACETYL CoA		

Brady[40] believes fatty acid synthesis to be dependent upon the activities of TPNH, after observing that citrate can stimulate fatty acid synthesis in the water soluble enzyme preparations of pigeon liver. Citrate is believed to generate TPNH with the aid of isocitrate dehydrogenase in the tricarboxylic acid cycle.

In Langdon's findings[41] using rat liver, crotonyl CoA was found to oxidize TPNH but not DPNH, suggesting that the specific function of TPNH

is to reduce unsaturated coenzyme A esters. Thus the step between crotonyl CoA and butyric acid was found to be TPNH dependent. Whether this reduction is catalyzed by a single enzyme or by a reductase which can transfer electrons from TPNH to the enzyme system, fatty acyl dehydrogenase, is at present not known.

This finding of the apparent need of both TPNH and DPNH is not supported by Popjak[42] who recently demonstrated that the mammary gland can use DPNH in both reductive steps of fatty acid synthesis. Possibilities suggested to explain the differences of findings are that the mammary gland differs from the liver in its nucleotide requirement or that the mammary gland possesses transhydrogenase activity.

That TPNH is required as a specific donor for fatty acid synthesis may explain some of the factors controlling the rate of fatty acid synthesis in certain biologic states. In conditions of a decreased rate of TPNH production or an increased rate of TPNH utilization, there may occur a decreased rate of fatty acid synthesis with little change in fatty acid oxidation. Conditions such as these may operate in the disease of diabetes.

In 1944 Stetten and Boxer[43] using deuterium oxide as a tracer in a diabetic rat found a great decrease in fatty acid synthesis. They gave evidence to show that the loss of depot fat in the diabetic was due to the decreased synthesis of fatty acids from glucose and also that glucosuria was the result of this decreased utilization of glucose.

In 1950 Brady and Gurin[44] using C^{14} acetate as a tracer again demonstrated an impairment of fatty acid synthesis in liver slices of the

alloxan diabetic rat and also in the depancreatized cats.

In the intact animal, Van Bruggen[45] demonstrated not only a similar decreased incorporation of acetate into fatty acids of the diabetic but also a decreased concentration of fatty acids in the carcass, skin, and gut, but some increase in concentration in the liver.

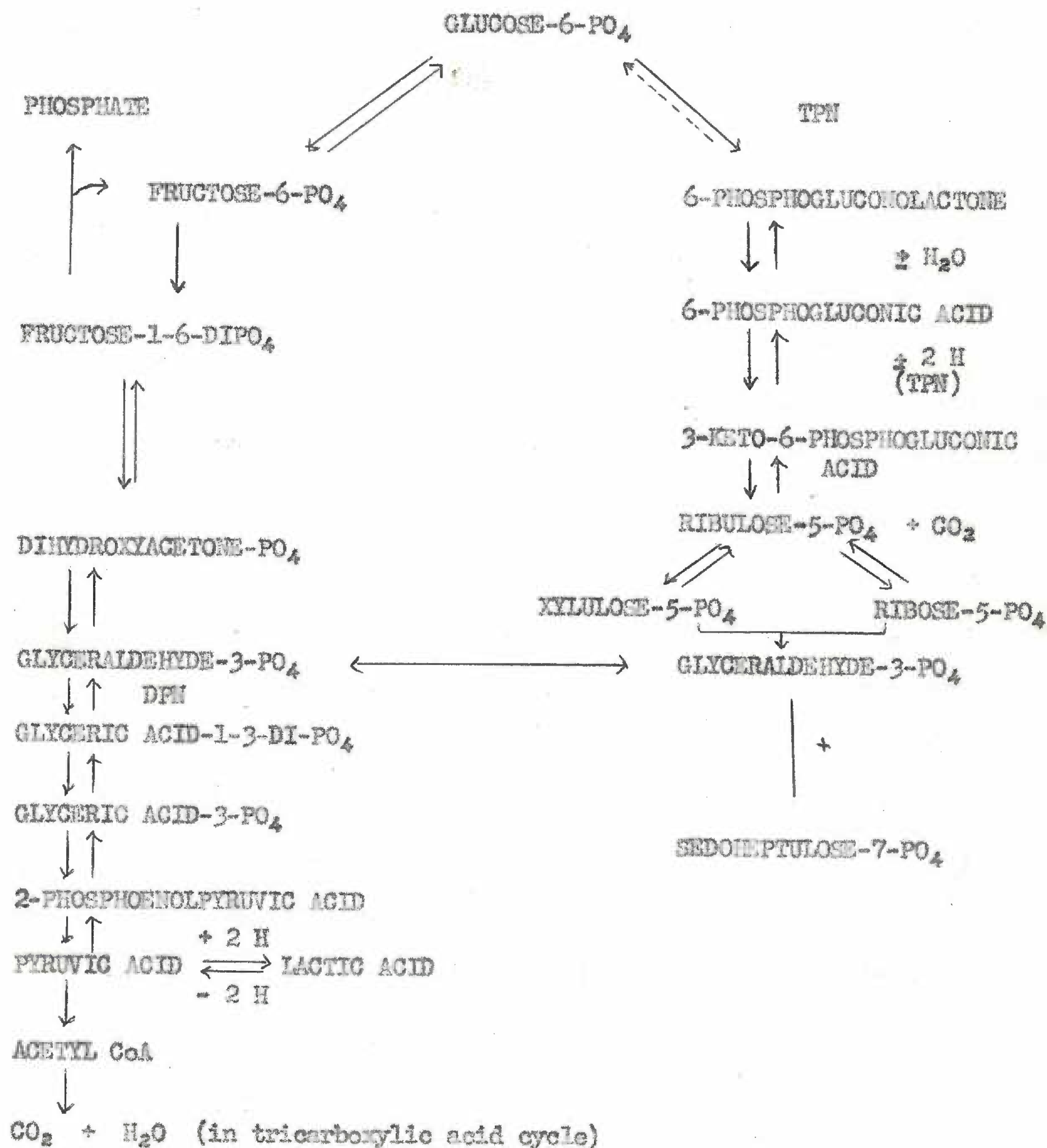
Although Renold[46] found that insulin does not repair the defective lipogenesis in the liver slice, it has been found that when insulin is administered to the diabetic animal before sacrifice it could restore glucose and pyruvate oxidation to normal[47,48]. Normal lipogenesis thus appears to be dependent upon normal carbohydrate metabolism.

The mechanism by which carbohydrate metabolism controls lipogenesis in the diabetic was first elucidated by Shaw et al.[49] who located the diabetic block to be at some point before the involvement of butyryl CoA. They observed that the supernatant fraction of the diabetic liver can not convert pyruvate, the precursor of acetate, to fatty acid unless the liver supernatant of a normal animal or butyryl CoA is added. Thus butyryl CoA is able to replace the normal supernatant fraction, suggesting that the supernatant of the diabetic probably lacks necessary cofactors for fatty acid synthesis.

The recent work of Siperstein[50] supports a proposed mechanism by which glycolysis controls fatty acid synthesis. Because this study embraces many of the factors discussed, it is described in some detail.

Glucose can be metabolized via two routes, the classical Embden Meyerhof pathway or the more recently described hexosemonophosphate pathway[51]. These two pathways of glucose metabolism are depicted in the following scheme.

TWO PATHWAYS OF GLUCOSE METABOLISM



To determine which of the pathways of glucose metabolism is responsible for the effects upon lipid synthesis in the normal and diabetic, Siperstein measured the rate of lipid synthesis from acetate under the influence of each of these pathways. His research approach was based upon the finding that the Embden Meyerhof pathway is enhanced by the addition of DPN, while oxidation via the hexosemonophosphate shunt is stimulated by TPN[52,53]. His system consisted of normal and diabetic rat liver cell-free homogenate and glucose-6-phosphate, the intermediate common to both pathways. It was found in the diabetic that, while the stimulation of the Embden Meyerhof pathway increased fatty acid synthesis slightly, stimulation of the hexosemonophosphate pathway caused a two hundred fold increase.

To explain this phenomenon, one is reminded of the finding that in the liver of the alloxan diabetic rat there is a decreased activity of the enzymes of the hexosemonophosphate pathway, namely, glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase[54] and also an increased activity of hepatic glucose-6-phosphatase[7], suggesting difficulty of metabolism via the hexosemonophosphate shunt. Since this pathway is an important source of TPNH in the body, it seems reasonable to assume that a decreased use of the hexosemonophosphate pathway leads to a decreased availability of TPNH for fatty acid synthesis. The alternate TPNH generating system, isocitrate and TPN, was also found to be able to correct depressed lipogenesis in the diabetic liver slice[50].

In summary, one of the current concepts for the depressed lipid synthesis seen in the diabetic is: first, there is decreased metabolism via the hexosemonophosphate pathway, thus resulting in a deficiency of TPNH;

and second, the primary block in fatty acid synthesis is at the site of action of TPNH, the step of reduction of crotonyl CoA to butyryl CoA.

These findings may also serve as a partial explanation of the accumulation of ketone bodies in the intact diabetic rat.

Synthesis of Cholesterol

The first evidence presented to show that acetate could be converted to cholesterol by animals was obtained by Bloch and Rittenberg[55,56] in 1942. Mice and rats ingesting deuterioacetate were found to produce deuteriocholesterol. It is now clearly established that acetate is the fundamental building block of the cholesterol molecule.

While it has been generally accepted that the liver is the chief site of cholesterol formation, recent work showed that, excepting for the mature nerve cell[57], every tissue is capable of cholesterol synthesis from acetate and also that cholesterol can be destroyed by most tissues. Thus *in vitro* studies of Srere *et al.*[58] have shown conversion of acetate to cholesterol to occur in the liver, adrenal, kidney, testis, small intestine, and skin. Popjak and his coworkers[59,60] have shown that cholesterol synthesis occurs in the mammary glands and the ovaries, and that fetal tissues are able to synthesize their own cholesterol from acetate.

Studies on the intracellular sites of synthesis of cholesterol include the successful fractionation of rat liver homogenates into a water soluble enzymic system capable of incorporating C^{14} acetate into cholesterol[61]. Bucher *et al.*[62], upon aerobically incubating the mitochondrial, microsomal, and soluble supernatant fractions of rat liver, demonstrated

each of these fractions to be inactive in the conversion of acetate to cholesterol. When these fractions were recombined in pairs, only the microsome plus soluble supernatant fractions together exhibited activity. Although the presence of mitochondria increased the amount of acetate being converted to cholesterol, the function of mitochondria was speculated to be maintainence of optimal pH.

The mechanism of cholesterol biosynthesis has involved much experimentation, but there is still no completely adequate proof to explain how the body builds acetate molecules into such a complex molecule as cholesterol.

Synthesis of cholesterol is, however, thought to occur in four stages[63]:

1. Formation of isoprenoid units from acetate.
2. Condensation of six isoprenoid units to squalene.
3. Cyclization of squalene, a sterol precursor, possibly to lanosterol[64].
4. Transformation of sterol precursor to cholesterol.

The disposition of methyl and carboxyl carbons of acetate in the cholesterol molecule has been studied mostly in two laboratories, Bloch's at Harvard in this country[65,66] and Popjak's at Hammersmith in England[67,68,69,70,71]. The origin of all 27 carbons of cholesterol biosynthesized from acetate is now known. Carbon numbers 1, 3, 5, 7, 9, 13, 15, 17, 18, 19, 21, 24, 26, and 27 originate from the methyl carbon, while carbon numbers 2, 4, 6, 8, 10, 11, 12, 14, 16, 20, 23, and 25 are from the carboxyl carbon.

The functions of cholesterol in the body are manifold, although practically no cholesterol is oxidized to carbon dioxide for energy. Cholesterol forms steroid hormones, bile acids, and bile salts, and may participate in immunological and detoxification reactions. Plasma cholesterol transports neutral fat via the lipoprotein molecules, and it may also be related to atherosclerosis.

The biosynthesis of cholesterol may be affected by factors such as age, nutritional state, and hormones. Cholesterol was found to be synthesized most rapidly in young animals[72]. The fasting of a rat has been found to result in a depressed hepatic cholesterogenesis[73]. This laboratory has found that the tissues, carcass, skin, gut and liver all show decreased cholesterogenesis from acetate upon fasting[74].

Cholesterol feeding also causes decreased synthesis of cholesterol. Tissues from dogs and rabbits maintained on high cholesterol diets for six weeks were found to have suppressed hepatic cholesterol synthesis. In vivo studies gave similar results[75,76]. The feeding of cholesterol-like substances, squalene, Δ-7 cholestenol, and 7-dehydrocholesterol were also found to decrease cholesterol synthesis[77]. This decrease was accompanied by a significant increase in total liver cholesterol.

The finding that a deficiency of panthothenic acid limits cholesterogenesis was attributed to a lack of coenzyme A, since pantothenic acid is a structural unit of coenzyme A. In pantothenic acid deficient rats, adrenal cholesterol was found to be depressed[78], as also was liver cholesterol[79]. In the latter work, Klein and Lipmann showed that decreased pantothenate results in decreased coenzyme A levels and decreased

cholesterol synthesis.

Deranged endocrine states are believed to influence cholesterol synthesis. The rate of incorporation of tritium from body water into cholesterol was found to be greater than normal in the hyperthyroid rat and below normal in the hypothyroid rat[60,61]. In the hypophysectomized rat, hepatic cholesterogenesis was found to be decreased by Tomkins and Chaikoff[62], but no change was found if an adequate carbohydrate diet was fed to these animals[63]. The effects of pancreatectomy and diabetes on the biosynthesis of cholesterol is pertinent to this thesis and is thus discussed in some detail.

Although insulin is known to be essential in the synthesis of fatty acids from acetate, its necessity in the synthesis of cholesterol from acetate has not been fully assessed.

Brady and Gurin[44] found cholesterogenesis of the liver of the alloxan diabetic rat and depancreatized cat to be unimpaired. In the severely diabetic an inhibition of synthesis was observed. Van Bruggen[45] confirmed this finding in the intact animal and showed that, in the intact animal, tissues other than the liver are also affected. Siperstein[50] reported no difference in cholesterol synthesis in the normal and diabetic rat liver slice, although production of cholesterol was found to be dependent upon the route of carbohydrate metabolism. Stimulation of the hexosemonophosphate pathway caused a great increase in synthesis (labelling) of this sterol as compared to the stimulation of the Embden Meyerhof pathway.

Hotta and Chaikoff[84] in their study of the diabetic liver found an increase of acetate conversion to cholesterol which could be decreased to normal by a diet of fructose[85]. It was postulated by these investigators that the restoration of glycolytic activity in the diabetic liver, induced by fructose, caused a shift of metabolism of the C-2 fragments from a pathway of cholesterol synthesis to other pathways such as lipogenesis.

Altman[86] in a study of perfused rat liver found that cortisone and insulin synergistically affect fatty acid synthesis but do not alter cholesterol synthesis, indicating an independence of the processes of cholesterol synthesis and fatty acid synthesis.

Mookerjea and Sadhu[87] fed excess pantothenate to diabetic rats before determining their lipid content. The pantothenate was found to increase the cholesterol concentration of the blood, liver, and adrenals. It was their belief that adequate amounts of pantothenate can guide the metabolism of acetoacetate toward increased synthesis of cholesterol.

TURNOVER OF LIPIDS

The study of metabolic turnover rates can indeed be a very complex subject as is evidenced by the highly mathematical and theoretical surveys of this subject by Reiner[88,89] and Wrenshall[90] and a somewhat more simplified treatise by Russell[91].

Although doubt is cast by some investigators[63] on the validity of measurements of turnover rates by tracer methods, these methods are still of great use in the study of dynamic aspects of metabolism. The usefulness of tracers may be due to the fact that they can be used under conditions

which do not alter the steady state of an animal.

In this paper, turnover of lipids is evaluated in terms of half-life. In the classical work of Schonheimer[92] there was presented the concept of a dynamic state of body constituents. Cell components are in a continued state of degradation and resynthesis. Half-life represents the time required for one-half of any component to be replaced. In one procedure used to determine half-life, a labelled precursor is administered to a group of animals, the animals are killed at various intervals, and the component is isolated and radioassayed. Because of the constant build up and breakdown, a steady decrease in isotope concentration with time is observed, and the time required for one-half of the isotope to disappear is defined as the half-life. In this method, there is assumed a homogeneity of mixing and a homogeneity of the manner in which the labelled material breaks down.

The turnover of lipids has been studied by a number of investigators and a wide variety of results are reported. This should not be surprising in view of the fact that turnover rates may be dependent upon many factors -- animal species, age, nutritional factors, etc. Thus no attempt is made to review all of the work reported on the turnover of lipids, but a few representative studies are presented instead.

The turnover of fatty acids in the rat is first considered. In an attempt to keep the radioacetate pool relatively constant, Pihl, Block, and Anker[93] fed radioacetate and a fat free diet to rats over a period of time and found the following half-lives for fatty acids in the various tissues:

TISSUE	HALF-LIFE OF SATURATED FATTY ACIDS	HALF-LIFE OF UNSATURATED FATTY ACIDS
Carcass	16 - 17 days	19 - 20 days
Liver	1 day	2 days

Hutchens, Van Bruggen, and West[94] obtained similar results using a single intraperitoneal injection of radioacetate. These are the results they reported:

TISSUE	HALF-LIFE OF FATTY ACID
Total Body	12.6 days
Carcass	16.8 days
Liver	2.3 days

Other studies have shown a half-life of 14.9 days for total body fat in a 150 gram rat[95] and a half-life of 18 days in the slower growing older rat[96].

It is the contention of some investigators that the use of deuterium and tritium tracers could better mirror the phenomenon of turnover of fatty acids in the body by insuring a constant supply of labeled small molecules, that is, in this case, water. However, other investigators feel that the interpretation of such results would be complicated by the greater possibility of isotopic exchange reactions, and that these may account for the somewhat slower turnover rates reported for the deuterium and tritium tracer studies. A D_2O study by Stetten and Boxer[97] showed a half-life of 1.9 days for total fatty acids of the liver. Using tritium

as a tracer, Thompson and Ballou[98] observed that the majority of body lipids, including two-thirds of both saturated and unsaturated fats, had half-lives of 70 days or longer. They postulated from these observations a predominantly non-dynamic state of body constituents in the rat.

Reports on the turnover of cholesterol in the rat are abundant and discordant. Typical studies were done by groups of investigators, as Pihl, Bloch, and Anker[93]; Hutchens, Van Bruggen, and West[94]; and Landon and Greenberg[99]. They reported half-lives for cholesterol in the various tissues of the rats as summarized below:

HALF-LIFE OF CHOLESTEROL

	INVESTIGATOR		
TISSUE	PIHL, BLOCH, AND ANKER	HUTCHENS, VAN BRUGGEN, AND WEST	LANDON AND GREENBERG
Total Body	--	8.8 days	--
Carcass	31-32 days	12 days	--
Liver	6 days	2 days	1.2 days

Liver cholesterol is thought to be synonymous with plasma cholesterol by some investigators, since plasma cholesterol is believed to be derived from the liver only. If such were the case, there is indeed a wide range of half-lives interpolated for plasma cholesterol, as is evidenced by the above values of 1.2 to 6 days.

The findings[63] of the different rates of cholesterol synthesis in the various types of cells and tissues, and the postulation of an influence of

specific proteins on the mobilization of cholesterol between cells and body fluid, all may be factors affecting the differences reported on turnover rates of cholesterol. In addition, these differences of results may also be attributed to the different species of animals used, and to the different conditions of experimentation employed by the various investigators.

In our studies of lipid turnover, we have attempted to minimize the many possible variables by keeping relatively constant the nutritional status, species, age, weight, and activity of the various groups of animals during experimentation.

II. EXPERIMENTAL

MATERIALS

Acetate-1-C^{14}

Semimicro quantities of labeled acetate were synthesized from $BaC^{14}O_3$ by the carbonation of the Grignard reagent, methyl magnesium iodide[100]. The acetate synthesized was diluted so that one milliliter contained approximately 1.96 micromoles of acetate in preparation Nos. 400 and 401, and 10.5 micromoles in Nos. 200 and 201. The radioactivity in each milliliter amounted to 33 microcuries, or 3.2×10^6 counts per minute, when assayed as follows: acetate was oxidized to carbon dioxide using potassium persulfate as the oxidizing agent; the carbon dioxide was absorbed in sodium hydroxide and precipitated as $BaCO_3$. A centrifugation technique[101] was used to prepare an infinitely thick $BaCO_3$ plate, which was counted under a D-47 micromil window gas flow counter equipped with an automatic sample changer and a Nuclear 183 scaler. This counter has an efficiency (counts/disintegrations) of ~~1.95%~~ 4.63% when counting infinitely thick $BaCO_3$ samples.

Alloxan Monohydrate

Commercial alloxan monohydrate was purified by a modification of a previously described method[102]. Twenty grams of alloxan were dissolved in 20 ml. of water and heated on a steam bath with a pinch of charcoal. While hot, this solution was filtered through a hot sintered glass funnel into 225 ml. of glacial acetic acid. After cooling overnight in a refrigerator, the crystals were filtered, washed with ether, and desiccated over

NaOH. The colorless crystals decomposed at 170^{0} C., the theoretical melting point.

PREPARATION AND CARE OF ANIMALS

Alloxan Diabetic Rats

Male albino rats of the Sprague-Dawley strain, weighing 200 grams, were fasted for 48 hours and then injected intramuscularly with a dose of 0.06 ml. of freshly prepared 10% alloxan monohydrate per 100 grams rat. Immediately, an intraperitoneal injection of 5 ml. of isotonic saline was administered. The rats were fed after one to two hours. Food *ad libitum* and a constant ample water supply were indispensable for the maintenance of these animals. This method of production of alloxan diabetic rats proved successful in 85% of the animals injected[103].

The effects of diabetes on these rats were manifested by an initial loss of body weight, followed by a maintenance of weight. The rats also had polyphagia, polyuria, and polydipsia. The progress of the disease was followed by body weight observations and periodic determinations of blood sugar. The 24 hour fasting blood sugars of these diabetic rats usually ranged from 300 to 400 mg. per cent. No animal with blood sugar values of less than 165 mg. per cent was used.

Most of the animals were maintained for six to seven weeks without insulin, but an occasional sick animal was given daily subcutaneous injections of four units of NPH Iletin (Lilly). Insulin therapy was always terminated four days prior to use of animals to eliminate any direct or immediate effects of insulin upon reactions being studied[104].

Pancreatectomized rats

Pancreatectomy was performed on male Sprague-Dawley rats weighing 200 grams and fasted 24 hours before surgery. The anesthesia used was an initial intraperitoneal injection of 35 mg. Nembutal (Na pentobarbital) per kilogram of rat and the periodic use of an ether mask. The surgical techniques of pancreatectomy were similar to that of Scow[12], using cotton swabs to strip off approximately 95% of the pancreas. That this much pancreas was removed was determined by the onset of diabetes[11] two to three weeks after surgery. The criterion for diabetes was a fasting blood sugar exceeding 165 mg. per cent.

Post operative care included an intramuscular injection of 60,000 units of penicillin immediately after surgery, followed by 30,000 units of penicillin daily for two consecutive days. The animals were fed ad libitum with ground Purina chow containing 1% Pancreatin, N.F. (Merck), and supplied with ample drinking water.

Animals were used six to seven weeks after surgery. When necessary, a subcutaneous insulin injection of two units of NPH Iletin was given daily until four days before the experiment. The mortality rate of this surgical procedure was approximately 30%.

For the supplementary studies of insulin treated pancreatectomized animals, two units of NPH Iletin were given subcutaneously daily for three days and two units of crystalline zinc insulin were given three hours before the start of the experiment.

For the studies of pantothenate-fed pancreatectomized animals, these animals were fed ad libitum with ground Purina Chow containing 40 mg. per

cent of calcium pantothenate.

EXPERIMENTAL PROCEDURE

The nutritional status of all animals was kept uniform, since it has been established that lipid metabolism is affected by fasting[105,106] and by fasting and refeeding[107]. Animals were fasted 24 hours, allowed to eat for one hour, and injected with 2 ml. of acetate-1-C^{14} one hour post-prandially.

To study lipid synthesis, an animal was placed into a metabolism chamber[108], Figure 1 (Page 30), immediately after the acetate injection. Respiratory carbon dioxide was first counted in the gas phase by a scaler connected to a thin end window GM tube, then the CO_2 was collected in NaOH. After 2 hours, the rat was lightly anesthetised with chloroform, and the blood was collected by means of heart puncture. The rat was dissected into the tissues, carcass, skin, gut, and liver, and after saponification of these tissues with alcoholic KOH the lipids were extracted.

To study lipid turnover, acetate-1-C^{14} injected rats were put into cage hood assemblies[109] to minimize radioactive contamination of the laboratory, fed ad libitum, and sacrificed at various time intervals of 2 to 28 days after acetate injections.

ANALYTICAL METHODS

Blood Sugar Determinations

The tail of the rat was warmed in hot water till hyperemic, dried with a towel, and the final 5 mm. cut off with a sharp pair of scissors.

Figure 1

The metabolism assembly consists of the following:

A. Soda lime tower

B. "C" clamp Flowrator

C. Polystyrene metabolism chamber

D. Water trap bottle

E. Geiger-Mueller tube assembly

F. Flow meter

G. Fractional CO_2 absorbers, containing 20 ml. each of 1 N NaOH solutions

H. Absorber containing 200 ml. of 1 N NaOH solution

I. Cartesian Manostat, a vacuum regulating device

J. Berkeley Decimal Scaler

K. Electric timer

L. Berkeley Counting Rate Computer

M. Esterline-Angus recorder

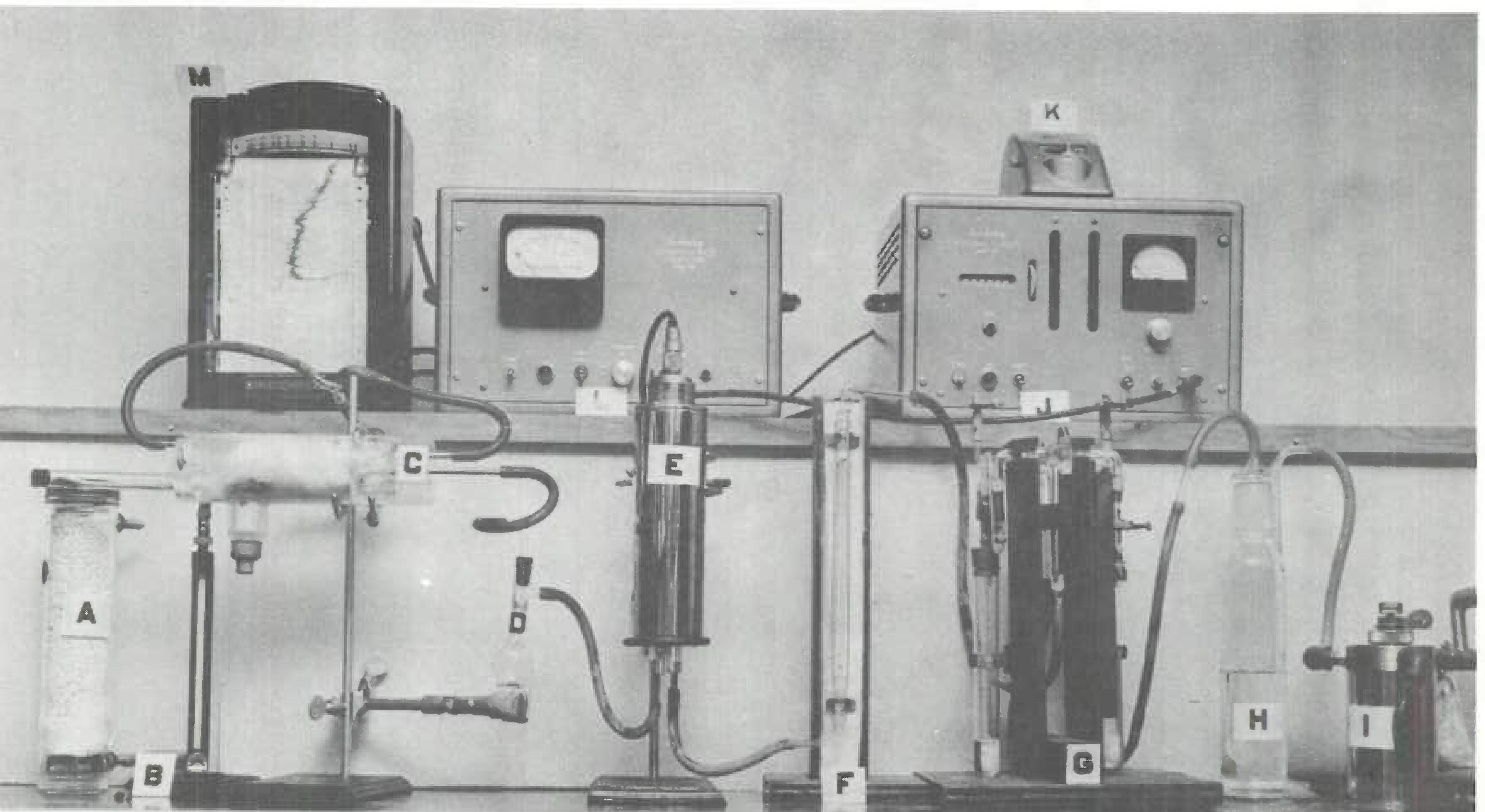


Figure 1

After collecting six to eight drops of blood on a spot plate containing potassium oxalate crystals, the cut surface of the tail was cauterized. To deproteinize the blood, 0.1 ml. of blood was added to a centrifuge tube containing 3.5 ml. water. To this were added 0.2 ml. each of 5% $ZnSO_4$ and 0.3 N $Ba(OH)_2$. After mixing and centrifugating for 20 minutes, 2 ml. of filtrate were added to 2 ml. of sugar reagent. The glucose was determined using the method of Somogyi[110].

Analysis of Respiratory Carbon Dioxide

An aliquot of the respiratory carbon dioxide collected in NaOH was plated as $BaCO_3$[101]. The weight of the $BaCO_3$ plate provided quantitative information on the total carbon dioxide output. The radioactivity of the infinitely thick plate was determined by counting with an end window GM counter with an efficiency factor (counts/disintegrations) of 1.95%.

Blood Cholesterol

Cholesterol was isolated from the blood according to the method of Abell *et al.*[111], by saponification of the blood with alcoholic KOH and extracting the cholesterol with petroleum ether. One-half ml. of serum and five ml. of alcoholic KOH (prepared by mixing 6 ml. of 33% KOH with 94 ml. of absolute alcohol) were mixed in a 25 x 150 mm. screw cap culture tube and incubated at 37-40° C. for 55 minutes. Five ml. water and 10 ml. petroleum ether were then added before shaking for five minutes in a mechanical agitator. After allowing this mixture to stand for approximately five minutes, two layers separated. Aliquots of the petroleum ether layer were immediately taken for color determinations of cholesterol and radioassay.

Isolation and Assay of Tissue Lipids

The tissues of the sacrificed rats were weighed and transferred to digestion flasks containing 25% alcoholic KOH solution in the following quantities:

Carcass	200 ml.
Skin	100 ml.
Gut	150 ml.
Liver	50 ml.

After two hours of digestion under reflux, the solutions were filtered while hot through glass wool plugs into graduated cylinders. These solutions were cooled, their volumes recorded, and aliquots of this solution were placed into 25 x 200 mm. screw cap culture tubes. These latter solutions were evaporated to half their original volumes and reconstituted to 20 ml. with water.

To extract the cholesterol, or non-saponifiable fraction, approximately 20 ml. of redistilled petroleum ether (B.P. 30 to 60° C.) were added to the saponified solution before shaking at high speeds in a mechanical shaker for five minutes. The petroleum ether phase was removed with a 30 ml. syringe fitted with an eight inch needle and transferred to a 250 ml. Erlenmeyer flask. The pooled petroleum ether extracts from five extractions were washed with KOH and water and dried over anhydrous Na_2SO_4 over night.

The aqueous phase remaining after cholesterol extraction was acidified with concentrated HCl to a Congo red endpoint. Five extractions with petroleum ether were carried out as described above. The pooled ether extracts were dried over anhydrous Na_2SO_4.

Both fatty acid and cholesterol residues were transferred in a similar manner. After drying, the ether solutions were filtered over a small glass wool plug into a wide mouth 500 ml. Erlenmeyer flask. The ether was evaporated by heating on a steam bath and blowing with a gentle stream o f nitrogen. Five successive five ml. portions of alcohol were added to dissolve the lipid, heated, cooled, then transferred with a pipet to a 25 ml. volumetric flask.

Quantitative assay of cholesterol was by the modified Sperry-Schonheimer method[112].

To determine the radioactivity, 0.5 ml. of the alcoholic solution of lipids was directly plated on a cupped aluminum planchet and dried with a heat lamp placed 12 inches away. Occasional dilutions of the solutions were necessary to prevent the infinitely thin samples from containing more than 0.3 mg. of solid material. Under our conditions of experimentation, this was found to eliminate any effects of self-absorption of radiation. The cholesterol was counted with a micromil window GM tube with an efficiency factor of 43.2%.

The fatty acid content was determined gravimetrically by evaporating an aliquot of the alcoholic solution and weighing the fatty acid residue. The radioassay was similar to that of cholesterol as described above. Counting efficiency of fatty acid at infinite thinness was 50%.

III. RESULTS AND DISCUSSION

Because of the many factors studied in this investigation, the results and discussion are presented in five major parts, as follows:

A. A study of the production of diabetes in rats using alloxan.

B. Information concerning animals used in the study.

C. Acetate-1-C^{14} oxidation.

D. Lipid synthesis.

E. Lipid turnover.

A STUDY OF THE PRODUCTION OF DIABETES IN RATS USING ALLOXAN

The production of experimental diabetes with alloxan has been found to be dependent upon many factors, among which are the dosage, the route of administration, the fasting period prior to injection of alloxan, and the sex of the animal. Although a number of different methods are reported in the literature on the production of experimental diabetes, no one method appeared adequate for producing a high incidence of diabetes and a low mortality in the male Sprague-Dawley rat. Thus a study of some of the methods was made and modified. The results of this study are summarized in Table I (Page 35).

Sturtevant[17] described a method in which the rats were not fasted but were injected intraperitoneally with a dose of 48 mg./100 gm. rat of a 1% alloxan solution administered in three divided doses of 16 mg./100 gm. rat every 48 hours. When this method was used in our laboratory, no deaths

TABLE I

The Production of Alloxan Diabetic Rats

Method	Method Number 1	2	3	4	5	6	7	8
ALLOXAN INJECTIONS								
Dosage (mg. per 100 gm. rat)	48	20	19.9	8	7	7	5	6
Concentration (% soln.)	1	10	2	10	10	10	10	10
Route of adminis-tration)	IP	SQ	SQ	IM	IM	IM	IM	IM
FASTING PERIOD PRIOR TO INJECTION (hours)	0	48	48	48	54	48	48	48
NO. OF RATS STUDIED	5	6	5	7	15	7	5	15
PER CENT DEATHS	0	100	60	86	100	86	0	0
PER CENT DIABETIC	20	0	40	14	0	14	0	85

resulted, and 20 per cent of the animals developed diabetes as determined by a fasting blood sugar exceeding 165 mg. per cent.

Subcutaneous injections as in methods 2[37] and 3[16], which differed only in the concentrations of the alloxan solution, resulted in 0 per cent or 40 per cent diabetics, respectively.

Method 4 was developed here by Dr. Clarissa Beatty for the production of diabetes in female rats. Using male rats and her conditions of drug administration, there was only a 14 per cent success in the production of diabetes. However, the intramuscular route of administration of her procedure seemed desirable to us. Thus, modifications of this technique were made and are shown in methods 5 through 8.

In method 5 the fasting time was increased to 54 hours and the dose decreased to 7 mg./100 gm. rat, resulting in death of all the animals. Upon returning to the use of the original fasting time of 48 hours and using the same dosage, 14 per cent diabetics were obtained in method 6. Further decrease of the dose to 5 mg., method 7, gave no deaths and no diabetics. Obviously a greater dosage was necessary, so in method 8 the dosage was changed to 6 mg./100 gm. rat and some other modifications were made, resulting in no deaths and 85 per cent diabetics. We believe this to be a highly effective method for the production of experimental diabetes in male Sprague-Dawley rats, having obtained equally good results with subsequent series of animals; this technique has become a standard method in our laboratory, and has already been described in a previous section of this thesis.

With the alloxan resistant rats, that is, those which were injected

but did not develop diabetes, reinjection using this same method was found to increase the fasting blood sugar, but only 50 per cent of these values exceeded 165 mg. per cent. This confirms the finding reported by other investigators that animals which have not responded to one injection of alloxan are frequently refractory to the reinjection of a similar dose.

INFORMATION CONCERNING ANIMALS USED IN THE STUDY

In Table II (Page 38) are given the animal weights, fasting blood sugars, and duration of diabetes of the animals used in the following studies. The blood sugar values were determined prior to acetate injection.

It may be seen that alloxan and pancreatectomized animals resemble each other in body weights, fasting blood sugars, and duration of the disease. The body weights of the diabetics were somewhat lower than the controls, although initially all the animals were of similar weights (150 to 200 grams) and of the same age. This loss of weight is to be expected since during the production of diabetes there is an initial loss of body weight which is followed by only a maintenance of body weight, rather than the normal weight gain. The body weights of the pancreatectomized animals which were treated with insulin or pantothenate appeared greater than the untreated pancreatectomized. This may be accounted for either by the drugs administered or by the greater duration of the disease.

Table III (Page 39) presents the weights of the tissues carcass, skin, gut, and liver of all the animals. Since these weights are dependent upon the weight of the rat, they are expressed as the per cent of the body

TABLE II

HISTORY OF ANIMALS USED IN THE STUDY

CONDITION	NO. RATS	BODY WEIGHT (grams)	FASTING BLOOD SUGAR (mg. %)	DURATION OF DIABETES (days)
Control	22	205 (183-235)	- -	- -
Alloxan	22	173 (136-225)	394 (202-505)	42 (35-67)
Pancreatectomized	17	177 (132-262)	365 (165-600)	46 (38-72)
Insulin Treated Pancreatectomized	3	269 (227-307)	505≠ (387-618)	78 (78-78)
Pantothenate Fed Pancreatectomized	2	208 (181-236)	440 (384-495)	105 (105-105)

Figures represent means and ranges of values.

≠ Blood sugar before insulin therapy

TABLE III

TISSUE WEIGHTS EXPRESSED AS PER CENT OF BODY WEIGHT

CONDITION	NO. RATS	CARCASS	SKIN	GUT	LIVER
Control	22	62.6 ±0.8	15.1 ±0.4	14.6 ± 0.5	3.7 ± 0.2
Alloxan	22	57.2 ± 0.8	10.8 ± 0.7	20.9 ±0.9	5.6 ± 0.2
Pancreatectomized	17	52.0 ±1.0	10.0 ±0.4	25.9 ±1.5	5.3 ±0.0
P for alloxan versus pancreatectomized		< 0.0025	>0.05	< 0.0025	>0.05
POOLED DIABETICS	39	54.9 ± 1.6	10.5 ± 0.4	23.1 ±0.9	5.5 ± 0.08
P for control versus pooled diabetics		< 0.0025	< 0.0025	< 0.0025	< 0.0025
Insulin Treated Pancreatectomized	3	55.0 ± 2.9	11.4 ± 1.4	19.1 ±1.6	5.6 ± 0.8
Pantothenate Fed Pancreatectomized	2	50.3 ± 2.6	15.3 ± 6.1	22.6 ± 0.0	5.6 ± 0.6

Figures represent averages ± standard error

P = probability level

weight to give a more meaningful comparison between animals and between groups of animals.

In the pancreatectomized animals, the carcass represented less total body weight, while the gut represented a greater portion of the body weight, as compared to the alloxan animals. There were no significant differences between the liver and skin fractions of the two preparations.

When considered as a unit, the diabetic animals differed significantly from the controls in the distribution of body weight. The diabetics had less carcass and skin and more gut and liver. The decreased carcass and skin may be due to a decrease of adipose tissue and subcutaneous fat resulting from the lesion of fatty acid synthesis in the diabetic or to a dehydration state which is usually thought to accompany severe diabetes despite the general polydipsia. The increased mobilization of fat in the diabetic may explain the increase of liver size, since the liver is thought to be the chief organ concerned with fat mobilization. The increased gut tissue quantitatively expressed the gross observation of protruding abdomens in the diabetic rats as a result of polyphagia, the weight of gut tissue representing the weight of tissue plus contents.

The supplementary studies of insulin treated and pantothenate fed animals were not statistically analyzed due to the small sample sizes involved.

The insulin treated animals resembled somewhat the pancreatectomized animals except for having a lower gut tissue weight. This gut tissue was still more than that of the control, however, indicating that insulin treatment for three days may be insufficient to grossly affect the

redistribution of body weight. It is interesting to note that these insulin treated animals resembled more closely the alloxan animals than the pancreatectomized animals.

The pantothenate fed pancreatectomized rats resemble the pancreatectomized rat in every tissue except the skin, which appears to resemble that of the control values. However, no great significance can be attached to this observation at present.

ACETATE-1-C^{14} OXIDATION

Certain of the results of the time course of $C^{14}O_2$ elimination are presented in Table IV (Page 42). Representative sampling times were chosen and the averages and ranges of values are reported for these times.

To aid in the understanding of this work, the mean values have been plotted in a fashion similar to the way the data was collected (see M, the Esterline Angus milliammeter recorder, on Figure 1, page 30). In Figure 2 (Page 43) are depicted the specific activity versus time curves of the $C^{14}O_2$ produced during the first thirty minutes of utilization of labeled acetate. This time interval was found to show the greatest changes.

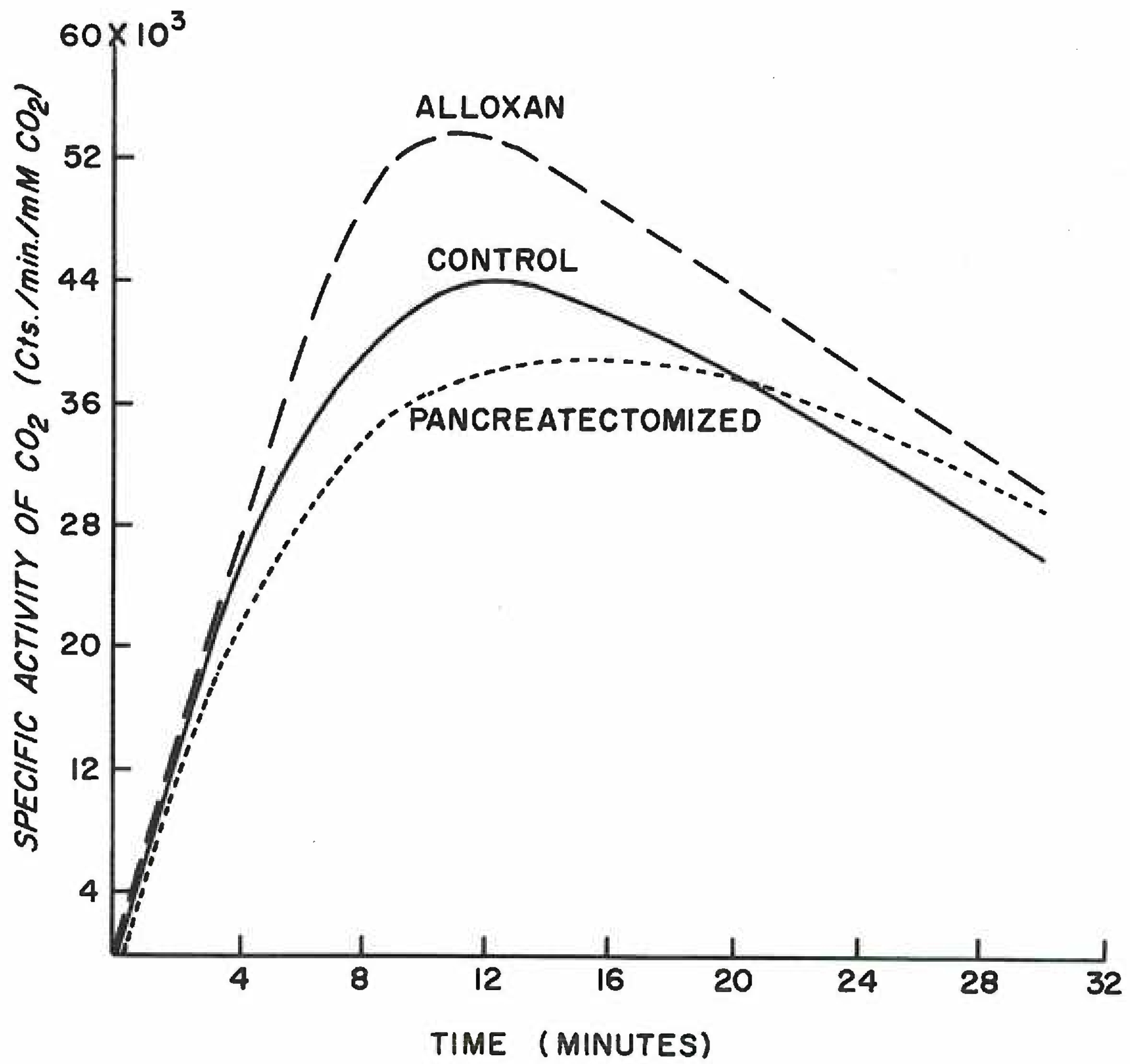
The control and alloxan curves resemble each other in shape, maximum specific activity being attained by both groups in approximately 12 minutes, but the specific activity of the alloxan curve was consistently higher than that of the control curve. The curve of the pancreatectomized animals seems to approach the control curve in magnitude of $C^{14}O_2$ specific activity, but the maximum specific activity appears to be prolonged over a longer period of time. Since the pancreatectomized animals converted

Figure 2

Specific activity versus time curves

of respiratory $C^{14}O_2$

FIGURE 2



pancreatectomized rats in all three measurements, i.e., millimoles of carbon dioxide output, specific activity, and per cent incorporation of label. For this reason these two groups of animals were taken as a unit and listed as pooled diabetics. The pooled diabetics produced the same amount of carbon dioxide as did the control, but have a statistically higher specific activity and per cent incorporation of label, as shown by P values of <0.05 and <0.025, respectively.

A visual evaluation of the specific activity-time curves of the control, alloxan and pancreatectomized animals confirms the rapidity of absorption, distribution, and metabolism of acetate, as well as the rapidity of transport and excretion of the $C^{14}O_2$ formed. There appears to be no major metabolic defect in the ability of the diabetic animals to oxidize tracer acetate to carbon dioxide.

The similarity of the shapes of the alloxan and control curves might indicate that in these preparations, labeled acetate was being metabolized at about the same rate. The greater maximum specific activity of the alloxan animals may indicate that the tracer acetate was less diluted by endogenous metabolites in its processes of activation or oxidation. The prolonged maximum specific activity and slower decay of $C^{14}O_2$ of the pancreatectomized animals may indicate that these animals have a decreased ability to activate acetate or that the labeled acetate was more diluted by the endogenous acetate. These suggestions may be compatible with the fact that pancreatectomized preparations are believed to become more ketotic than alloxan preparations[10].

At the end of the two hour period, the alloxan and pancreatectomized

animals did not differ from each other in any of the parameters measured, showing that, although they may have metabolized the acetate differently at first, they oxidized the same amount after a period of time.

The diabetic animals are seen to produce carbon dioxide of a higher specific activity than that of the control group, which leads to an increase in the per cent incorporation of label. Since the total amounts of carbon dioxide produced by the diabetic and control groups were similar, these increases cannot be attributed to any changes in the overall production of carbon dioxide. The depressed lipogenesis previously found in diabetic animals[45] may lead one to interpret this phenomenon as a shift of metabolism of acetate from synthesis to oxidation. However, the amount of labeled acetate used for lipogenesis is so small that it cannot account for the increase in the amount of labeled acetate oxidized to $C^{14}O_2$ in the diabetic. It would appear, then, that acetate-1-C^{14} activation and dilution should account for these findings.

In the diabetic animal the accelerated formation of ketone bodies may increase the supply of coenzyme A by means of the two reactions:

1. condensation of acetyl CoA to acetoacetyl CoA

2 Ac-CoA ⟶ Acetoacetyl-CoA + CoA

2. deacylation of acetoacetyl CoA by the liver

Acetoacetyl CoA ⟶ Acetoacetate + CoA

In the light of these reactions, we believe it reasonable to postulate that the increased production of labeled carbon dioxide by the diabetic may be due to an increase of coenzyme A availability which would facilitate oxidation of more acetate. Potential substantiation of this

hypothesis may be present in the comment of Brady[40] that there is a substantial increase of coenzyme A in the liver of the diabetic, and the finding of Brady that there is a stimulation of fatty acid oxidation in the liver by high concentrations of coenzyme A.

LIPID SYNTHESIS

An attempt was made to evaluate the comparative rates of synthesis of cholesterol and fatty acids by measuring the following three parameters: weights of lipids in the various tissues, specific activity, and the per cent of C^{14} acetate incorporated into the lipids.

In Table VI there is presented the data on the amounts of cholesterol and fatty acids in the tissues (page 49). By examining lines 2 and 3 and lines 7 and 8, it is seen that the alloxan and pancreatectomized animals resemble each other very closely in both fatty acid and cholesterol content. If, on the other hand, the diabetic preparations are compared with the control animals, it is seen that, although there are no gross differences in the cholesterol content of the tissues, there are some differences in the fatty acid content. The carcass, skin, and gut of the diabetic animals have less fatty acids than the control, while the liver of the diabetic has approximately one and one-fourth times as much fatty acid as does the control liver.

The reasons for these differences are not obvious at present, since it is believed that the lipid content of the various tissues may be in part dependent upon the size of the animal. These values have then been normalized by a calculation of the unit concentrations of lipids, that is,

TABLE VI

WEIGHTS OF LIPIDS IN TISSUES

OF CONTROL AND DIABETIC RATS

CONDITION	NO. RATS	CARCASS	SKIN	GUT	LIVER
		GRAMS CHOLESTEROL PER TISSUE			
Control	22	0.22 ± .01	0.14 ± .008	0.05 ± .002	0.02 ± .001
Alloxan	22	0.16 ± .01	0.11 ± .007	0.06 ± .007	0.03 ± .004
Pancreatectomized	17	0.15 ± .02	0.10 ± .009	0.04 ± .007	0.02 ± .002
Insulin Treated Pancreatectomized	3	0.27 ± .02	0.21 ± .02	0.09 ± .01	0.02 ± .004
Pantothenate Fed Pancreatectomized	2	0.24 ± .03	0.14 ± .02	0.09 ± .008	0.03 ± .003
		GRAMS FATTY ACID PER TISSUE			
Control	22	7.0 ± .27	3.9 ± .31	1.1 ± .09	0.24 ± .01
Alloxan	22	3.7 ± .38	0.86 ± .17	0.62 ± .06	0.28 ± .02
Pancreatectomized	17	3.1 ± .59	0.70 ± .22	0.70 ± .08	0.34 ± .03
Insulin Treated Pancreatectomized	3	7.1 ± .08	2.3 ± .50	1.4 ± .06	0.40 ± .04
Pantothenate Fed Pancreatectomized	2	4.4 ± .33	0.35 ± .05	0.47 ± .08	0.33 ± 1.6

Figures represent means ± standard error

the milligrams of lipids per gram of tissue. These are presented in Table VII (page 51).

Statistical analysis of the results shows a decreased concentration of cholesterol in the gut and liver and a decreased concentration of fatty acid in the skin of the pancreatectomized animals when compared to the alloxan animals.

When the cholesterol concentrations of the diabetic preparations are compared to the control values, it is noted that only the skin fraction of the diabetic animals differs from that of the control. The finding of a greater concentration of cholesterol in the skin of the diabetic might lead one to postulate an increased mobilization of cholesterol to the skin or a decreased turnover of cholesterol in the skin, since the previous findings of our laboratory[45] showed no apparent increase of synthesis of skin cholesterol in the diabetic.

A comparison of the fatty acid concentration of the control and diabetic animals indicates a significant decrease of fatty acid concentrations in the carcass, skin, and gut of the diabetic, but no difference in that of the liver. Since the carcass and skin fractions contain the major lipid depots, i.e., the adipose tissue and the subcutaneous fat, respectively, and since the diabetic is thought to be more dependent upon lipid catabolism for energy, one might expect to find these decreases of fatty acids in the carcass and skin. An accompanying decreased formation of fatty acids in the diabetic would also result in low stores of fat.

The liver is believed to be an important site of degradation of fatty acids. Consequently, in a state of increased fatty acid utilization (as

TABLE VII

CONCENTRATION OF LIPIDS IN CONTROL AND DIABETIC RATS

CONDITION	NO. RATS	CARCASS	SKIN	GUT	LIVER
		MILLIGRAMS CHOLESTEROL PER GRAM TISSUE			
Control	22	1.48 ± .07	4.12 ± .18	1.57 ± .08	2.15 ± .10
Alloxan	22	1.62 ± .13	5.69 ± .33	1.54 ± .21	2.18 ± .06
Pancreatectomized	17	1.62 ± .15	5.48 ± .42	0.93 ± .14	1.78 ± .21
P for alloxan vs. pancreatectomized		>0.10	>0.10	<0.05	<0.05
POOLED DIABETICS	39	1.62 ± .07	5.57 ± .41	1.27 ± .12	2.00 ± .07
P for control vs. pooled diabetics		>0.10	<0.005	>0.10	>0.10
Insulin Treated Pancreatectomized	3	1.88 ± .27	7.02 ± 1.1	1.83 ± .25	1.49 ± .32
Pantothenate Fed Pancreatectomized	2	2.36 ± .17	7.34 ± 2.6	1.87 ± .12	2.53 ± .22
		MILLIGRAMS FATTY ACID PER GRAM TISSUE			
Control	22	47.0 ± 2.6	106.6 ± 2.1	31.7 ± 2.3	26.5 ± 1.5
Alloxan	22	36.2 ± 2.3	40.5 ± 5.8	16.9 ± 0.6	28.2 ± 1.6
Pancreatectomized	17	29.3 ± 3.9	25.5 ± 3.0	14.9 ± 1.0	29.8 ± 1.7
P for alloxan vs. pancreatectomized		>0.10	<0.05	>0.05	>0.10
POOLED DIABETICS	39	33.2 ± 2.3	33.9 ± 3.7	16.0 ± 0.3	28.9 ± 1.1
P for control vs. pooled diabetics		<0.005	<0.005	<0.005	>0.10
Insulin Treated Pancreatectomized	3	47.8 ± 1.3	72.0 ± 1.7	32.4 ± 0.4	27.6 ± 1.6
Pantothenate Fed Pancreatectomized	2	20.9 ± 1.0	18.8 ± 5.0	10.0 ± 0.9	28.3 ± 3.5

Figures represent means ± standard errors

seen in diabetes), one would not expect to find a decrease in fatty acid concentration in the liver.

It may be recalled that in Table III (page 39) it was shown that the diabetic animals undergo a redistribution of their body weights so that the carcass and skin tissues represent smaller proportions of the body weights, as compared to control animals. From the above findings of changes in lipid concentration of the diabetic animals it may be concluded that the redistribution of body lipids is in part responsible for the redistribution of body weights.

The effects of insulin were studied in a few animals to give an indication of its effects on lipid metabolism in the diabetic. Although the results were not treated statistically, it can be seen (Table VII, page 51) that insulin causes a slight increase of cholesterol concentrations of the carcass, skin, and gut and a decrease in the liver of the pancreatectomized animals. Insulin also causes an increase in fatty acid concentrations of the carcass, skin, and gut and a slight decrease in the liver of the pancreatectomized rats, resulting in fatty acid levels resembling those of the control rats. Insulin has been found to be capable of restoring deranged carbohydrate metabolism in the diabetic to normal. Here its effect on fatty acid metabolism is demonstrated.

By feeding pantothenate, presumably to increase levels of coenzyme A, Mookerjea and Sadhu[87] found an increase in cholesterol concentrations in the blood, liver, and adrenals of the diabetic rat. Thus they postulated that a depressed cholesterologenesis in the diabetic was due to an insufficient supply of coenzyme A in the diabetic. We have similarly fed

TABLE VIII

SERUM CHOLESTEROL LEVELS

(mg./100 ml. serum)

CONDITION	NO. RATS	SERUM CHOLESTEROL LEVELS		MEANS ± STANDARD ERROR
Control	6	75	92	
		81	92	87.5 ± 3.2
		89	96	
Alloxan	12	70	105	
		78	106	
		82	148	
		86	170	
		88	186	
		99	206	118.7 ± 13.4
Pancreatectomized	16	61	130	
		75	130	
		90	135	
		90	135	
		90	142	
		94	150	
		104	170	
		110	195	119.0 ± 9.0
P for alloxan vs. pancreatectomized				> 0.10
POOLED DIABETICS	28			116.6 ± 7.3
P for control vs. pooled diabetics				> 0.05
Insulin Treated Pancreatectomized	3	80		
		80		
		70		77.0 ± 3.8
Pantothenate Fed Pancreatectomized	1	105		

P = probability level

were the result of hypercholesteremia, then our findings of a similarity of cholesterol levels in the pancreatectomized and alloxan preparations would not support their findings.

Katz and his coworkers[115] showed that chickens fed a cholesterol-oil diet developed coronary vessel lesions which regressed when the cholesterol-oil feeding was terminated. However, when insulin was administered after the cessation of cholesterol-oil feeding, the usual regression of coronary vessel lesions did not occur; thus they postulated an atherogenic-potentiating effect of insulin. Although we have studied only three animals, we find that the diabetic animals treated with insulin do not have elevated blood cholesterol levels but slightly depressed levels. This decrease of cholesterol levels in the blood is accompanied by a decrease in liver cholesterol levels also. Since blood cholesterol is thought to be derived from liver cholesterol, such a parallelism of findings is in order.

The specific activities of the isolated lipids of the tissues are presented in Table IX (page 56). Upon examination of this table it is found that the cholesterol specific activity of the carcass of the pancreatectomized animals is slightly higher than that of the alloxan animal, while the cholesterol specific activities of the rest of the tissues of the alloxan and pancreatectomized preparations are of similar magnitudes. The pooled diabetic animals have lower concentrations of radiocholesterol than the control animals. This decrease in radioactivity may indicate a decreased cholesterol synthesis since the decrease cannot be due entirely to changes in pool size and dilution of labeled cholesterol. As shown in Table VII (page 51), the only diabetic tissue differing from the control in cholesterol concentration is the skin.

TABLE IX

SPECIFIC ACTIVITIES OF LIPID FRACTIONS
OF CONTROL AND DIABETIC ANIMALS

CONDITION	NO. RATS	CARCASS	SKIN	GUT	LIVER
		SPECIFIC ACTIVITY OF CHOLESTEROL FRACTIONS			
Control	10	51.6 ± 4.4	50.4 ± 7.5	266.4 ± 10.7	460.8 ± 60.7
Alloxan	5	23.3 ± 2.4	15.9 ± 3.3	154.0 ± 24.5	85.9 ± 20.0
Pancreatectomized	6	37.3 ± 4.2	24.7 ± 3.6	175.0 ± 17.0	217.0 ± 90.6
P for alloxan vs. pancreatectomized		< 0.025	> 0.10	> 0.10	> 0.10
POOLED DIABETIC	11	31.0 ± 3.2	20.7 ± 2.7	165.4 ± 14.8	157.1 ± 52.7
P for control vs. pooled diabetic		< 0.005	< 0.005	< 0.005	< 0.005
Insulin Treated Pancreatectomized	3	30.3 ± 5.0	13.9 ± 3.3	158.0 ± 31.2	442.0 ± 147.4
Pantothenate Fed Pancreatectomized	2	30.3 ± 3.1	15.6 ± 4.8	142.0 ± 4.0	295.0 ± 123.3
		SPECIFIC ACTIVITY OF FATTY ACID FRACTIONS			
Control	10	34.4 ± 5.5	16.9 ± 1.9	171.8 ± 24.0	87.5 ± 16.0
Alloxan	5	8.4 ± 2.8	10.2 ± 4.4	126.2 ± 19.6	28.8 ± 5.2
Pancreatectomized	6	9.3 ± 2.1	10.8 ± 2.4	105.8 ± 19.1	29.9 ± 7.7
P for alloxan vs. pancreatectomized		> 0.10	> 0.10	> 0.10	> 0.10
POOLED DIABETICS	11	8.9 ± 1.6	10.5 ± 2.3	115.1 ± 13.5	29.4 ± 4.3
P for control vs. pooled diabetics		< 0.005	< 0.05	> 0.05	< 0.005
Insulin Treated Pancreatectomized	3	18.8 ± 5.1	13.1 ± 7.5	92.6 ± 33.6	79.8 ± 18.3
Pantothenate Fed Pancreatectomized	2	9.2 ± 0.7	13.0 ± 0	104.0 ± 22.1	18.0 ± 4.6

Figures represent means ± standard error

While this finding of a lack of increase in specific activity of cholesterol in the liver of the diabetic is contrary to the ten fold increases in the liver slice of the diabetic reported by Hotta and Chaikoff[84], our finding is more in accord with that of Brady and Gurin[44], Siperstein[90], and Van Bruggen[45].

The specific activities of the fatty acids in the alloxan and pancreatectomized animals do not differ. In comparing the fatty acids of the diabetic animals to the control animals it is seen that, whereas the gut of the diabetic is not statistically different from the control, the carcass, skin, and liver of the diabetic have lower fatty acid specific activities than those of the control.

The specific activities of the serum cholesterol of the diabetic and the control animals, as shown on Table X (page 58), indicate no differences. However, the serum cholesterol of the pancreatectomized animals have a higher specific activity than the alloxan animals. It is interesting to note that the apparent decreased synthesis of cholesterol by most tissues of the diabetic do not grossly affect the blood cholesterol concentration and specific activities. It is possible that serum cholesterol levels may be dependent not only upon synthesis but also upon the degradation and transport of cholesterol.

Insulin administered to the pancreatectomized animals influences the cholesterol specific activity level of the liver only, elevating it to control values. Depressed fatty acid specific activities in the diabetic carcass and liver are also returned to normal by insulin injections. The insulin treated animals have serum cholesterol of a higher specific activity than either the untreated pancreatectomized or the control animals.

TABLE X

SPECIFIC ACTIVITIES OF SERUM CHOLESTEROL

CONDITION	NO. RATS	CHOLESTEROL SPECIFIC ACTIVITY	MEANS ± STANDARD ERROR
Control	2	86 132	109.4 ± 23.7
Alloxan	3	42 82 94	72.3 ± 6.6
Pancreatectomized	4	103 125 146 180	138.3 ± 17.8
P for alloxan vs. pancreatectomized			< 0.025
POOLED DIABETIC	7		108.6 ± 17.0
P for control vs. pooled diabetic			>0.10
Insulin Treated Pancreatectomized	3	176 271 297	248.0 ± 31.0
Pantothenate Fed Pancreatectomized	1	328	

P = probability level

Since this high specific activity serum cholesterol is accompanied by a decrease in cholesterol concentration in the serum, the effects of pool size and decreased dilution must also be considered here.

The per cent of C^{14} acetate label incorporated into lipids, which is the resultant of the specific activity times the total mass of lipid in the tissue, gives a measure of lipid synthesis which minimizes the effects of variations in lipid concentration. The per cent incorporation of labeled acetate into cholesterol and fatty acids as shown in Table XI (page 60) indicates that excepting for the carcass cholesterol the alloxan and pancreatectomized groups do not differ statistically in any of the tissue examined in both types of lipids. A comparison of the control and diabetic animals indicates a decreased per cent incorporation of label into cholesterol and fatty acids in all tissues.

Although insulin does not seem to increase the ability of the skin of the diabetic to incorporate label into cholesterol, it does return the incorporation of acetate into cholesterol of other tissues to control levels. Fatty acid radioactivity is also increased in all tissues following insulin treatment.

Due to the small number of animals studied, the following presentation of the influence of pantothenate feeding on cholesterolegenesis is presented to give evidence of a trend of the results of such a study. Pantothenate feeding does not affect the per cent incorporation of label into cholesterol in any of the tissues except liver. The fatty acid activity is also unaffected or decreased. Mookerjea and Sadhu[87] used a quantitative analysis of cholesterol to indicate increased cholesterogenesis. Although in the two animals we have studied there is an

TABLE XI

PER CENT INCORPORATION OF C^{14}-ACETATE INTO LIPID FRACTION

CONDITION	NO. RATS	CARCASS	SKIN	GUT	LIVER
		PER CENT INCORPORATION INTO CHOLESTEROL			
Control	10	0.14 ±.01	0.087 ±.01	0.19 ±.02	0.11 ±.01
Alloxan	5	0.042 ±.009	0.025 ±.007	0.064 ±.02	0.023 ±.004
Pancreatectomized	6	0.099 ±.03	0.042 ±.006	0.14 ±.04	0.066 ±.03
P for alloxan vs. pancreatectomized		<0.05	>0.05	>0.10	>0.10
POOLED DIABETICS	11	0.073 ±.01	0.034 ±.005	0.10 ±.03	0.046 ±.02
P for control vs. pooled diabetics		<0.005	<0.005	<0.05	<0.025
Insulin Treated pancreatectomized	3	0.11 ±.02	0.039 ±.01	0.19 ±.02	0.14 ±.05
Pantothenate Fed pancreatectomized	2	0.10 ±.01	0.031 ±.01	0.17 ±.06	0.12 ±.04
		PER CENT INCORPORATION INTO FATTY ACID			
Control	10	2.36 ± .33	0.54 ± .04	1.63 ± .30	0.24 ±.01
Alloxan	5	0.42 ± .11	0.09 ± .04	0.90 ± .21	0.12 ±.03
Pancreatectomized	6	0.45 ± .05	0.12 ± .015	0.95 ± .09	0.11 ±.03
P for alloxan vs. pancreatectomized		>0.10	>0.10	>0.10	>0.10
POOLED DIABETICS	11	0.44 ± .06	0.11 ± .02	0.92 ± .12	0.11 ±.02
P for control vs. pooled diabetics		<0.005	<0.005	<0.05	<0.005
Insulin Treated Pancreatectomized	3	1.68 ± .31	0.31 ± .11	1.63 ± .54	0.40 ±.11
Pantothenate Fed Pancreatectomized	2	0.27 ± .05	0.06 ± .007	0.65 ± .08	0.082 ±.04

Figures represent means ± standard error
P = probability

increased cholesterol concentration in the carcass, skin, gut, and liver, only the liver has an increased per cent incorporation of label into its cholesterol. Since the latter measurement of per cent incorporation better reflects synthetic activity, we agree with these investigators that pantothenate may increase the cholesterogenesis of the liver, but we find that cholesterogenesis is not increased in the carcass, skin, and gut. It is difficult at this time, however, to accept the idea that an increased cholesterogenesis results from the oral supplement of coenzyme A in the form of pantothenate feeding. If coenzyme A were indeed lacking in the diabetic, and if pantothenate did replenish the supply, one would expect the other tissues to respond similarly. Also one would expect the synthesis of fatty acids and the oxidation of acetate to be increased, since these reactions are also known to be coenzyme A dependent. The fatty acids are not affected, however, and our carbon dioxide data suggest, if anything, an excess of coenzyme A in the diabetic.

To better illustrate per cent incorporation figures, two histograms of the results are presented in Figures 3 and 4 (pages 62 and 63). One can readily picture the lowered values of the alloxan and pancreatectomized groups as compared to the control animals in both fatty acid and cholesterol. One does not find, however, any direct correlation of mass of tissue with the amount of incorporation of label. The gut incorporates the most label into cholesterol, whereas if a direct correlation with mass existed, the carcass should be greatest since it has a greater mass. For the fatty acids, the carcass and liver tissues of the control animals incorporate the most label, whereas in the diabetic the gut and

Figure 3

Incorporation of Acetate-1-C^{14}

into Cholesterol of the Various Tissues

FIGURE 3

INCORPORATION OF ACETATE -1-C^{14} INTO CHOLESTEROL

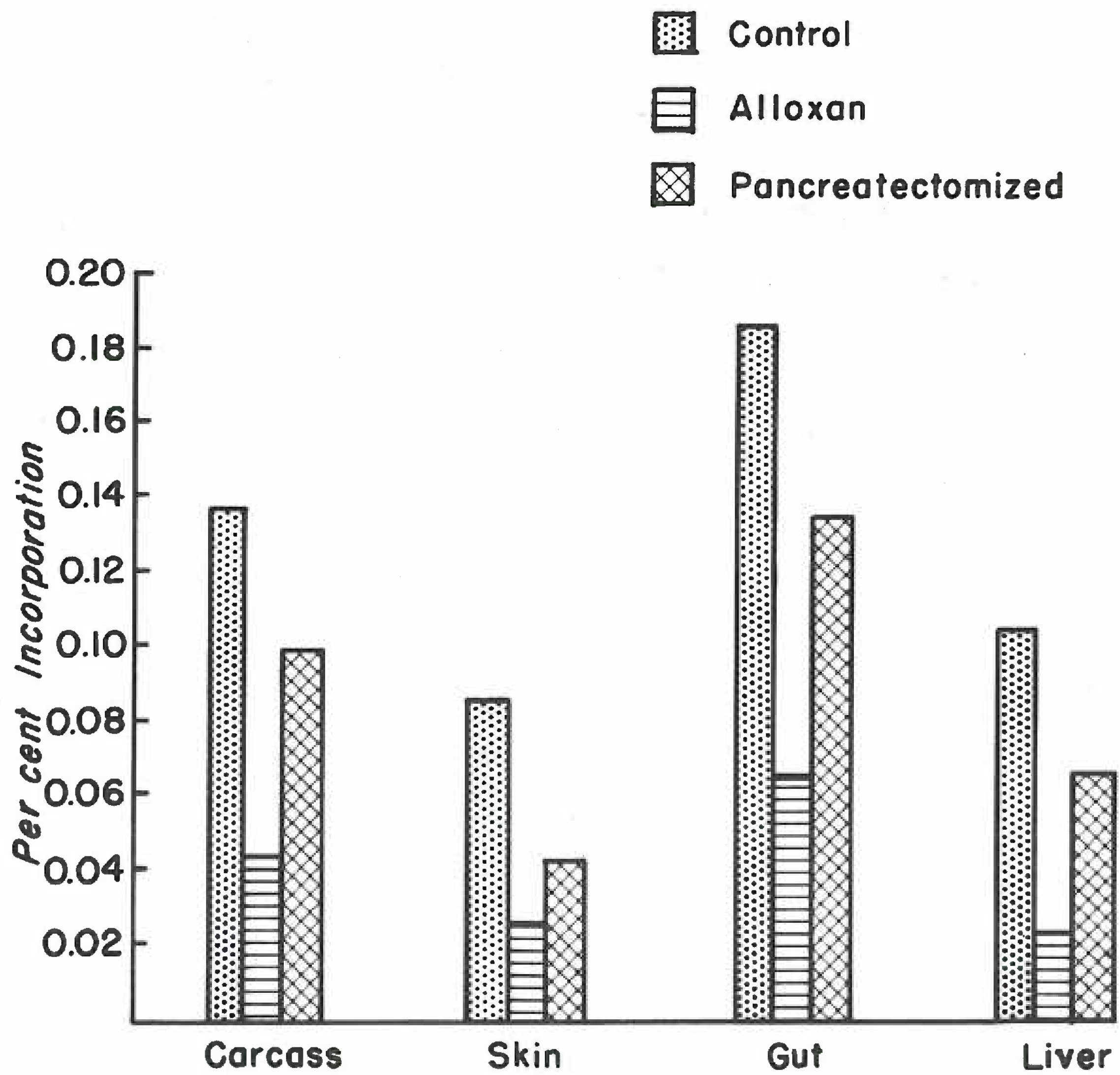


Figure 4

Incorporation of Acetate-1-C^{14}

into Fatty Acids of the Various Tissues

FIGURE 4

INCORPORATION OF ACETATE -1- C^{14}
INTO FATTY ACID

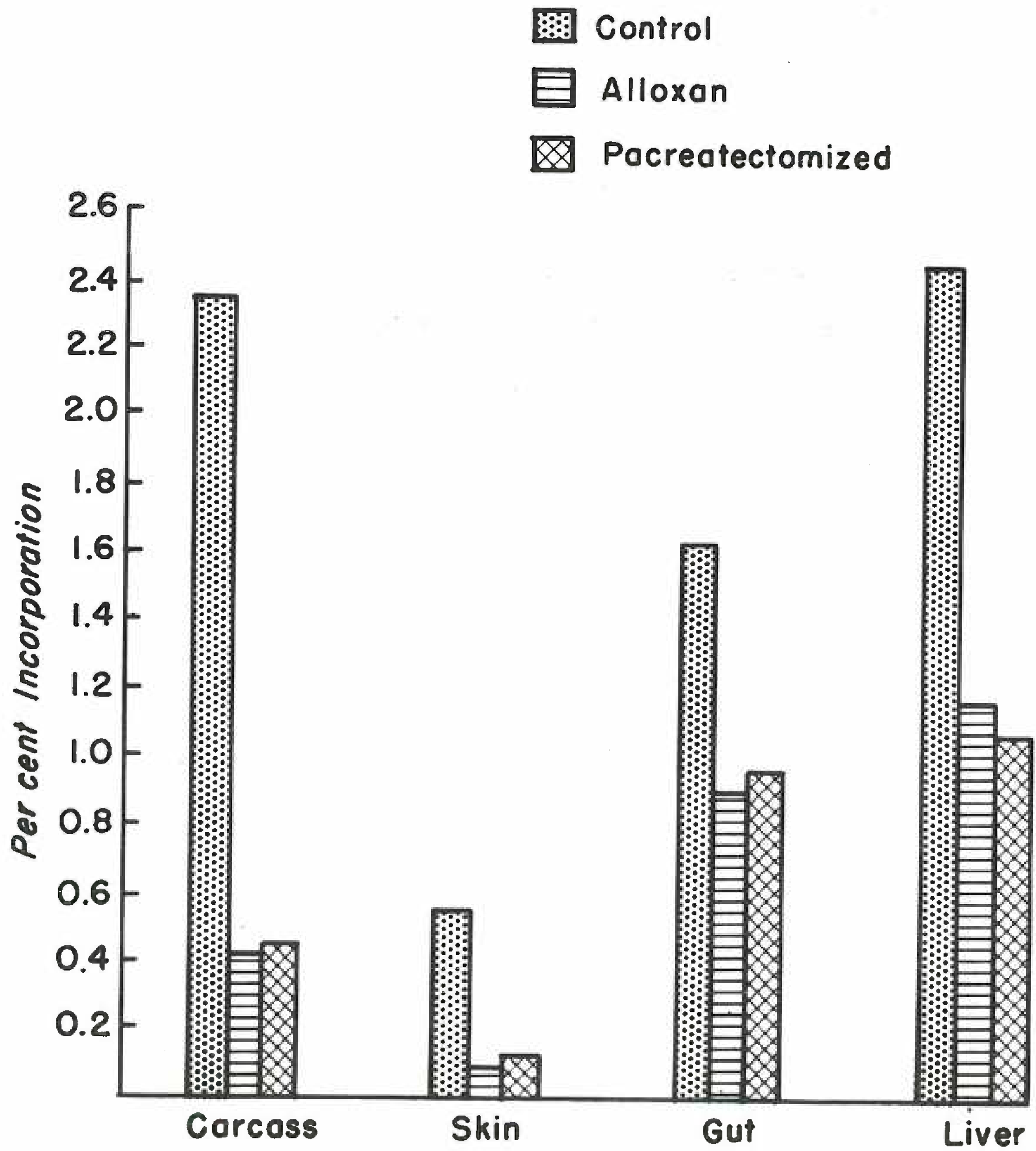


TABLE XII

SPECIFIC ACTIVITIES OF LIPIDS

OF THE CARCASS FRACTIONS

AT VARIOUS TIME INTERVALS

TIME (days)	CONTROL			ALLOXAN			PANCREATECTOMIZED		
	NO. RATS	CHOL.	F.ACID	NO. RATS	CHOL.	F.ACID	NO. RATS	CHOL.	F.ACID
0	10	51.6 (36.2-85.3)	34.4 (17.4-77.9)	5	23.3 (19.0-32.5)	8.4 (2.7-18.7)	6	37.3 (27.1-53.5)	9.3 (2.1-17.0)
2	2	72.0 (35.0-109)	59.0 (56.4-60.8)	4	36.9 (27.4-49.4)	6.1 (2.7-12.0)	4	29.5 (18.6-38.9)	12.9 (10.0-17.2)
4	2	56.8 (45.4-68.2)	24.6 (19.8-29.3)	4	23.4 (18.7-28.3)	4.1 (2.6-6.6)	4	37.7 (26.5-47.3)	8.5 (6.2-11.3)
7	2	31.2 (27.6-34.7)	27.4 (26.8-28.0)	5	17.6 (12.1-22.4)	2.8 (1.1-8.7)	3	15.4 (11.2-18.7)	6.9 (3.3-10.4)
14	2	20.4 (19.7-21.2)	16.0 (12.0-20.0)	2	9.5 (8.9-10.0)	<2.0			
21	2	15.2 (13.7-16.7)	19.9 (19.1-20.6)	2	8.6 (1.2-16.0)	<2.0			
28	2	10.4 (7.9-13.0)	6.8 (5.7-8.0)						

Figures represent means $\pm$ ranges of values.

TABLE XIII

SPECIFIC ACTIVITIES OF LIPIDS

OF THE SKIN FRACTIONS

AT VARIOUS TIME INTERVALS

TIME (days)	CONTROL NO. RATS	CONTROL CHOL.	CONTROL F.ACID	ALLOXAN NO. RATS	ALLOXAN CHOL.	ALLOXAN F.ACID	PANCREATECTOMIZED NO. RATS	PANCREATECTOMIZED CHOL.	PANCREATECTOMIZED F.ACID
0	10	50.4 (26.4-97.6)	16.9 (9.0-24.6)	5	15.9 (9.7-28.4)	10.2 (5.4-17.0)	6	24.7 (15.7-36.9)	10.8 (7.7-25.5)
2	2	53.2 (46.3-60.1)	23.7 (23.3-24.0)	4	26.2 (17.7-43.6)	6.0 (1.8-9.6)	4	33.4 (8.4-72.1)	30.6 (9.0-63.9)
4	2	74.5 (34.0-115.0)	18.0 (13.4-22.7)	4	28.1 (18.2-36.2)	13.2 (4.2-27.8)	4	21.9 (18.1-24.9)	8.6 (4.3-11.2)
7	2	36.8 (33.9-39.6)	8.8 (8.1-9.4)	5	36.8 (15.9-73.7)	9.0 (5.5-13.4)	3	17.7 (13.5-23.4)	8.5 (8.0-9.0)
14	2	11.1 (10.7-11.5)	8.4 (7.3-9.6)	2	9.4 (6.1-12.7)	--			
21	2	6.7 (5.5-7.9)	5.8 (4.6-7.1)	2	14.8 (13.0-16.6)	--			
28	2	5.8 (5.7-6.0)	3.5 (3.1-3.9)						

Figures represent means ± ranges of values

TABLE XIV

SPECIFIC ACTIVITIES OF LIPIDS

OF THE GUT FRACTIONS

AT VARIOUS TIME INTERVALS

TIME (days)	CONTROL NO. RATS	CONTROL CHOL.	CONTROL F.ACID	ALLOXAN NO. RATS	ALLOXAN CHOL.	ALLOXAN F.ACID	PANCREATECTOMIZED NO. RATS	PANCREATECTOMIZED CHOL.	PANCREATECTOMIZED F.ACID
0	10	266.4 (122.8-435.0)	171.8 (61.2-320.0)	5	154.0 (101.0-244.0)	126.2 (64.8-180.0)	6	175.0 (118.0 226.0)	105.8 (29.1-167.0)
2	2	92.3 (89.3-95.3)	212.0 (157.0-266.0)	4	101.0 (86.4-107.0)	41.7 (37.6-45.9)	4	82.1 (60.5-124.5)	46.6 (29.4-69.2)
4	2	54.2 (29.6-78.8)	81.2 (23.4-139.0)	4	31.6 (23.5-36.4)	6.2 (3.4-7.8)	4	43.0 (29.2-61.1)	13.0 (8.3-20.2)
7	2	27.6 (25.1-30.1)	107.0 (86.5-127.0)	5	28.4 (18.2-36.7)	4.8 (1.5-10.8)	3	19.8 (18.1-20.9)	11.6 (8.1-13.5)
14	2	24.7 (22.2-27.2)	36.1 (28.2-44.0)	2	6.2 (5.5-7.0)				
21	2	18.1 (17.4-18.8)	60.0 (35.9-84.1)	2	5.6 (2.8-8.4)				
28	2	10.6 (9.7-11.4)	24.3 (18.9-29.7)						

Figures represent means ± ranges of values

TABLE XV

SPECIFIC ACTIVITIES OF LIPIDS

OF THE LIVER FRACTIONS

AT VARIOUS TIME INTERVALS

	CONTROL			ALLOXAN			PANCREATECTOMIZED		
TIME (days)	NO. RATS	CHOL.	F.ACID	NO. RATS	CHOL.	F.ACID	NO. RATS	CHOL.	F.ACID
0	10	460.8 (140.0 760.0)	87.5 (18.0-198.0)	5	85.9 (39.9-149.0)	28.8 (15.5-43.7)	6	217.0 (20.0-653.0)	29.9 (7.2-52.7)
2	2	62.9 (57.1-68.7)	102.0 (88.0-115.0)	4	79.4 (54.0-113.0)	12.6 (10.9-15.2)	4	63.6 (41.6-75.9	17.8 (16.3-19.4)
4	2	57.5 (27.2-87.8)	22.3 (19.0-25.0)	4	42.1 (30.7-56.7)	5.5 (4.6-6.1)	4	51.0 (32.2-71.4)	11.3 (8.1-14.0)
7	2	39.0 (27.3-50.6)	19.7 (13.0-26.5)	5	18.9 (5.4-30.9)	3.8 (1.2-8.0)	3	15.6 (8.1-25.2)	5.5 (3.8-6.6)
14	2	16.8 (11.6-22.1)	14.8 (7.6-22.0)	2	9.1 (7.5-10.7)				
21	2	11.4 (11.3-11.6)	12.8 (12.7-13.0)	2	8.5 (5.9-11.1)				
28	2	6.6 (4.9-8.3)	8.6 (6.8-10.4)						

Figures represent means ± ranges of values

TABLE XVI

SPECIFIC ACTIVITIES

OF SERUM CHOLESTEROL

AT VARIOUS TIME INTERVALS

	CONTROL		ALLOXAN		PANCREATECTOMIZED	
TIME (days)	NO. RATS	SPECIFIC ACTIVITY	NO. RATS	SPECIFIC ACTIVITY	NO. RATS	SPECIFIC ACTIVITY
0	2	109.4 (86.4-131.9)	3	72.3 (41.5-83.6)	4	138.3 (102.9-180.0)
2	2	78.3 (77.0-79.6)	4	83.2 (54.3-131.7)	4	69.9 (59.6-88.3)
4	2	61.6 (25.6-97.6)	3	50.2 (39.3-57.0)	4	56.5 (47.5-66.1)
7			2	13.5 (13.0-14.1)	3	17.6 (16.5-19.1)

Figures represent means ± ranges of values

time is dependent upon the amount of activity at zero time (t_0) and the rate of turnover of the lipids. For any one certain starting activity, (activity at t_0) the activity at time t (t_t) is proportional to the turnover of the lipid. For a steady state system in which the amount of lipid does not change, that is, synthesis is equal to decay, the activity in the lipid will decrease as a first order reaction, similar to the radioactive decay of nuclides[116]. This change in activity or decay is represented as a straight line function when the semilog of the activity is plotted against time. On the other hand, a mixture of nuclides, each decaying at its own rate, will give a hyperbolic curve which shows two or more components. By analogy, if one finds such a non-linear curve for biological data, it would appear that the assumed mono-molecular first order decay of the radioactivity may not be taking place. To understand the role of significance of the various reactions present in the mixture, this curve may be resolved into its components. The longest component is usually removed first by approximating the straight line to which the curve is asymptotic; the remaining components are then obtained by successive subtractions.

When the mean specific activities given in the tables were plotted as a function of time, we obtain the representative curves seen in Figures 5 through 13 (pages 71 - 75 and 81 - 84). A straight line was drawn wherever possible; otherwise curves were drawn to best fit the plotted points. Since it was difficult to predict the number of components in each curve, we have extracted only the two longest components from each curve and have interpreted these turnover curves to be composed predominantly of two rate components, a rapid and a slow component.

Figure 5

Specific Activity-Time Curves

of Carcass

Cholesterol

FIGURE 5

CARCASS CHOLESTEROL

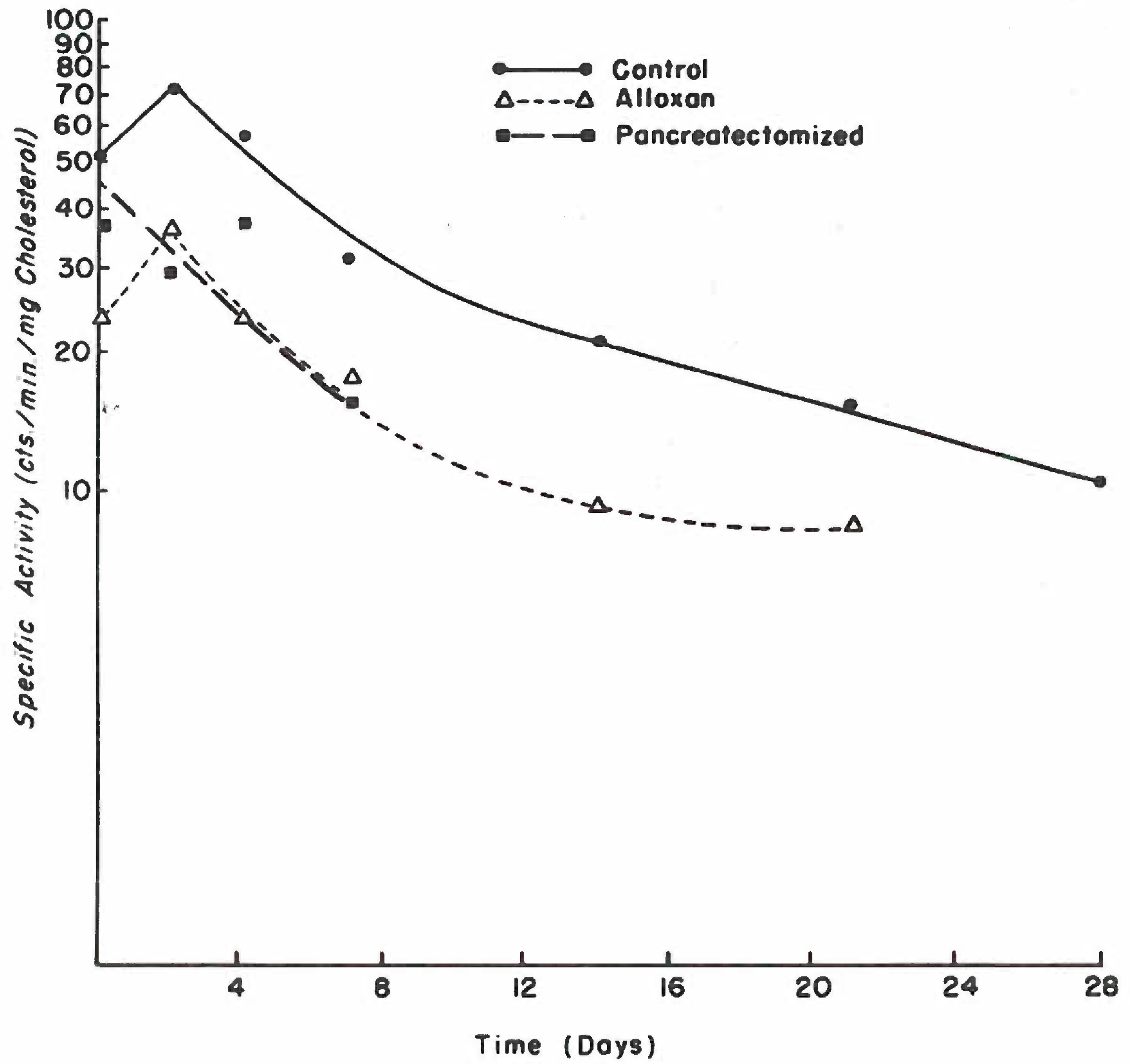


Figure 6

Specific Activity-Time Curves

of Skin

Cholesterol

FIGURE 6

SKIN CHOLESTEROL

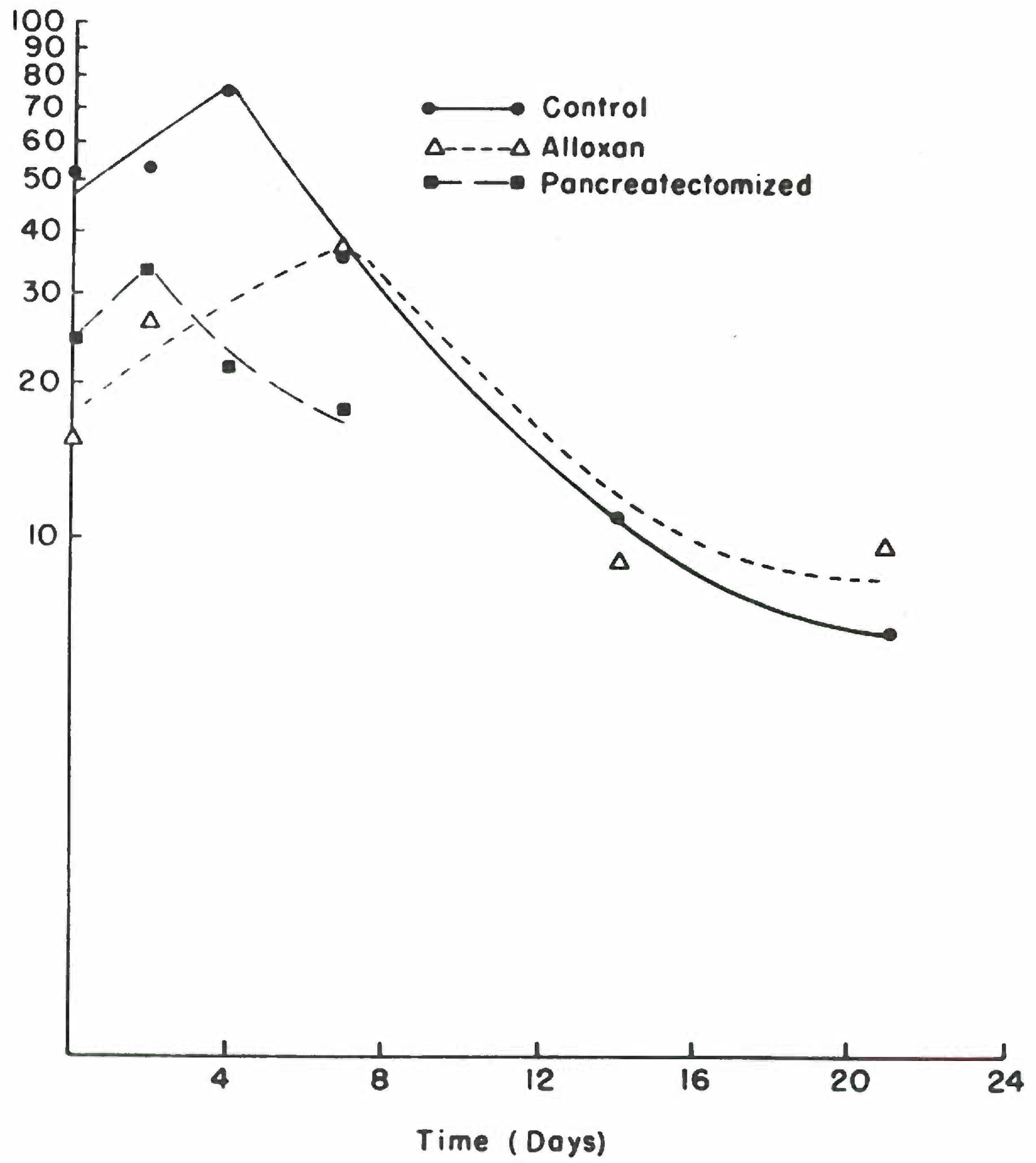


Figure 7

Specific Activity-Time Curves

of Gut

Cholesterol

FIGURE 7

GUT CHOLESTEROL

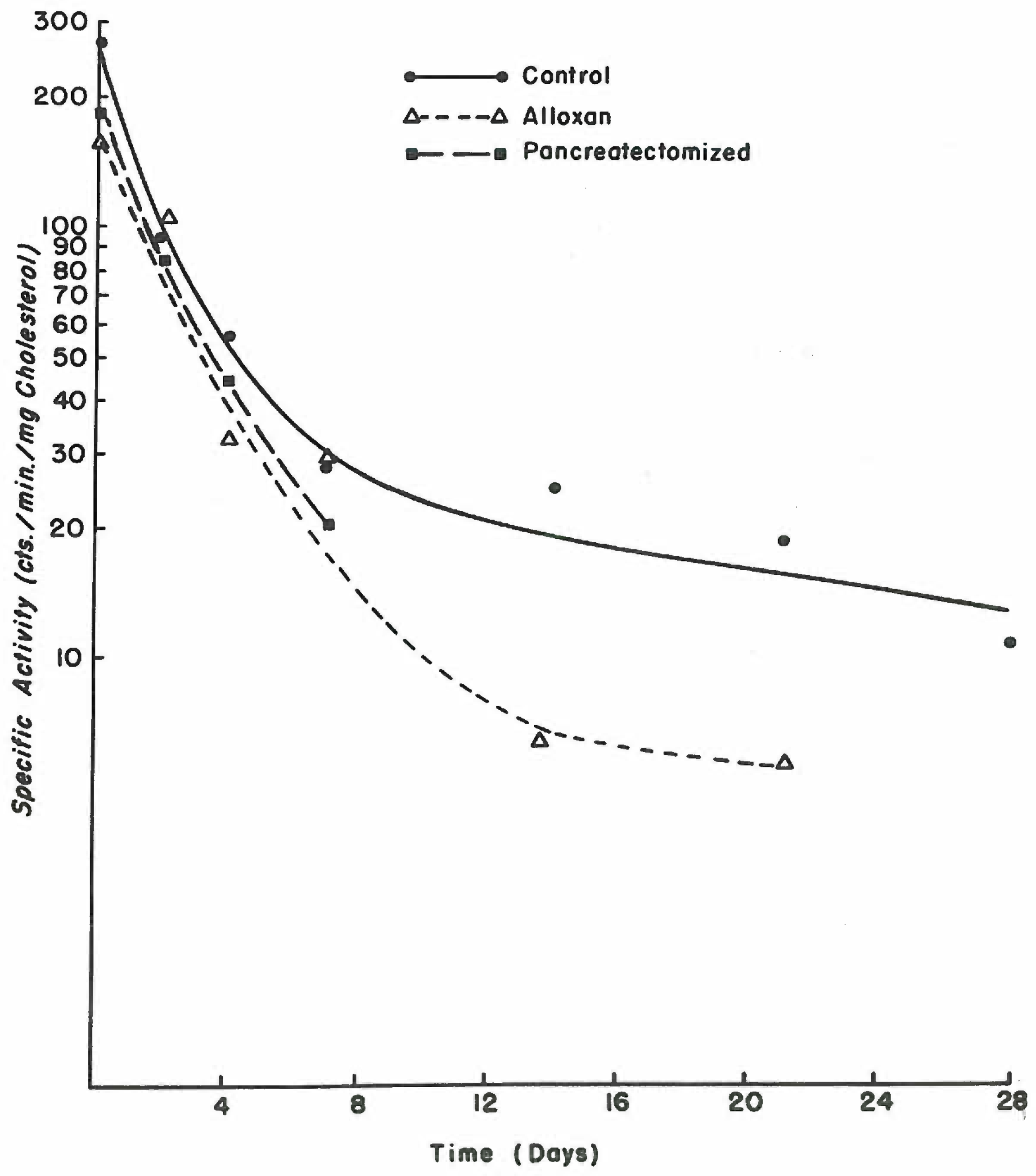


Figure 8

Specific Activity-Time Curves

of Liver

Cholesterol

FIGURE 8

LIVER CHOLESTEROL

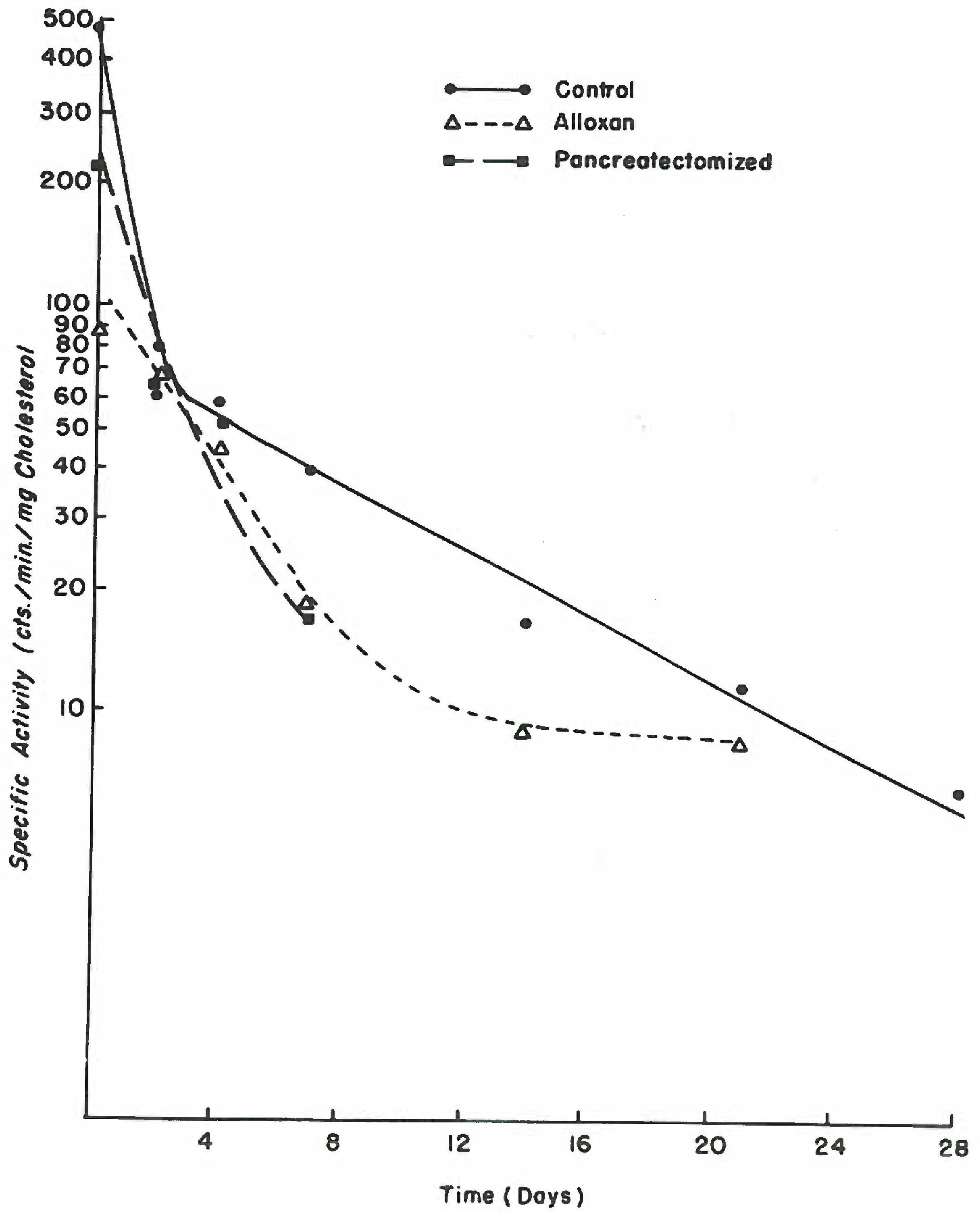


Figure 9

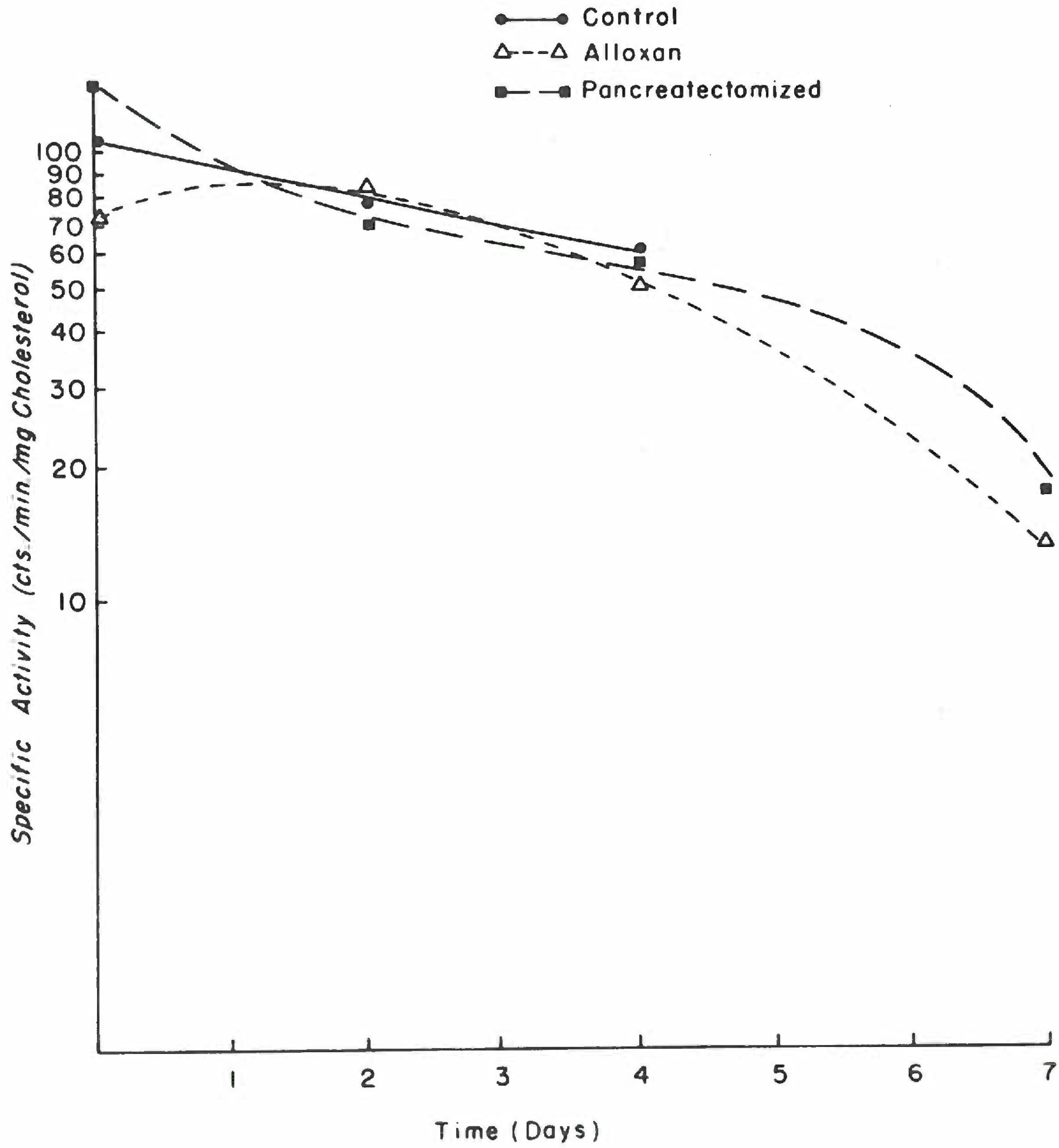
Specific Activity-Time Curves

of Serum

Cholesterol

FIGURE 9

SERUM CHOLESTEROL



skin for replacement of the cholesterol that is "turning over", and if the labelled cholesterol brought into the carcass and skin had a higher specific activity, then a net increase in carcass and skin cholesterol specific activities should result. If, on the other hand, the cholesterol brought into the carcass and skin had a lower specific activity, a decrease in carcass and skin cholesterol specific activities should result. The observed latent increase of specific activity of carcass and skin cholesterol fractions seems to indicate that labelled cholesterol of high specific activity was being transported to and deposited in these particular tissues.

A possible source of this high specific activity cholesterol is suggested. From an inspection of the tables it is clear that the liver and gut cholesterol specific activities are five to eight times higher than the carcass and skin. Thus both liver and gut might be sources of carcass and skin cholesterol. However, in relating the high specific activities of the gut and liver cholesterol to the latent increase in carcass and skin, one must also consider the fact that all cholesterol transported to the carcass and skin must pass by way of the blood. Thus the direct precursor of carcass and skin cholesterol would be blood cholesterol. The cholesterol levels recorded in Tables 12, 13, and 16 (pages 65, 66, and 69, respectively) indicate that the serum cholesterol activity levels are higher than those of the carcass and skin, but the difference in levels is not too great. It is conceivable that an investigation of free, ester, and lipoprotein cholesterol activity might reveal one of these forms to be the active transport form of cholesterol, having a specific activity high enough to account for the high specific activity precursor.

Another possible mechanism to explain the latent increase of carcass and skin cholesterol would be a conversion of other radioactive substances to cholesterol at these sites, but data on such reactions is lacking.

In the liver (Figure 8, page 74) the resemblance of all three curves is good during the first four days only. Figure 9 (page 75) shows a close correlation of the blood cholesterol levels of the alloxan and pancreatectomized animals. Figures 8 and 9 are considered together since liver and blood cholesterol are thought by many to be directly related. Leroy[118] has even considered the liver-plasma system as a large compartment with free cholesterol rapidly exchanging between the two tissues. If this were true, then one might expect similarly shaped curves for liver and blood cholesterol.

When comparing the liver and blood cholesterol curves, one need bear in mind that the blood cholesterol curves represent a time interval of one week only and that they may not reflect any differences which might occur in the liver after a week. An inspection of the following table (page 80), which was abstracted from Tables XV and XVI, reveals that at zero time the specific activities of the liver cholesterol is higher than the blood cholesterol in the control group (461 vs. 109), in the alloxan group (86 vs. 72), and in the pancreatectomized group (217 vs. 138).

SPECIFIC ACTIVITIES OF CHOLESTEROL

TIME	CONTROL		ALLOXAN		PANCREATECTOMIZED	
(days)	Liver	Serum	Liver	Serum	Liver	Serum
0	461	109	86	72	217	138
2	63	78	79	83	64	70
4	57	62	42	50	51	56

These data indicate that plasma cholesterol may be derived from the liver. However, after two and four days the blood cholesterol specific activities are higher than the liver cholesterol at the corresponding times, introducing some doubt on the sole hepatic control of blood cholesterol. It may be possible that blood is receiving a supply of cholesterol from other tissues and, as already implicated, other tissues may be receptors of liver cholesterol.

The fatty acid curves as depicted in Figures 10 through 13 (pages 81 - 84) are now considered. The continuous low specific activity levels of fatty acids in the diabetic, the result of a decreased synthesis of fatty acids, makes interpretation of long term turnover of fatty acid in the diabetic merely speculative. In Figure 10 (page 81), concerning the carcass fatty acids, there is an initial rise of specific activity in the control and pancreatectomized curves. In the skin, Figure 11 (page 82), all three curves again have this initial rise in specific activity and

Figure 10

Specific Activity-Time Curves

of Carcass

Fatty Acid

FIGURE 10

CARCASS FATTY ACID

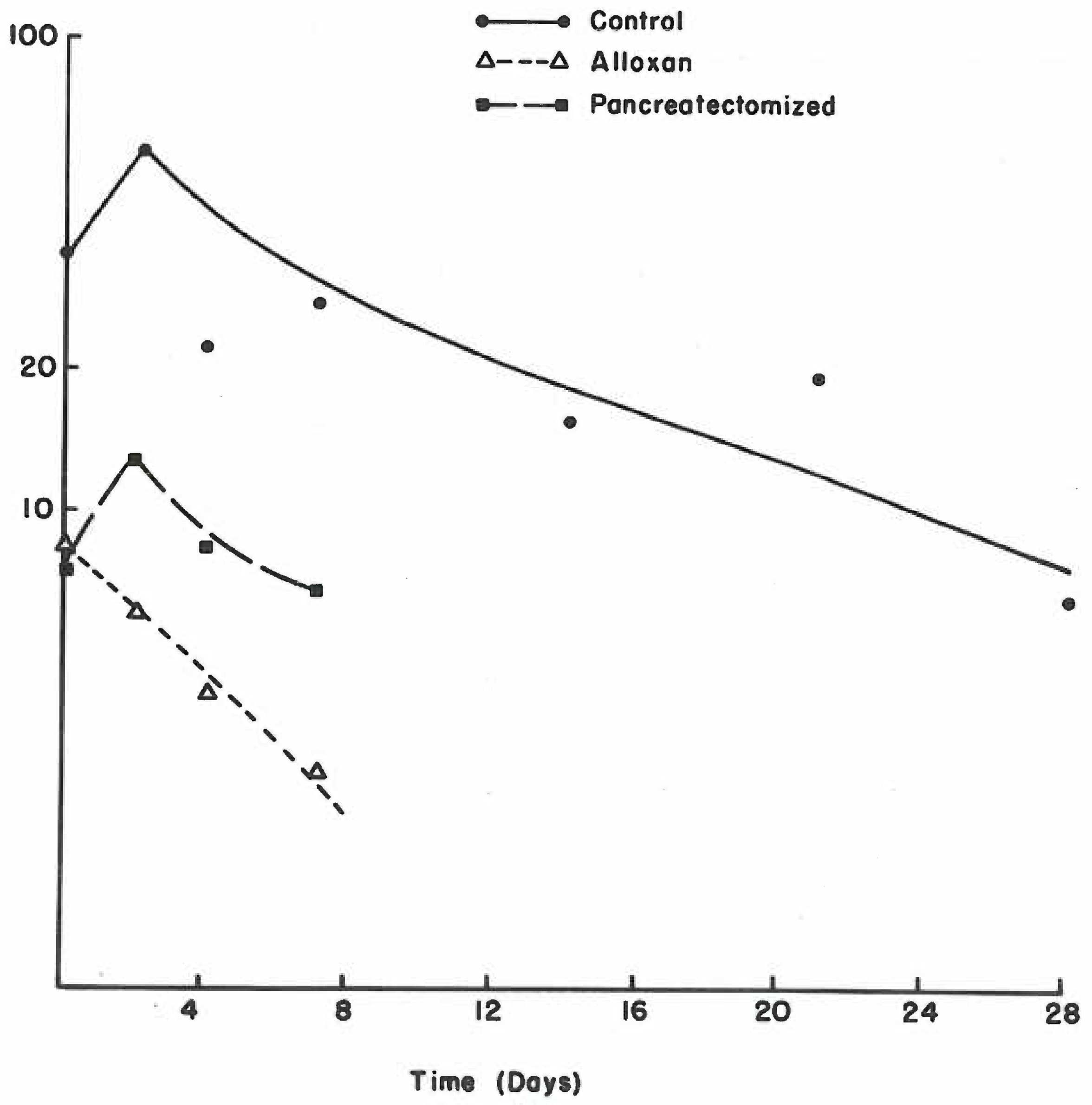


Figure 11

Specific Activity-Time Curves

of Skin

Fatty Acid

FIGURE 11

SKIN FATTY ACID

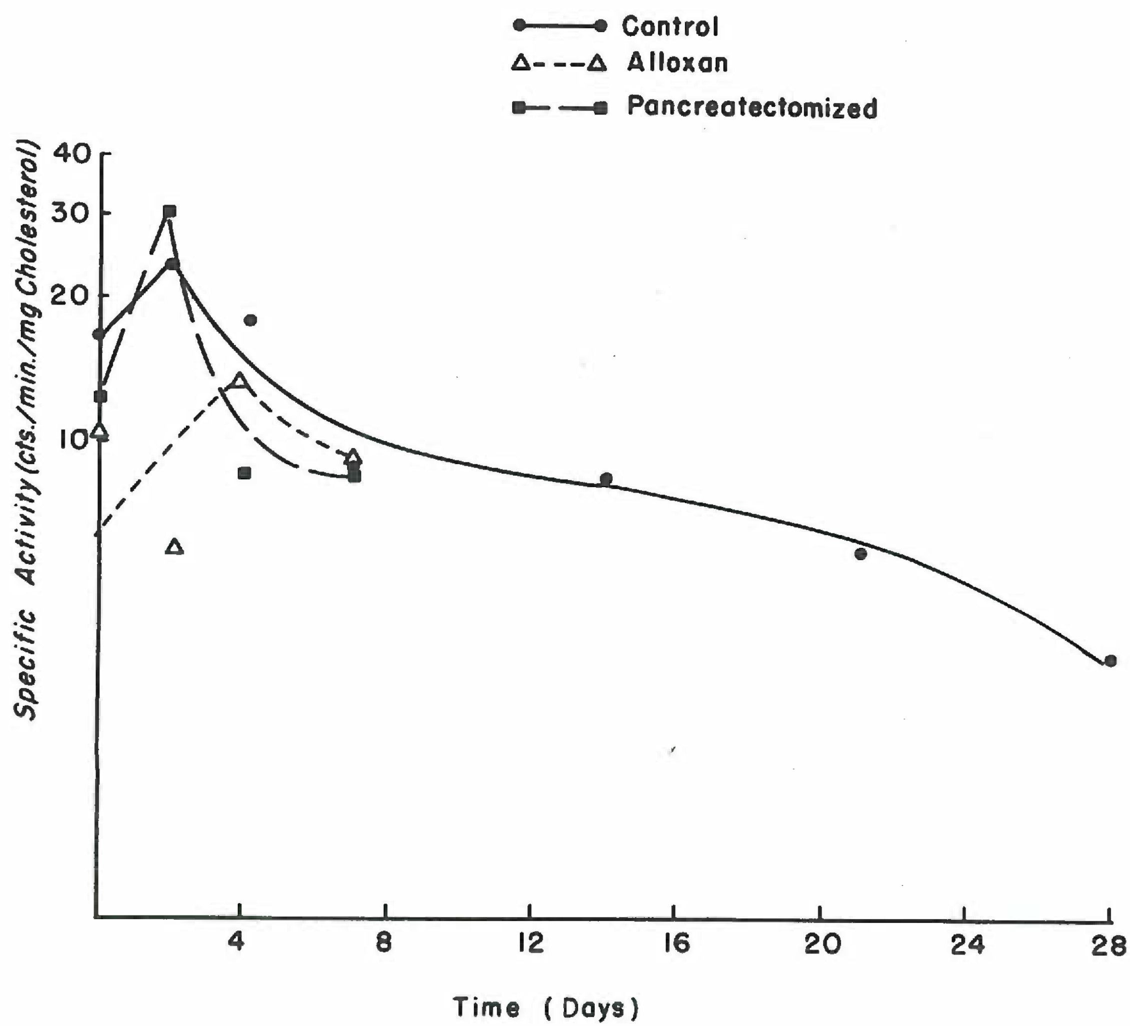


Figure 12

Specific Activity-Time Curves

of Gut

Fatty Acid

FIGURE 12

GUT FATTY ACID

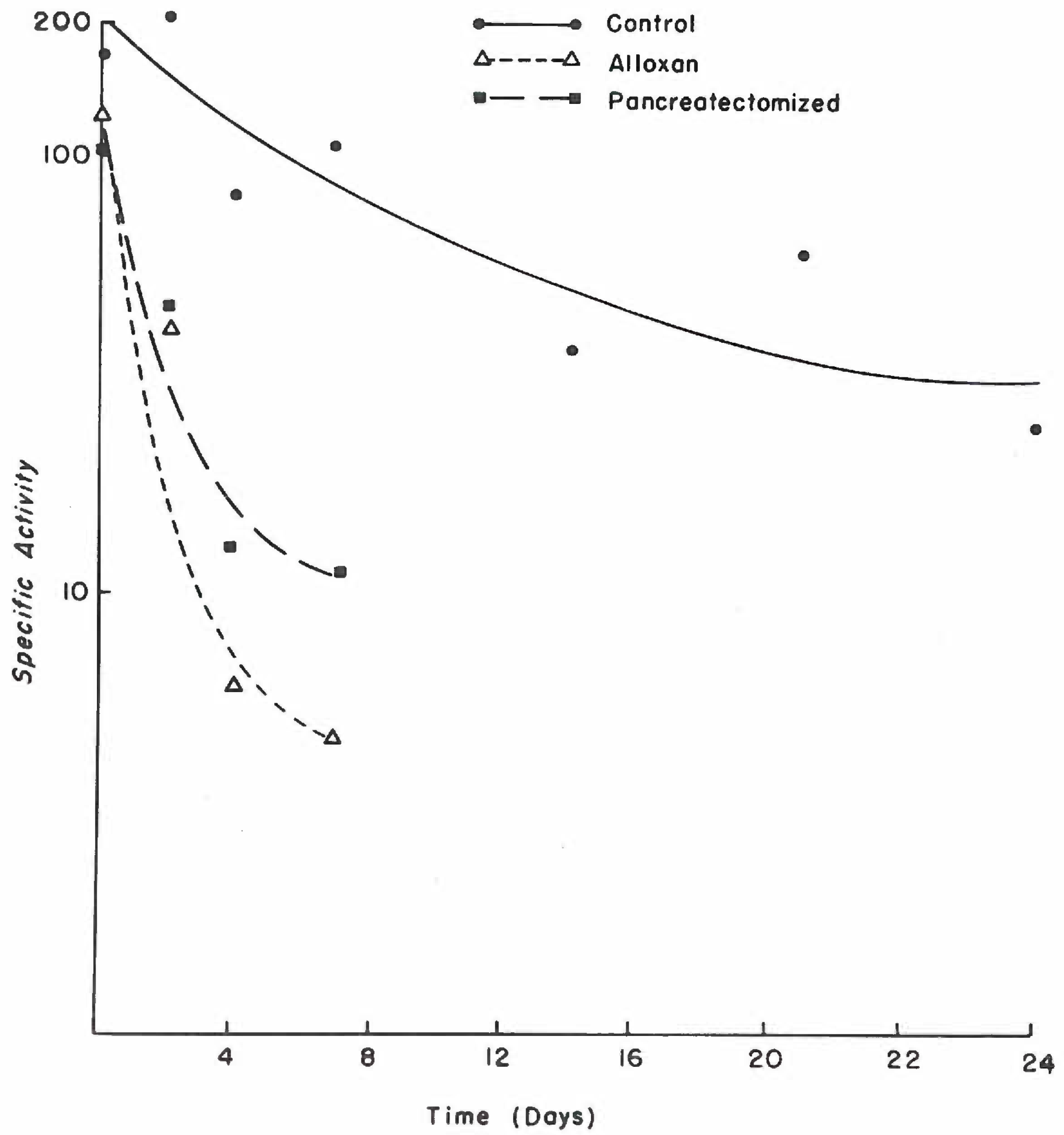


Figure 13

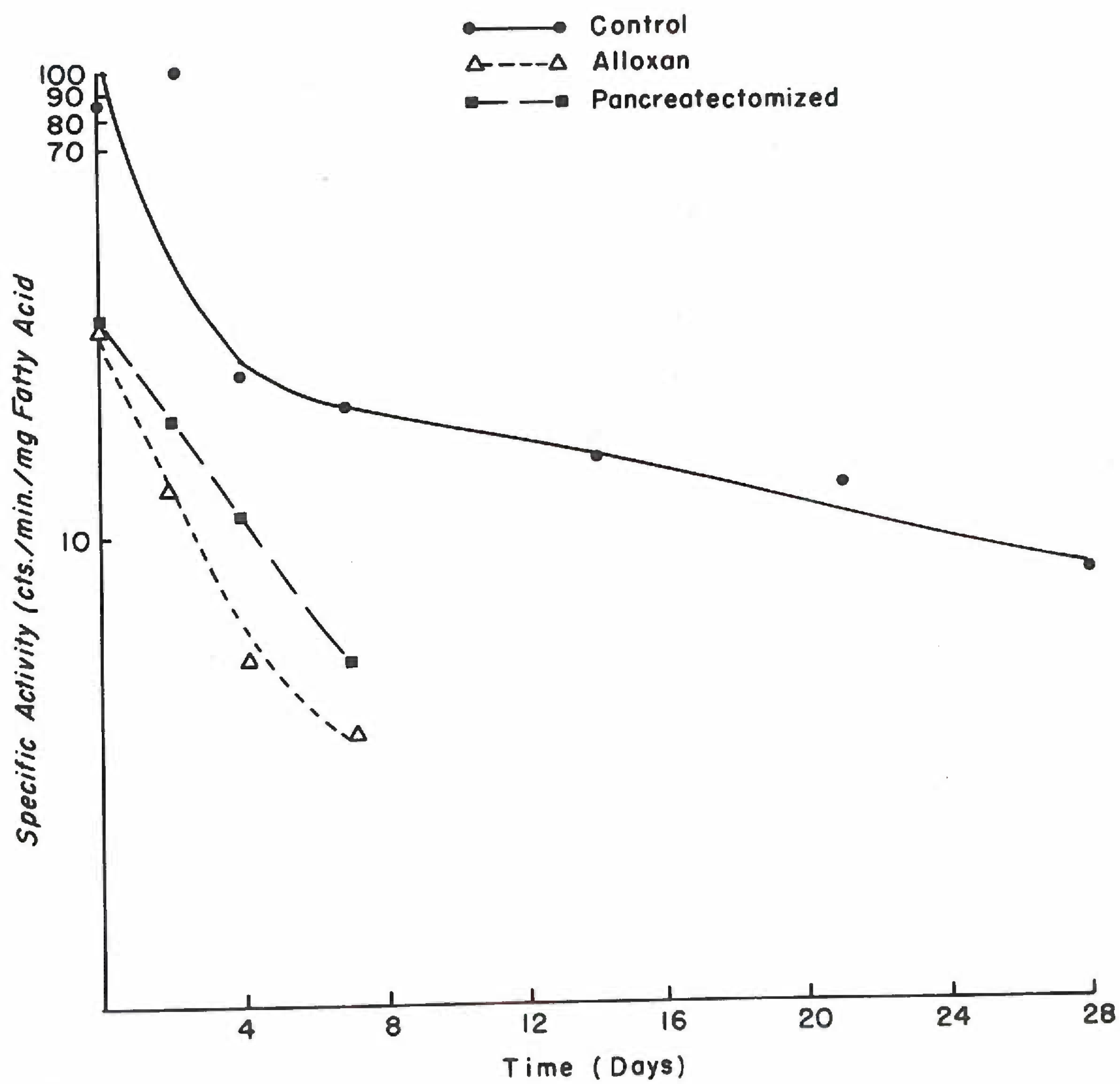
Specific Activity-Time Curves

of Liver

Fatty Acid

FIGURE 13

LIVER-FATTY ACID



all three resemble each other in slope. Like cholesterol, this latent rise of specific activity of fatty acids may be due to a transport from other sites of synthesis or a synthesis from labeled precursors derived from other already formed labeled compounds.

The gut fatty acid curves, Figure 12 (page 83), show that alloxan and pancreatectomized animals behave similarly. The diabetic differs from the control in having a more rapid decay even though both preparations start at a similar level.

The liver fatty acids as shown in Figure 13 (page 84) show the control curve to be resolvable into two components. The alloxan and pancreatectomized curves resemble each other but differ slightly from the control.

Better to illustrate lipid turnover, we have quantitated our observations by presenting in Table 17 (page 86) five parameters of turnover, the first of which was derived from Table 3 (page 62), and the other four from the turnover curves. These parameters are defined since there exists some confusion of nomenclature in the literature.

1. Lipid level: represents the mg. of lipid in 100 gm of tissue (wet weight).

2. Half-life ($T_{1/2}$) refers to the time necessary for one half of a component to disappear. This can be obtained from the graph directly or calculated using equations for first order reactions, as given:

TABLE XVII

TURNOVER OF LIPIDS IN TISSUES

	CHOLESTEROL			FATTY ACID		
	Control	Alloxan	Pan-x	Control	Alloxan	Pan-x
CARCASS						
Lipid level - mg. %	148	162	162	4700	3620	2930
Half-life - days						
a. rapid component	3.6	4.4	4.4	10	4.2	4.4
b. slow component	14.0	27.6				
Turnover time - days						
a. rapid component	5.2	6.3	6.3	14.4	6.0	6.3
b. slow component	20.2	39.7				
Per cent turnover/day						
a. rapid component	0.19	0.16	0.16	0.07	0.17	0.16
b. slow component	0.05	0.025				
Turnover rate - mg %/day						
a. rapid component	28.1	25.9	25.9	324	615	469
b. slow component	7.4	4.0				
SKIN						
Lipid level - mg. %	412	563	548	10,660	4050	2550
Half-life - days						
a. rapid component	3.8	4.0	5.4	11.2	6.0	4.0
b. slow component	9.2	16.0				
Turnover time - days						
a. rapid component	5.5	5.8	7.8	16.1	8.6	5.8
b. slow component	13.2	23.0				
Percent turnover/day						
a. rapid component	0.18	0.17	0.13	0.06	0.12	0.17
b. slow component	0.08	0.04				
Turnover rate - mg %/day						
a. rapid component	74.2	95.7	71.2	661	486	434
b. slow component	33.0	22.5				
GUT						
Lipid level - mg. %	157	154	93	3170	1690	1490
Half-life - days						
a. rapid component	2.0	2.2	2.0	10	1.2	1.8
b. slow component	16.0	24.0				
Turnover time - days						
a. rapid component	2.9	3.2	2.9	14.1	1.7	2.6
b. slow component	23.0	34.6				
Per cent turnover/day						
a. rapid component	0.35	0.31	0.35	0.07	0.59	0.38
b. slow component	0.04	0.03				
Turnover rate - mg %/day						
a. rapid component	55	47.7	32.6	222	997	566
b. slow component	6.3	4.6				

(continued on next page)

TABLE XVII TURNOVER OF LIPIDS IN TISSUES (continued)

	CHOLESTEROL			FATTY ACID		
	Control	Alloxan	Pan-x	Control	Alloxan	Pan-x
LIVER						
Lipid Level - mg. %	215	218	178	2650	2820	2980
Half-life - days						
a. rapid component	0.7	2.8	1.8	1.6	2.0	2.8
b. slow component	7.6	33.0		17.6		
Turnover time - days						
a. rapid component	1.0	4.0	2.6	2.3	2.9	4.0
b. slow component	10.9	47.5		25.3		
Per cent turnover/day						
a. rapid component	1.0	0.25	0.38	0.43	0.35	0.25
b. slow component	0.09	0.02		0.04		
Turnover rate - mg %/day						
a. rapid component	215	54.5	67.6	1140	987	745
b. slow component	19.4	4.6		106		
BLOOD						
Lipid level - mg. %	87.5	113.4	119.0			
Half-life - days	4.4	2.7	2.7			
Turnover time - days	6.3	3.9	3.9			
Per cent turnover/day	0.16	0.26	0.26			
Turnover rate-mg %/day	14.0	29.5	29.5			

$A_t = A_o e^{-kt}$ and $T_{1/2} = \ln 2/k$

A_t = specific activity at time t

A_o = specific activity at time zero

k = fractional turnover constant

3. Turnover time (T_t): refers to the time required for the turnover of an amount of material equal to the pool size. This value is obtained by multiplying the half-life by 1.44. Since T_t is the reciprocal of the fractional turnover (see below), it is equal to the following:

$$T_t = T_{1/2}/\ln 2 = T_{1/2}/0.693 = 1.44 \times T_{1/2}$$

4. Per cent turnover/day or fractional turnover: is the fraction of a given pool turned over per unit time. It is equal to:

$$k = \ln 2/T_{1/2}$$

Since k = the reciprocal of T_t, and since T_t had already been calculated as shown above in 3, k was obtained by the following:

$$k = 1/T_t$$

5. Turnover rate or absolute turnover: is the quantity of material turned over per unit time. Here it is expressed as the mg. per cent per day.

For cholesterol there are no differences between the half-lives of the rapid component of the alloxan and pancreatectomized animals in every tissue studied. In comparing the rapid component of each tissue of the

control and diabetic animals, only the liver and blood tissues differ. The cholesterol of the diabetic liver has a lower turnover rate than the control liver, being 54.5 mg. per cent per day for the alloxan and 67.6 mg. per cent per day for the pancreatectomized animals as compared to the 215 mg. per cent per day for the control animals. The turnover of only one-fourth as much cholesterol per day by the diabetic liver as compared to the controls may result from a lesion in cholesterol turnover by the diabetic liver. In the presence of a decreased turnover one would expect an accumulation of cholesterol in the liver. We find no accumulation of cholesterol in this tissue, but find a decreased rate of synthesis of cholesterol. Thus in the diabetic liver of the rat there may be present a decreased synthesis and a decreased degradation of cholesterol, resulting in no net accumulation.

The turnover of blood cholesterol evaluated from the shape of the curves of Figure 9 (page 75) is tabulated in Table XVII (page 87), but at this point little significance is attributed to the differences that are seen. It is apparent, however, that the hypercholesteremia associated with diabetes cannot be attributed to a grossly decreased turnover of cholesterol in the blood, since this was not found to be true.

In analyzing the slow component of cholesterol turnover, a comparison is made between the control and alloxan animals, since no values are available for the pancreatectomized animals. The tissues of the alloxan animals again have a longer half-life of cholesterol than the control, suggesting a decreased turnover of cholesterol. This decreased turnover by the diabetic probably would affect the decreased synthesis we also observed in the diabetic.

For fatty acids, the half-lives of the fatty acids of the alloxan and pancreatectomized animals show no differences, but the control and diabetic carcass, skin, and gut differ in that the turnover was much faster in the diabetic, resulting probably from the increased utilization of fatty acids by the diabetic. The rapid component of the control liver is similar to that of the diabetic.

IV. SUMMARY

The following conclusions are drawn from the study of lipid synthesis and turnover in alloxan, pancreatectomized, and control rats, using acetate-1-C^{14} as the radioactive tracer.

1. A method is described for the production of experimental diabetes with alloxan, which produced permanent diabetes in 85 per cent of the animals injected.

2. Experimental diabetes was found to cause a redistribution of body weights of the rats so that there are more gut and liver and less carcass and skin in the diabetic as compared to the control rats.

3. Radioacetate injected into a rat was rapidly metabolized as shown by the attainment of maximum specific activity of respiratory carbon dioxide within the first thirty minutes after injection. After two hours, most of the acetate was metabolized. The carbon dioxide expired by the alloxan and pancreatectomized rats in two hours did not differ in total amount, specific activity, and per cent incorporation of label, although the mechanism of acetate oxidation during the first thirty minutes was shown to be somewhat different. When the pooled diabetic animals were compared to the controls, it was seen that, although both groups produced the same amount of carbon dioxide, the diabetic group produced carbon dioxide of higher specific activity and thus incorporated a higher per cent of the labeled acetate.

4. From the measurements of concentrations of lipid, specific activity, and per cent incorporation of label into lipids, it was found that the alloxan and pancreatectomized animals did not differ in their ability to synthesize lipids. Compared to the control animals, the diabetic animals did not differ in tissue cholesterol concentrations, but had a decreased fatty acid concentration. The diabetic rats also had a decreased specific activity of lipids and a decreased per cent incorporation of label into the lipids, indicating an impaired ability to synthesize fatty acid and cholesterol.

5. The alloxan and pancreatectomized animals did not differ from each other in the measurements of lipid turnover. The cholesterol of the diabetic rats had a longer half-life than that of the control, while the fatty acids of the diabetic tissues, excepting the liver, had a shorter half-life than those of the control. Postulated mechanisms of lipid transport and turnover are discussed.

6. The results of this investigation lead us to propose the following theory of cholesterol metabolism in the diabetic. We believe there is a defect in the mechanism of cholesterol degradation in the diabetic rat. According to one of the fundamental concepts of physiology, as proposed by Claude Bernard, any disturbance of the physiological state of an animal would result in the activation of certain physiological mechanisms to reinstate homeostasis. Thus our finding of a decreased cholesterol synthesis would indicate certain compensatory mechanisms activated in the direction of decreased degradation, and our finding

of normal levels of cholesterol in the diabetic rat would indicate the re-establishment of homeostatic states.

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