METABOLISM OF THE ISOLATED SURVIVING GUINEA PIG HEART

by

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LIST OF ABBREVIATIONS

ATP	-	adenosine-5'-triphosphate
CP	-	creatine phosphate
CoA	-	coenzyme A
DHAP	-	dihydroxyacetone phosphate
DNP	-	2,4-dinitrophenol
F6P	-	fructose-6-phosphate
G6P	-	glucose-6-phosphate
HMP	-	hexose monophosphate
mu	-	milli-units (insulin)
NAD	-	nicotinamide adenine dinucleotide (oxidized)
NADH	-	nicotinamide adenine dinucleotide (reduced)
NADP	-	nicotinamide adenine dinucleotide phosphate
S.E.M.	-	standard error of the mean
TCA	-	trichloroacetic acid
UDPG	-	uridine diphosphate glucose

CHAPTER 1

INTRODUCTION

"What hinders the different parts of the body from having a merely accidental relation in nature? As the teeth, for example, grow by necessity, the front ones sharp, adapted for dividing, and the grinders flat, and serviceable for mastication of food; since they were not made for the sake of this, but it was the result of accident. And in like manner as to other parts in which there appears to exist an adaptation to an end. Wheresoever, therefore, all things together (that is all the parts of the whole) happened like as if they were made for the sake of something, these were preserved, having been appropriately constituted by an internal spontaneity; and whatever things were not thus constituted, perished and still perish."

Aristotle (1)

"Natural selection implies the preservation of such variations as arise and are beneficial to the being under its conditions of life - - - - If we take as the standard of high organization, the amount of differentiation and specialization of the several organs in each being when adult, natural selection clearly leads toward this standard: for all physiologists admit that the specialization of organs, inasmuch as in this state they perform their functions better, is an advantage of each being."

Charles Darwin (2)

The principle of natural selection was applied by Darwin to the individual organ as well as to the organism. Each organ may be considered the result of selections through perhaps many millions of generations, arriving at its present level of development and specialization primarily by way of natural selection. The mammalian heart is a striking example of an organ which has evolved to do a specific task with a high degree of efficiency. The primary function of the heart is that of pumping fluid nutritive material to the organism. As this enormous task must be performed continuously throughout the life of the organism, it would be expected that such a function would require a highly versatile and specialized organ. The heart is capable of deriving the energy for its contraction from every fuel available from the blood (3). It regenerates its power of contraction in a very brief increment of time, may work independently of nervous influences, and possesses a regulatory mechanism for varying its force of contraction. Although the purpose of the present work is to explore some of the metabolic sequences which take place in the heart, a few particulars concerning the architecture of cardiac muscle will be discussed in an attempt better to understand how these metabolic events may occur, and how the energy thus derived is utilized to do work.

THE STRUCTURE OF CARDIAC MUSCLE

Stenson (4), in 1664, first propounded the muscular nature of the heart. Later studies using the light microscope confirmed the structural similarity between heart and skeletal muscle. Heart muscle fibers have transverse and longitudinal striations which resemble very closely those seen in skeletal

muscle. Cardiac muscle fibers are less regular in shape than are those of skeletal muscle, and when sectioned longitudinally they are seen to give off branches, effecting anastomoses with adjacent fibers (5). The branching nature of cardiac muscle fibers has in the past led to the belief that the whole heart muscle formed a syncytium, i.e. a mass with no discernible cell boundaries. Recent findings made with the electron microscope have brought about a change of view (6). Cohn suggested as early as 1932 (7) that cardiac muscle fibers possess a sarcolemma or muscle cell membrane, but that it is much finer and more difficult to demonstrate than in voluntary muscle. Separating the cardiac muscle fibers into segments, each of which usually contains a single nucleus, are very thin transverse bands known as intercalated discs. The significance of these structures as cell boundaries has been made doubtful, however, by the fact that they appear late in the development of the organism and increase in number with age - unaccompanied, it is believed, by corresponding cell division (5). The sarcosomes are present in far greater abundance in heart than in skeletal muscle (8). Apart from the above-mentioned characteristics, heart muscle does not differ extensively from skeletal muscle (9).

HEART MUSCLE MITOCHONDRIA - THE SARCOSOMES

Holmgren (10) and Bullard (11) made detailed studies of the granules present in heart muscle. Holmgren classified striated muscle as falling into two types, depending on the distribution and concentration of these granules, and suggested that these two classes correspond to two physiological types. Muscles with granules at the level of the isotropic bands

of the myofibrils are those with intermittent activity, such as skeletal muscle of many vertebrates and invertebrates. Granules at the level of the anisotropic bands are found in muscles required for sustained activity, such as the flight muscles of birds and insects, and the heart muscle of vertebrates. The latter type of muscle was found to contain more and larger granules than the former type. More recently, Holmgren's classification has been shown to have a biochemical basis. Keilin (12) showed that the muscles of the former type are rich in glycolytic enzymes, while the metabolism of the latter is predominantly aerobic.

In 1909 Regaud (13) concluded, on the evidence of staining reactions, that sarcosomes were identical with mitochondria. Differential centrifugation, introduced by Bensley and Hoerr (14) and by Claude (15) made it possible for later workers to show conclusively that muscle sarcosomes from many sources were microscopically identical with mitochondria (16) and that they contained the respiratory enzymes characteristic of mitochondria (17,18,19).

The respiratory activities of different muscles were compared with their mitochondrial densities by Paul and Sperling (20). Their results show that white muscle containing relatively few mitochondria has a low respiratory activity, while red muscle which is rich in mitochondria has a high respiratory activity. The red color was shown to be due to myoglobin, which aids in the transfer of oxygen from the blood to the respiratory enzymes in the mitochondria. Heart muscle contains a greater number of mitochondria (8) and more myoglobin and cytochrome oxidase (21) than most other types of muscle. The only muscles that have respiratory activity equal to that of heart muscle are the breast muscles of certain flying birds (20). These data, taken collectively, clearly show that heart muscle possesses the necessary enzymes for oxidative metabolism concomitant with its function.

MUSCULAR CONTRACTION

An enormous body of evidence has accumulated, pointing to the fact that actomyosin is the main protein involved in muscular contraction, and that muscular contraction derives from the interaction of actomyosin with ATP (22, 23). In resting muscle the structural proteins of the fibril, actin and myosin, exist side by side in a dissociated form separated from one another by the strong positive ionic atmosphere provided by potassium ions. When a stimulus causes the ionic balance of the muscle to change, actin and myosin combine to form the contractile protein, actomyosin, which in turn forms a complex with high energy phosphate. Thus, excitation results in actomyosin formation. It has been shown that the hydrolysis of ATP is required for relaxation to occur. Actomyosin, as well as myosin is capable of splitting ATP (23). The interactions between ATP, actomyosin and ions have been demonstrated in experiments using reconstructed models of the contractile apparatus (22, 23).

The direct source of energy for contraction was once thought to be the fermentation of lactic acid (24). This view gradually gave way to that first proposed by Lundsgaard, who maintained that CP was the immediate source of contractile energy (25). Refinements in the knowledge of the formation of high energy phosphate bonds (26, 27) have led to the present belief that ATP is the immediate source of energy for contraction and that CP is utilized to replace ATP. Although the exact nature of the coupling of chemical and mechanical energy is not fully understood, all the current hypotheses (22, 28-32) regarding the contractile mechanism agree on the probability of an interaction of actin, myosin and ATP. Most of these hypotheses have been developed from studies of skeletal muscle, but appear to be applicable to cardiac muscle as well.

THE METABOLISM OF HEART MUSCLE

Several methods have been used to study cardiac metabolism. In vivo studies have demonstrated that the heart is able to extract a large variety of foodstuffs from the blood and that in certain disease states cardiac metabolism is altered. In heart-lung preparations and, more recently, in the isolated saline-perfused heart more precise determinations of substrate extraction and fate have been carried out. The latter experimental method is adaptable to studies of the quantitative relationships of foodstuffs utilized by the heart and the effect of humoral factors on cardiac metabolism and function. Purified preparations from heart tissue have revealed the presence of many enzyme systems required for the stepwise oxidation of foodstuffs. In the following sections an attempt will be made to correlate the information that has been gained concerning cardiac metabolism by the several methods which have been employed.

FUELS OF RESPIRATION IN VIVO

The substances which are extracted and oxidized by the normal human heart have been determined by Bing, Siegel, Ungar and Gilbert (3, 33). The results taken for the postabsorptive state show that more than half of the oxygen consumption is due to the oxidation of fatty acids. Most of the remaining oxygen uptake is accounted for by oxidation of glucose and lactate. The oxidation of ketone bodies and of amino acids each contributes but a few percent to the total oxygen consumption.

Carbohydrate Utilization in vivo

The extraction of glucose and of lactate are highly concentration dependent over the range of concentrations normally found in the blood (3, 34). An almost insignificant amount of glucose is extracted by the heart at glucose concentrations 20 to 30 percent below normal blood levels. If complete oxidation of carbohydrate is assumed, the total aerobic metabolism in normal man and dog does not account for more than 35 percent of the total myocardial extraction of oxygen (34).

Studies using coronary sinus catheterization have failed to demonstrate any significant extraction of fructose (35). In addition, Hers (36), using C^{14} - labelled fructose, has shown that little or no cardiac muscle glycogen is derived from direct utilization of fructose.

Fatty Acid Utilization in vivo

The utilization of fatty acids by the heart was demonstrated by means of coronary catheterization. It was shown that about 60 percent of the energy of the heart was obtained by oxidation of fatty acids (33). Gordon (37) and Dole (38) reported that the principal fraction of plasma concerned with transport and metabolism of fatty acids by the heart is the plasma non-esterified or free fatty acid fraction. Gordon (37) demonstrated that the heart uses a considerable amount of free fatty acids. Further investigations revealed, however, that in the fasting human the mean extraction of fatty acids accounts for only 40 percent of the total fatty acid extraction, the esterified fraction making up the remaining 60 percent (39). The high myocardial extraction of esterified fatty acids is consistent with the finding that more than half of chylomicron triglycerides found in blood are directly oxidized (40). Lipoprotein lipase

present within or on the surface of the heart cell may hydrolyze the triglycerides at the site of their oxidation (40). On the basis of the work of Fredrickson and Gordon (41), it is unlikely that phospholipids or cholesterol esters are metabolized by the heart.

In recent studies by Rothlin and Bing (42) the myocardial extraction of individual free fatty acids was investigated. Apparently, although both human and dog hearts extract all individual free fatty acids, there are significant differences in their myocardial uptake. Oleic acid was reported to be extracted at a higher rate than other fatty acids studied (42). The measurement of arteriovenous differences, which was employed in these studies, gives a net result of uptake and turnover of fatty acids and may not be a true picture of myocardial utilization. The preferential uptake of an individual fatty acid may be considered unlikely in the light of work by Neptune (43), in which he showed that there is no significant difference in the rate of uptake of C^{14}-labelled fatty acids into striated muscle.

Considering the high myocardial uptake of esterified fatty acids, and the large amount of lipoprotein lipase present in the heart muscle, the likelihood of exchange between triglycerides and free fatty acids in heart muscle is great. It therefore seems likely that triglyceride fatty acids constitute a significant percentage of the fuel of respiration for the heart.

The Utilization of Ketone Bodies in vivo

Ketone bodies are utilized by the human and dog heart, normally accounting for about 5 percent of the total oxygen extraction (33). The diabetic dog heart utilizes a considerably larger quantity of ketone bodies (44).

It has been suggested that the utilization of ketone bodies by the heart is governed by their arterial concentration and by the amount of carbohydrate available (33). Early work by Waters, Fletcher and Mirsky (45) indicates that carbohydrate is used in preference to ketones.

The Utilization of Amino Acids in vivo

Human and dog hearts *in situ* extract considerable quantities of amino acids from the coronary blood (33). The complete oxidation of all the amino acids extracted would account for only about 5 percent of the fuel of respiration. After infusion of amino acids, as much as 40 percent of the cardiac oxygen consumption can be accounted for by aerobic oxidation of amino acids. An increase in the blood amino acid level of 30 percent produces a disproportionate rise in myocardial amino acid extraction of about 250 percent (33). However, the fate of the amino acids thus extracted was not followed in these experiments. It is likely that the myocardial balance of amino acids is influenced by continuous exchange between exogenous and endogenous amino acids, both of which can be used for protein synthesis (3).

CONDITIONS AFFECTING MYOCARDIAL METABOLISM IN VIVO

Metabolism of the Hypoxic Heart

A number of pathological states result in hypoxia of cardiac muscle, with a concomitant shift toward anaerobic metabolism. Myocardial anoxia is accompanied by a negative balance of lactate and pyruvate. This has been

reported in cases of hemorrhagic shock, ventricular tachycardia and in fibrillation (46, 47, 48, 49). In all cases reported, anoxia leads to the disappearance of glycogen and to an increase in the concentration of lactate and G6P in heart muscle. Myocardial anoxia also leads to a transitory increase in the ratio of active phosphorylase (48). This is in agreement with the early finding of Cori and Cori (50) in skeletal muscle: when the gastrocnemius muscle of the rat is stimulated at a high rate, there is a regular increase in the level of active phosphorylase.

A fall in oxygen tension in heart muscle would logically suggest a complementary shift in the oxidation-reduction systems toward a more reduced state. The extent to which individual members of the electron carrier system shift toward the reduced form is determined by the redox potential of the system. As pointed out by Hewitt (51), NAD has the lowest potential in the carrier system. When the oxygen potential becomes low enough metabolic systems become involved; the first NAD-coupled system to be reversed should be the one with the potential closest to that of NAD to NADH, i.e., the lactic dehydrogenase system: pyruvate + NADH $\overset{\text{LDH}}{\longrightarrow}$ lactate + NAD (52, 53). An increase of NADH would, by mass action, shift the equilibrium to the right, with production of lactate and oxidation of NADH. Bing (53) has suggested that this reaction may act as a safety valve in the presence of hypoxia, permitting other metabolic oxidative systems to continue to function. Indeed, it is considered likely, from the studies of Lochner, Mercker and Nasseri (54), that in anoxia fatty acids continue to contribute to the energy requirements of heart muscle.

Heart muscle has a very high resistance to anoxia. For example, it has been shown that rapid resynthesis of glycogen and ATP can take place in heart muscle during reperfusion of the coronaries after a brief period of anoxia (55). The amount of glycogen which disappears anaerobically in heart muscle can be grossly accounted for as the sum of HMP and lactic acid, indicating that no other glycolytic intermediates accumulate in the anoxic heart (55). This assumption is further substantiated by the low concentrations of FDP and DHAP which are found in the anoxic heart. These observations suggest that the rate at which glycogen is broken down to HMP in the anoxic heart is greater than the rate of lactate formation from HMP. Contraction of striated muscle, under anaerobic conditions, causes the same intermediates to accumulate (50).

Increased or atypical heart contractions, as seen in ventricular tachycardia and in fibrillation, fails to result in glycogenolysis or in the accumulation of glycolytic intermediates so long as coronary circulation is maintained (48).

Myocardial Infarction

There is little doubt that anoxia of the myocardium is the cause of the biochemical, as well as the dynamic changes resulting from myocardial infarction. In experimental myocardial infarction, extraction of oxygen, pyruvate and lactate is reduced, while glucose extraction remains unaltered (49, 56). Apparently, anaerobic glycolysis still proceeds in heart muscle under these conditions. The release of enzymes such as transaminases, aldolase, phosphohexoisomerase and malic dehydrogenase from the heart

is probably related to anoxia. According to Hess (57), the cause of the increase in these enzymes in plasma following myocardial infarction is increased permeability of the cell membrane, resulting from deficient cellular respiration.

Metabolism of the Heart in Diabetes

A number of metabolic alterations have been shown to occur in the hearts of humans with diabetes mellitus and in dogs with alloxan diabetes (44,58). The utilization of carbohydrate is quite markedly decreased and the utilization of non-carbohydrate material is increased (44). Evans (59) found that the diabetic dog heart utilizes less than one-fourth as much glucose as the normal. Myocardial lactate and pyruvate usage by the diabetic human heart is also greatly reduced (44). The addition of insulin increases the glucose extraction, even at the lowered blood glucose concentrations which ensue, but the defect in lactate utilization is apparently unaffected (44,58).

METABOLISM OF THE HEART IN VITRO

Although studies of cardiac metabolism in the intact animal have the advantage of being carried out under physiological conditions, the complexity of the method makes it impossible to have exact knowledge of all factors influencing metabolism. Preparations of the completely isolated surviving heart have been employed extensively in recent years. Most of the data cited in the following pages will refer to results obtained with isolated mammalian hearts, employing either the heart-lung preparation or preparations in which the heart is perfused with a physiological saline medium to which known nutrients have been added.

Carbohydrate Metabolism in the Isolated Heart

It has been established that the isolated heart utilizes glucose, pyruvate and lactate, and that the rate of utilization of pyruvate and lactate is a function of the initial concentration of substrate (60). More recently, reports by Morgan, Henderson, Regen and Park (61), Post, Morgan and Park (62) and Opie, Shipp, Evans and Leboeuf (63) have demonstrated a concentration dependence for glucose utilization as well. In the perfused rat heart, glucose (5 mM) and lactate (5 mM), when each is added as the sole substrate, contribute 24 and 52 percent respectively to the total fuel of respiration (64). Glucose is utilized even more rapidly at higher concentrations (63). Pyruvate oxidation is also very high in the rat heart (65, 66).

Control of Carbohydrate Metabolism in the Isolated Heart

The Effect of Substrate Concentration

Glucose utilization by the perfused rat heart was determined by Morgan et al. (61), employing a wide range of perfusate concentrations. Extracellular transfer of glucose from the capillaries to the cell membrane is shown to be very rapid. In the absence of added insulin, glucose transport through the mucous membrane is the major limiting step for uptake in the normal range of blood glucose concentrations. At higher concentrations, phorphorylation becomes limiting. With the addition of insulin, glucose uptake is increased. Under these conditions, phorphorylation is limiting even at very low external glucose concentrations.

The rate of glucose uptake by the isolated rat heart is increased by anoxia (61). Transport is predominant rate-limiting for uptake at low external glucose concentrations and phosphorylation at higher concentrations. With anoxia plus insulin, phosphorylation becomes the only important limiting step, and near maximal rates of uptake are obtained even at very low external glucose concentrations (61).

Opie et al. (63) studied the uptake and exodation of glucose-U-C^{14} by the isolated rat heart. In the absence of added insulin, glucose uptake increases sharply with increased perfusate concentrations to about 10 mM. with a lesser rise from 10 to 40 mM. Oxidation to $C^{14}O_2$ accounts for 60 percent of the glucose taken up at 1.25 mM glucose, while only about 25 percent of the total glucose uptake is accounted for as $C^{14}O_2$ at glucose concentrations above 40 mM. Although the percent of total glucose uptake which is oxidized to $C^{14}O_2$ decreases with increasing concentrations of glucose, the absolute amount of glucose oxidized increases until the external glucose concentration is as high as 40 mM (63). There is a net glycogen breakdown in the presence of glucose at concentrations up to 10 mM, and a significant increase in glycogen at glucose concentrations above 40 mM. Lactate accumulates somewhat at all concentrations of glucose, but reaches very high values at high glucose concentrations (63). These data indicate that, in the absence of added insulin, glycolysis is not limiting, even at glucose concentrations far in excess of those normally found in the blood.

The Effect of Diet and Diabetes on Carbohydrate Metabolism of the Perfused Heart

Newsholme and Randle (67, 68) and Garland, Newsholme and Randle (69)

compared the concentrations of hexose phosphates in isolated rat hearts after perfusion in the presence of added insulin and either glucose or pyruvate. Three conditions applicable to the present discussion were used: (a) hearts from fed rats; (b) hearts from diabetic rats; and (c) hearts from starved rats. The rates of uptake were also studied. A decrease in the amount of FDP and an increase in F6P were noted in (b) and (c) as compared to (a). These results were interpreted as indicative of an inhibition of the phosphofructokinase reaction by starvation or diabetes. Similar results were obtained using diaphragm muscle (67, 68). The uptake, both of glucose and pyruvate, is suppressed as a result of starvation or diabetes (68, 69). It has been suggested that this effect is due to enhanced respiration of fatty acids under conditions of decreased utilization of glucose and pyruvate (68).

Williamson (64) compared the metabolism of glucose and lactate by the perfused hearts of rats fed a stock laboratory diet, with those of animals fed a high carbohydrate diet and of animals which were fasted for 24 hours. When added to 5 mM concentration, glucose is taken up at a rate of 70μ moles/gm. dry wt./hr. No detectible lactate is formed, and there is no net change in cardiac glycogen during perfusion. Addition of insulin increases glucose uptake approximately four-fold. A large percentage of insulin-stimulated glucose uptake is accounted for as lactate and net glycogen synthesis. However, taking into account lactate accumulation and glycogen synthesis, insulin increases the contribution of glucose to the fuel of respiration from 24 percent to 57 percent (64). In the absence of insulin, starvation decreases glucose uptake about 40 percent, and by

extrapolation of the data it appears that glucose oxidation is reduced to about the same degree by starvation. These data are in accord with those reported by Garland et al. (69), who observed a similar effect of starvation or diabetes on glucose uptake by rat heart. Insulin increases the rate of glucose uptake by hearts from starved rats; most of this increased glucose uptake accumulates as lactate (64). The contribution of glucose to the total fuel of respiration by hearts from starved rats appears to be restored to near normal values by the addition of insulin (64).

Lactate uptake, while accounting for more than 50 percent of the total fuel of respiration by the heart of fed rats, is reduced by about half by a day of fasting. Taking into account net glycogenolysis by hearts from starved rats, the net disappearance of lactate is reduced about 30 percent by starvation (64). Insulin has no significant effect on lactate utilization.

Feeding rats on a high carbohydrate diet has no significant effect on the carbohydrate metabolism of their hearts in vitro (64).

The changes in the metabolism of carbohydrate by muscle tissues from starved animals bear some resemblance to the alterations in these tissues resulting from diabetes. Evans et al. (59), using isolated hearts from diabetic dogs, and Goodale, Olson and Hackel (70) and Ungar et al. (44), in humans with diabetes mellitus, observed decreased utilization of lactate and glucose. Morgan, Cadenas, Regen and Park (71) noted a similar effect by diabetes in isolated rat hearts. The indication is that the changes occurring during starvation and diabetes may affect carbohydrate and fat metabolism of cardiac and skeletal muscle by a similar mechanism.

The Effect of Fatty Acids and Ketone Bodies on Carbohydrate Metabolism by the Perfused Heart

Shipp, Opie and Challoner (72) recently studied the effect of long-chain fatty acids on the metabolism of glucose-U-C^{14} by the perfused rat heart. With glucose-U-C^{14} (5 mM) as the only substrate, 17 percent of the uptake appears as $C^{14}O_2$. There is definite incorporation of label into glycogen, and lactate production accounts for nearly 60 percent of glucose uptake. When free fatty acids are added (0.4 mM), glucose oxidation is strikingly reduced. Under these conditions the uptake of glucose is slightly less, more residual glycogen is found after perfusion and more radioactivity from glucose-U-C^{14} is recovered in glycogen. Although both glucose and fatty acid concentrations used are within the physiological range, fatty acids have a very pronounced effect on glucose oxidation, while glucose has no significant effect on the oxidation of palmitate-1-C^{14}. On the basis of these results it was suggested that fatty acids are oxidized in preference to glucose by the rat heart, and that in the presence of free fatty acids more glucose is diverted to the synthesis of cardiac glycogen. Since fasting and diabetes result in the mobilization of free fatty acids from adipose tissue, these findings may explain the fact that cardiac glycogen increases with fasting (73) and with diabetes (74).

Bowman (75) perfused rat hearts with glucose in the presence and absence of albumin-bound long-chain fatty acids. In the presence of insulin, the latter author found that palmitate, oleate and linoleate at concentrations

of 0.3 to 2.0 mM increase intracellular free glucose two- to three-fold while slightly decreasing glucose uptake. Contrary to the results of Shipp et al.(72), in the absence of insulin these effects are not observed. From these results Bowman suggested that long-chain fatty acids decrease the rate of glucose phosphorylation; this effect is seen only in the presence of insulin due to low glucose uptake rates in non-insulinized hearts.

Newsholme, Randle and Manchester (76) measured the concentrations of hexose phosphates in rat hearts. After the addition of acetoacetate, octanoate or pyruvate, the amounts of G6P and F6P are increased, while that of FDP is decreased. The changes which these substrates induce in the hexose phosphate concentrations in the hearts of normal animals are very similar to those found in the hearts of starved or diabetic rats in the absence of these substrates (67, 77). These authors (76) maintain that the phosphofructokinase reaction in rat heart is inhibited in the presence of ketone bodies and fatty acids, and that a similar inhibition in perfused hearts from starved or diabetic rats may result from increased oxidation of endogenous fatty acids or ketone bodies.

Octanoate, acetoacetate and β-hydroxybutyrate (76) decrease somewhat the uptake of glucose by the perfused rat heart. Newsholme et al. (76) found that glucose accumulates within the cell in the presence of these substrates and, on the basis of this finding, suggested that these substances inhibit glucose uptake by inhibiting the intracellular phosphorylation of glucose by hexokinase.

In the perfused rat heart, pyruvate uptake is impaired by the addition of fatty acids or ketone bodies, or by starvation or alloxan diabetes (69).

Similarly, pyruvate uptake and oxidation is suppressed in heart slices (78) and diaphragm muscle (79) from diabetic rats. In no case reported by Garland et al. (69) is pyruvate uptake decreased by more than 50 percent, even after addition of very high concentrations of acetoacetate or fatty acids. It is further stated by the latter authors that the addition of ketone bodies or fatty acids has no significant effect on lactate formation by the perfused rat heart.

Williamson and Krebs (80) found that in the absence of insulin acetoacetate causes no change in the rate of glucose uptake by the perfused rat heart, but affects the fate of glucose in that less glucose undergoes oxidation; instead, almost all of the glucose removed is converted into glycogen or lactate. Ottaway and Sarker (81) also recorded an increased lactate formation by rat hearts perfused with acetoacetate. These workers believe that the additional lactate arises directly from acetoacetate. In the presence of insulin, glucose uptake is approximately halved by acetoacetate, this decrease being due almost entirely to the suppression of glucose oxidation. The amounts of lactate and glycogen formed from glucose in the presence of insulin are not affected by acetoacetate (81).

The Utilization of Fatty Acids by the Perfused Heart

Cavert and Johnson (82) made a comparative study of the metabolism of short-chain carboxyl-labelled fatty acids to $C^{13}O_2$ and to glycogen in the isolated blood-perfused dog heart. Assuming that the rates of combustion of all carbon atoms in each compound are equal, acetate, propionate, butyrate and octanoate contribute 41, 15, 13 and 25 percent to the total fuel of respiration respectively. These high values were obtained only at extremely high levels of substrate (12 to 14 mM). Formate does not

appear to be readily converted to CO_2. Carboxyl carbon from none of the labelled fatty acids contributes detectibly to cardiac glycogen. Lorber and Cook (83) traced the metabolism of butyrate-3-C^{14} by the blood-perfused cat heart and in heart slices and homogenates. The results in all three preparations are consistent with the reactions of the citric acid cycle as the main route of butyrate oxidation. In no case is detectible label from butyrate found in cardiac glycogen.

Palmitate-1-C^{14} is rapidly taken up and oxidized to $C^{14}O_2$ by the perfused rat heart. Shipp et al. (72) found that, when present at o.4 mM concentration, approximately half of the palmitate taken up is found as $C^{14}O_2$. A significant amount of palmitate is incorporated into tissue fatty acids. Addition of glucose (5 mM) to the perfusate has no detectible effect on the uptake or fate of palmitate-1-C^{14}. Gausios, Felts and Havel (84) have shown that 1-C^{14}-labelled palmitate is extracted and oxidized to $C^{14}O_2$ by the perfused rabbit heart. Labelled palmitate as the triglyceride is utilized to about the same extent as the free fatty acid. The dietary state of the animal has no significant effect on fatty acid utilization.

Olson (85) studied the effect of high concentrations of pyruvate and acetoacetate on the utilization of albumin-bound palmitate-1-C^{14} by the perfused rat heart. About 75 percent of the palmitate taken up is oxidized to $C^{14}O_2$, accounting for about 40 percent of the oxygen consumption of the heart. About 10 percent of the palmitate extracted appears in lipid fractions, and this is equally distributed between neutral lipids and phospholipids. In the presence of 10 mM pyruvate or acetoacetate, the uptake of palmitate is reduced approximately 50 percent and the

oxidation of palmitate to $C^{14}O_2$ is reduced to only 25 percent of that in controls. Fatty acid incorporation into neutral lipids is increased about five-fold in the presence of pyruvate or acetoacetate, while the incorporation of fatty acids into the phospholipid fraction is unchanged (85). It would appear that in heart muscle the reactions in the metabolism of these two major classes of lipids are independent of one another either through compartmentalization or by novel biochemical pathways. It is known that both pyruvate and acetoacetate are highly preferred substrates for oxidation by heart mitochondria (86) and that, in the perfused heart, they presumably aid in the preservation of a reasonable concentration of glycolytic intermediates including -glycerol phosphate (76). Under these conditions, it would not be surprising that fatty acids are rejected for oxidation and incorporated into triglyceride.

Fatty Acid Synthesis in Heart Muscle in vitro

There have apparently been no attempts to demonstrate fatty acid synthesis by the isolated heart. Hulsmann (87) and Christ and Hulsmann (88) have demonstrated the synthesis of long-chain saturated and unsaturated fatty acids from acetate-1-C^{14} by rabbit-heart sarcosomes in vitro. The presence of ATP, CoA, NADP and a citric acid cycle intermediate is required for maximal incorporation. Malonate is incorporated into long-chain fatty acids more readily than acetate. Hulsmann therefore concluded that fatty acid synthesis in heart sarcosomes proceeds through malonyl-CoA.

Utilization of Ketone Bodies by the Perfused Heart

Acetoacetate is rapidly oxidized by the perfused rat heart (80, 89).

At concentrations above 4 mM about half of the acetoacetate removed from the perfusate is recovered as β-hydroxybutyrate. At low concentrations (below 0.25 mM) acetoacetate disappears rapidly but little is reduced to -hydroxybutyrate (80). Added β-hydroxybutyrate is as readily oxidized by the isolated rat heart as is acetoacetate. In the absence of insulin or glucose, acetoacetate (5 mM) may contribute as much as 80 percent to the total fuel of respiration. Insulin with or without glucose somewhat lowers the rate of oxidation of acetoacetate. Acetoacetate is reported by the latter authors to reduce the contribution of glucose to the fuel of respiration from 24 to 4 percent in the absence of insulin, and from 57 to 15 percent in the presence of insulin. These conclusions are based on indirect measurements of O_2 consumption and on changes in the perfusate concentrations of glucose, acetoacetate, β-hydroxybutyrate and lactate. The fact that in the absence of glucose insulin decreases the oxidation of acetoacetate suggests that it has a direct stimulatory effect on the combustion of endogenous material. It is further suggested from these data that acetoacetate is oxidized in preference to glucose and endogenous substrates (80, 89). In well-fed rats the concentration of ketone bodies in the blood is below 0.5 mM. A concentration of ketone bodies of 1.0 mM is regarded as mildly ketotic and in severe ketosis the value can rise to 20 mM (90, 91). The results cited therefore indicate that ketone bodies serve as fuel for the heart at concentrations that occur in vivo.

Utilization of Amino Acids by the Perfused Heart

Gaddie, Clark and Stewart (92) and Cruickshank and McClure (93) were unable to find evidence of ammonia formation by isolated frog hearts when

amino acids were added to the perfusing medium. Lorber and Olsen (94) added C^{13}-carboxyl-labelled glycine to an isolated cat heart and found a barely significant increase in the isotope content of the respiratory CO_2. More recently, Clarke (95) found that carboxyl-labelled C^{14}-alanine, valine, leucine and glutamic acid are metabolized to $C^{14}O_2$ by the perfused rat heart. Less than 4 percent of the respiratory CO_2 is contributed by each of the former three amino acids, while glutamic acid contributes only about 0.5 percent to the total fuel of respiration. The relatively low values found for glutamic acid are surprising in view of the specific transaminases found in cardiac muscle (96). The results of Awapara (97) strongly indicate that glutamic acid is oxidized by homogenates of rat heart only via transamination and subsequent oxidation in the citric acid cycle. It is of interest that leucine and valine should be decarboxylated at the same rate as alanine. There is no evidence of direct decarboxylation of amino acids by heart muscle. Deamination is thought always to precede decarboxylation and to be brought about either by means of a transamination reaction or by oxidative deamination (98). In such a series of reactions, the keto acids formed from leucine and valine would be metabolized at the same rate as pyruvate.

At least twenty-five amino acids have been shown by Cammarata and Cohen (96) to participate in transamination reactions catalyzed by extracts from pig heart. Each transamination reaction appears to be due to a different transaminase. The reactions involving glutamate, aspartate, valine, leucine, isoleucine and alanine show the highest activities. In the perfused heart, however, Hicks and Kerly (99) were able to demonstrate reversible transamination between aspartate and glutamate, but no

detectible transamination occurs between any other pairs of amino and keto acids. The glutamic/aspartic transaminase activity is only 3 to 4 percent of that observed by Cohen and Hekhuiss (100) in rat heart homogenates. The inability to demonstrate any activity of the large number of transaminases shown to be present in heart muscle is not due to the inability of the acids to penetrate the heart cells, for many of the amino and keto acids studied are found to accumulate within the cell.

THE RESPIRATORY ACTIVITY OF THE PERFUSED HEART

Fisher and Williamson (101) have made a detailed study of the oxygen consumption of the saline-perfused rat heart. The polarigraphic method first described by Laitinen and Lolthoff (102,103) was employed. In the absence of added substrate, the level of uptake of oxygen by the perfused rat heart is 39μ l/mg. dry wt./hr. This value is much in excess of that obtained in heart slices (104), even though in the perfusion experiments the hearts were doing no external work. Two types of experiments were carried out to determine adequacy of oxygen in the perfusion medium: (a) erythrol tetranitrate, which produces an increase in coronary flow, was added to the perfusate in the presence of glucose as substrate, and (b) DNP was added to the perfusate at concentrations that increase oxygen consumption. In (a) the oxygen uptake is unaffected although the coronary flow rate is considerably increased, while in (b) the oxygen consumption is elevated without any significant change in the perfusion rate. The normal oxygen tension is therefore not so low as to limit oxygen uptake so long as the perfusion rate is maintained.

The Effect of Nutrients and Insulin on Oxygen Consumption by the Perfused Heart

For the first 40 minutes of perfusion, the oxygen uptake of the perfused rat heart is not significantly increased by the addition of exogenous substrates. In the absence of added substrates, the oxygen consumption drops significantly after about 40 minutes, but can be maintained constant up to three hours in the presence of nutrients. Insulin has no significant effect on oxygen uptake, either in the presence or absence of added substrates (105). According to the latter authors, the glucose uptake in the presence of insulin could account for all of the oxygen uptake if it were completely oxidized. Since the uptake of glucose is much greater in the presence of insulin than in its absence, it appears that the percent contribution by exogenous glucose to the total fuel of respiration is increased in the presence of insulin.

The Effect of Adrenaline on the Oxygen Consumption of the Perfused Heart

Adrenaline greatly increases the oxygen consumption of the isolated heart, having an optimal effect on the perfused rat heart at a concentration of 10^{-6}M; at this concentration of adrenaline the oxygen consumption is almost doubled, and the perfusion rate is increased proportionately. The response to adrenaline lasts for about 30 minutes, after which time the rate of oxygen uptake falls to that observed in the absence of added adrenaline. Initially, the ability to respond to adrenaline is independent of the presence of added glucose or insulin; however, the rat heart fails to respond to the addition of adrenaline after about 45 minutes of perfusion in the absence of substrate, but is sensitive to the addition of adrenaline for at least 160 minutes if glucose is present in the perfusing medium (105).

CHAPTER II

MATERIALS AND METHODS

CHEMICALS

All common laboratory chemicals were of "Reagent grade" and were used without additional purification. D-glucose, sodium acetate, l-adrenaline bitartrate and zinc insulin were obtained from Fisher Scientific Company, Montreal. Propionate, butyrate, pentanoate, hexanoate, and heptanoate, used in the form of their potassium salts, were prepared by Professor P.G. Scholefield of this Institute. Beef heart lactic dehydrogenase was purchased from Worthington Biochemical Company, Freehold, N.J., and NAD from Nutritional Biochemical Company, Cleveland, Ohio. Radioactive compounds were obtained from the following sources: sodium DL-lactate-1-C^{14}, sodium DL-lactate-2-C^{14}, sodium acetate-1-C^{14}, and sodium butyrate-1-C^{14} from New England Nuclear Corporation, Boston, Mass., glucose-U-C^{14} from the Radiochemical Centre, Amersham, England, and sodium propionate-1-C^{14} from the Atomic Energy of Canada Ltd., Ottawa.

PREPARATION OF SOLUTIONS

Solutions of glucose, lactate and fatty acids were made up in distilled water to a concentration of 1 M and were kept at -20°C. Radioactive compounds without added carrier were diluted with water to a concentration of 10 μc/ml. and stored at -20°C until immediately before use.

Perfusion Medium

Perfusions were carried out with Krebs-Henseleit bicarbonate buffer (106) with reduced amounts of calcium and magnesium as suggested by Bleehen and Fisher (107). Isotonic stock solutions of each salt were prepared and stored for periods up to several days at room temperature.

Perfusion medium was regularly made up fresh from these solutions. When fatty acids were added as their potassium salts, the potassium content of the perfusion medium was correspondingly reduced.

APPARATUS

A heart perfusion apparatus was designed which would permit the use of radioactive isotopes and the subsequent measurement of metabolites which accumulate in the perfusion medium. It was further desired to measure the rate of $C^{14}O_2$ production from exogenous C^{14}-labelled substrates during the course of the experiment. The basic unit described by Bleehen and Fisher (107) was modified to meet the above requirements.

A photograph of the apparatus is shown in Figure II.1a, and a schematic representation in Figure II.1b. In the description which follows reference is made to the latter.

Perfusion fluid is measured into the heart chamber (A in Figure II.1b) and cycled by means of a peristaltic pump (B) through a Soxhlet filter (C) and into the oxygenating chamber (D). Before entering the oxygenating chamber the fluid must pass through a glass spreader (E). This device is moulded in the shape of a disc, the edge of which has 0.05 mm. holes around its periphery. In passing through the spreader the perfusing fluid is forced to flow along the inner surface of the oxygenation chamber. A large surface contact between the moving gas phase and the incoming perfusion fluid is maintained in this manner.

The perfusion fluid is continuously oxygenated by a flow of water-saturated O_2-CO_2 (95-5) going into the oxygenating chamber at F and

FIGURE II.1a

PHOTOGRAPH OF PERFUSION APPARATUS

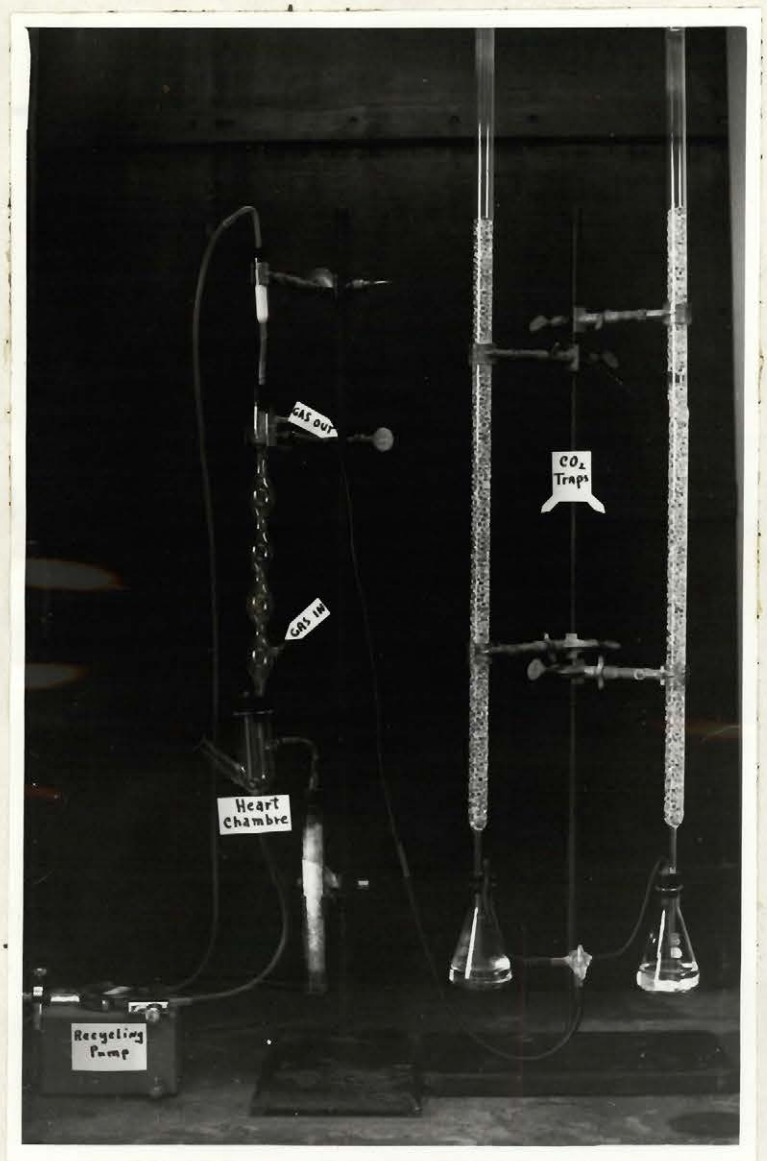


FIGURE II.1b

SCHEMATIC OF PERFUSION APPARATUS

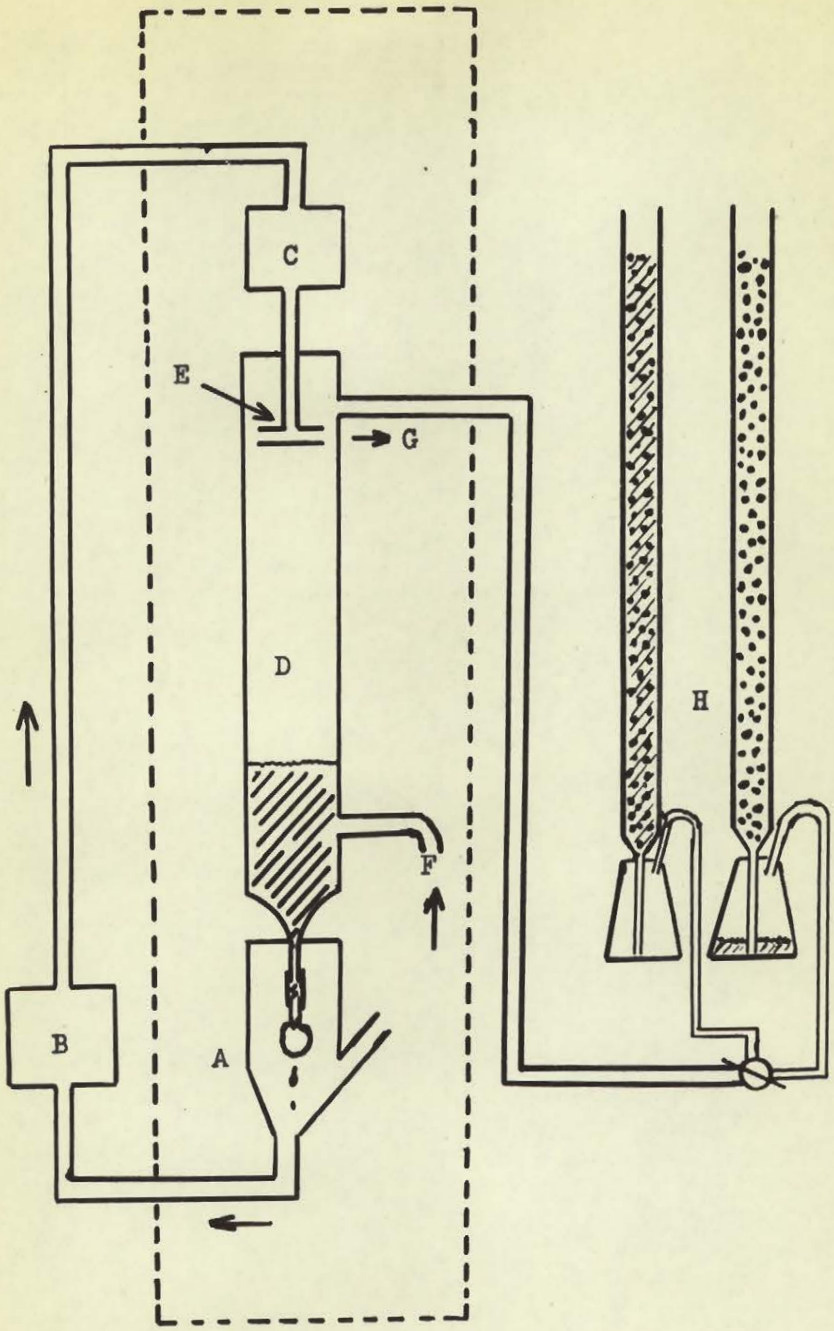


FIGURE II.1b

LEGEND

A - heart chamber

B - Peristaltic pump

C - Soxhlet filter

D - oxygenating chamber

E - spreader

F - inlet of gas

G - outlet of gas

H - columns containing glass beads

The part of the apparatus enclosed within dotted lines is maintained at 37°C.

out at G (Figure II.1b). The gas phase bubbles through the reservoir of perfusion fluid in the chamber and flows counter to that of the perfusion fluid re-entering the oxygenation chamber. The oxygenating gas then passes into an Erlenmeyer flask containing 50 ml. of 10 percent KOH, and into which is inserted a column filled with glass beads (H). The tip of the column is extended to the bottom of the flask so that the flow of gas into the flask forces the KOH solution into the column. As the gas bubbles through the column the CO_2 is trapped quantitatively. The column serves two purposes: (a) a constant pressure of 60 cm. of H_2O is maintained to ensure coronary circulation, and (b) the rate of $C^{14}O_2$ production by the heart from C^{14}-labelled substrates can be monitored throughout the experiment. Samples of CO_2 may be taken at regular intervals by changing columns. The apparatus, exclusive of the peristaltic pump and columns containing glass beads is enclosed in a constant-temperature chamber kept at 37°C.

PREPARATION AND PERFUSION OF HEART

100 ml. of perfusion fluid, to which was added the desired concentration of substrate was equilibrated with O_2-CO_2 (95-5) at 37°C. The apparatus was rinsed with about 200 ml. of distilled water followed by about 30 ml. of perfusion fluid. A Soxhlet thimble was inserted into the small glass container provided for this purpose. 60 ml. of perfusion fluid were measured into the heart chamber and cycled through the apparatus for about 5 minutes to remove any small particles which might interfere with coronary circulation. Recirculation was interrupted and the apparatus opened by displacing the heart chamber.

A guinea pig was injected intraperitoneally with 5 mg. of sodium heparin and killed 15 minutes later by a blow on the head and by decapitation. The thorax was opened and the heart removed and placed in a bath of perfusion fluid at room temperature. The great vessels, pericardial sac and connective tissue were cut away and the aorta was trimmed just proximal to the junction of the innominate artery. A glass cannula filled with perfusion fluid was inserted into the aorta and secured with a piece of suture. The heart and attached cannula were then connected to the perfusion apparatus at the tip of the oxygenation chamber. The recycling pump and gassing mixture were turned on immediately and 20 ml. of perfusion fluid were allowed to perfuse through the heart in order to remove residual blood. The apparatus was closed and after a period of equilibration (1 to 2 minutes), C^{14}-labelled substrate was added through a side-arm in the heart chamber (Figure II.1a).

The rate of flow of O_2-CO_2 was carefully adjusted to a pre-determined rate of 340 ml./min. The flushing gas was directed through a column containing KOH as previously described. Gas flow was diverted to an alternate column at intervals of 6 minutes. The first column was rinsed with boiled water and another flask containing KOH attached to the column. In this manner $C^{14}O_2$ flushed from the perfusate was trapped continuously and at regular intervals, and the rate of its formation throughout the period could be determined.

Determination of Suitability of Perfused Heart Preparations

Several parameters were used to determine empirically the suitability of a given preparation. These measurements were beat rate, intensity and regularity of beats, and perfusion rate.

In the absence of added adrenaline the beat rate, after 2 to 5 minutes of equilibration, was 190± 10 beats/minute. The beat intensity was observed only visually, but atypical or irregular beats, determined in this manner, usually led to degeneration of the preparation. Perfusion rate was estimated by counting the rate of formation of drops coming from the heart, and was usually in the range of 12 to 14 ml./minute.

When adrenaline was added, the beat rate was increased to 260 to 270 beats/minute, and the perfusion rate was nearly doubled. Lactate, at the concentration used, increased the perfusion rate to about 25 ml./minute, but had no significant effect on the beat rate or beat intensity. The addition of glucose or short-chain fatty acids had no noticeable effect on the perfusion rate or on the beat of the heart.

Perfused heart preparations which failed noticeably, as judged by the above-mentioned measurements, were discarded.

Estimation of Lag Time in the Transfer of $C^{14}O_2$ from the Perfusion Medium

To determine the time required to flush $C^{14}O_2$ out of the perfusion medium and the completeness of this transfer, the following experiment was carried out:

A heart was perfused with 40 ml. of bicarbonate buffer which contained 5 mM D-glucose and 5 μc of D-glucose-U-C^{14} as tracer. The gas flowed through the system at a rate of 340 ml./min., and samples of

CO_2 were collected by changing columns every 3 minutes. 45 minutes after the beginning of perfusion the flow of fluid to the heart was interrupted by clamping the short piece of rubber tubing which was used to attach the cannula to the oxygenation chamber. 60 minutes after the beginning of perfusion, HCI was added to bring the pH of the buffer to below 2, and CO_2 samples were taken for an additional 15 minutes.

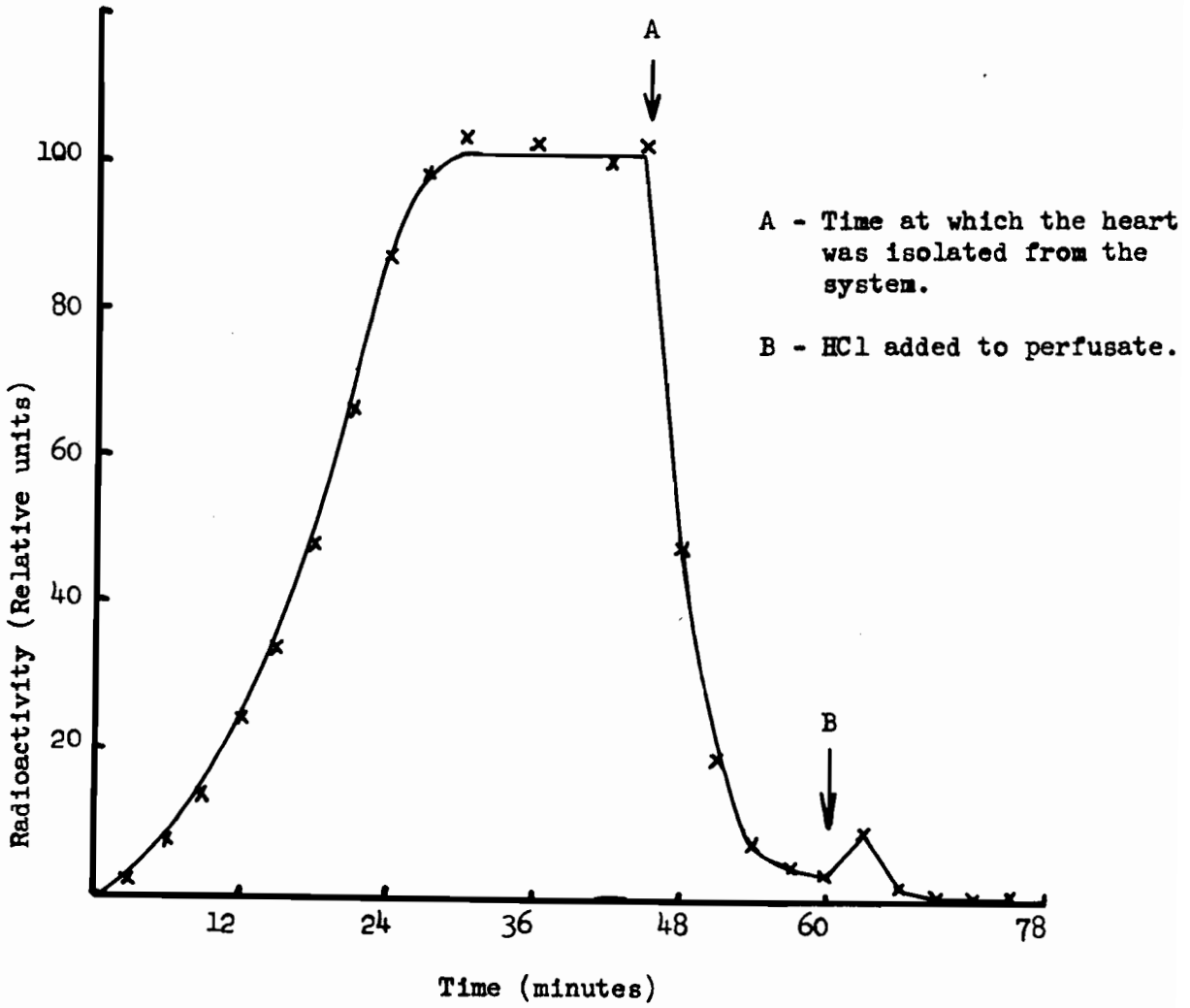
1 ml. aliquots of each 3-minute CO_2 sample were precipitated as $BaCO_3$, plated on aluminum planchettes and counted as described in a subsequent section. Figure II.2 is a plot of the relative amounts of $C^{14}O_2$ collected in 3-minute intervals for 75 minutes. The rate of $C^{14}O_2$ production from glucose-U-C^{14} became constant after about 25 minutes of perfusion. Within 3 minutes after isolating the heart from the perfusate the rate of removal of $C^{14}O_2$ was decreased by more than 50 percent, and the amount of $C^{14}O_2$ removed during the third 3-minute interval was almost negligible. The addition of HCI to the system released only a very small amount of radioactivity, indicating that there was very little $C^{14}O_2$ retention by the bicarbonate in the buffer.

Determination of Radioactivity in CO_2

1. 1 ml.aliquots from each 6-minute sample were added to 15 ml. centrifuge tubes.
2. 0.5 ml. of 2 M NH_4Cl was added to each tube, followed by distilled water to a total volume of 10 ml.
3. 1 ml. of 20 percent $BaCl_2$ was added to each tube and the tubes were centrifuged.

FIGURE II.2

DETERMINATION OF LAG TIME FOR $C^{14}O_2$ TRANSFER FROM PERFUSION MEDIUM



4. The $BaCO_3$ precipitates were washed twice with 10 ml. of distilled water and once with 10 ml. of acetone.
5. Finally, the $BaCO_3$ precipitates were suspended in 0.6 ml. volumes of acetone, plated on aluminum planchettes with pasteur pitettes, dried under a heat lamp and counted, using a thin window flow counter attached to a Tracerlab scaler.
6. Correction for self-absorption by the $BaCO_3$ was determined by comparing the count obtained from a standard amount of radioactive material added without carrier to planchettes containing $BaCO_3$ to that obtained for the same amount of radioactive material on planchettes as an infinitely thin layer. Since the amount of $BaCO_3$ plated was always the same, self-absorption was a constant in all determinations.

Extraction of Amino Acids

Extraction of amino acids from heart tissue was carried out according to the method of Kini and Quastel (108): at the termination of an experiment, a slice of ventricle weighing approximately 150 mg. was rinsed with perfusion fluid, blotted, weighed and homogenized in 3.5 ml. of 80 percent ethanol. After two hours the suspension was centrifuged and the supernatant decanted and saved. The residue was re-extracted twice with 3.5 ml. of 80 percent ethanol. The supernatants were combined and dried under a stream of air at room temperature. The residue was redissolved in 0.5 ml. of 80 percent ethanol for chromatography.

Chromatography of Amino Acids

Suitable aliquots of the ethanol-soluble extract were spotted on

25 cm. squares of Whatman no.1 paper. Two-dimensional ascending chromatography was carried out using secondary butanol-formic acid-water (100: 15: 25, by volume) for the first phase, and tertiary butanol-methyl ethyl ketone-ammonia-water (50: 50: 15: 25, by volume) for the second phase. Identity of amino acids was established by comparing the position and color of ninhydrin-positive spots with those obtained using authentic amino acids.

Radioautography

The position of radioactive spots on chromatograms was determined by placing them in contact with Kodak "No Screen" Medical X-ray film for periods up to 10 days depending on the radioactivity of the original spot.

Determination of Radioactivity on Paper Chromatograms

Areas on the chromatograms which coincided with the radioactive spots were counted by placing a Geiger-Müller tube, separated by a 1 mm.-thick lead disc, directly over the spot. The disc was situated so that it shielded the Geiger-Müller tube from any other radioactive areas which might be in the vicinity of the spot being counted.

The radioactivity of the individual amino acids on the filter paper was corrected for self-absorption by the paper and for the difference in efficiency between the Geiger-Müller tube and the thin-window flow counter. The factor used for these corrections was determined in the following way: equal aliquots of labelled amino acids without carrier were spotted on filter papers and at infinite thinness on aluminum planchettes. The amino acids on the filter paper were chromatographed

and the radioactive spots located in the usual way and counted with a Geiger-Müller tube. The planchettes were counted with a thin-window flow counter. The correction factor was found to be 9.8.

Determination of Radioactive Lactate

Aliquots of perfusate which were taken at the termination of each experiment were spotted on 25 cm. strips of Whatman no.1 filter paper and subjected to one-dimensional ascending chromatography in secondary butanol-formic acid-water (100: 15: 25, by volume). Lactate had an R_f of about 0.86. The radioactive spot corresponding to lactate was located by radioautography and counted in the same manner as the amino acids. No significant amount of lactate was lost by evaporation.

Enzymatic Determination of Lactate

The method described by Horn and Bruns (109) was employed for the determination of total lactate. This assay is dependent on the oxidation of lactate to pyruvate, with the concomitant formation of NADH.

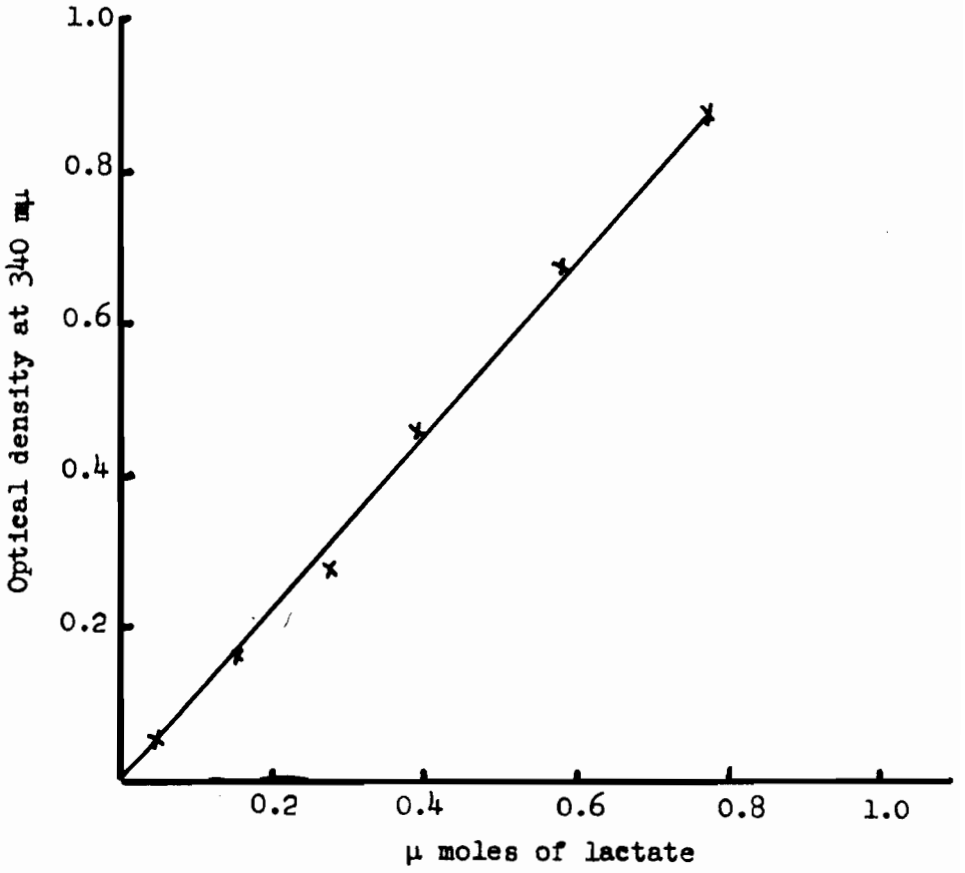
Appropriate amounts of perfusate taken at the end of an experiment were added to the following incubation mixture:

2.05 ml. glycine-NaOH buffer, pH 10.5
0.05 ml. NAD (2.24×10^{-2} M, initial concentration)
0.30 ml. LDH (0.4 mg. crystalline horse heart LDH)

The solutions were added to a cuvette in the order listed, and optical density at 340 mμ noted at the time of addition of LDH and after 120 minutes of incubation at room temperature. The reaction was near equilibrium within 60 minutes, but better reproduceability could be attained if allowed to proceed for 120 minutes. A separate standard curve was used for each set of determinations. The increase in optical density at 340 mμ was proportional to the amount of lactate present (Figure II.3).

FIGURE II.3

TYPICAL LACTATE STANDARD CURVE
(Enzymatic Assay)



Isolation of Glycogen

Glycogen was isolated as described by Wiener and Steyn-Parve (110). The final steps of glycogen purification were adapted from Somogyi's method (111). The procedure was as follows:

1. About 1 gm. of heart was weighed, homogenized in 20 ml. of 30 percent KOH and heated 5 hrs. at 100°C.
2. While the digest was still warm, 12 ml. of 95 percent ethanol were added, and the mixture was allowed to settle overnight at room temperature. After centrifugation the supernatant fluid was set aside for fatty acid isolation.
3. The sediment was washed once with alcoholic KOH and twice with 60 percent ethanol.
4. The residue was extracted three times with 10 percent TCA. The TCA extracts were combined and their volume was made up to 5 ml. with TCA.
5. Appropriate aliquots of the TCA extract were taken at this step before final purification in order to obtain the desired amounts of purified glycogen for separate determinations of total glycogen and radioactivity.
6. An equal volume of 95 percent ethanol was added to each aliquot. The samples were allowed to stand for three hours at 37°C. to ensure complete precipitation; they were then centrifuged. The supernatant fluid was discarded and the glycogen samples were sealed and stored at -20°C. until a convenient number of samples had accumulated for colorimetric determination of glycogen.

Colorimetric Estimation of Glycogen

Total glycogen was estimated as described by Carroll, Longley and Roe (112). Anthrone reagent was prepared immediately before each set of determinations.

1. Aliquots containing up to 200 μ gm. of purified glycogen were dissolved in 2 ml. of water. Blanks contained distilled water, and standards containing glucose at concentrations bracketing the amounts of glycogen which were to be estimated were prepared.
2. 5 ml. of Anthrone reagent were added to each tube. The tubes were mixed vigorously, tightly capped with an air condenser and placed in cold water. The air condensers were prepared by inserting a four-inch length of thin glass tubing into the rubber stoppers which were used to cap the tubes.
3. After the tubes had attained a uniform temperature in the cold water bath they were immersed in boiling water for 15 minutes to a depth above the level of the liquid in the tubes. The tubes were cooled to room temperature in a cold water bath. The optical density was read at 620 mμ in a Beckman Model DU spectrophotometer.

Determination of Radioactivity of Glycogen

Aliquots containing 4 to 25 percent of the total extracted glycogen were dissolved in water and plated on aluminum planchettes and counted. Correction for self-absorption was not necessary, since the amount of glycogen on the planchettes never exceeded 5 mg.

Extraction of Long-Chain Fatty Acids

The method for fatty acid extraction was adapted from the method described by Hülsmann (87):

1. The supernatants from step 2 in the glycogen purification were extracted twice with 1.3 vol. of petroleum ether, and the petroleum ether fractions were discarded.
2. 6 M HCl was added until the pH was below 2.
3. The acidified mixtures were cooled and extracted twice with 1.2 vol. of n-hexane, which extracts only long-chain fatty acids (113).
4. The combined hexane fractions were extracted with 2 vol. of water. The water was removed, and the hexane phase was evaporated at room temperature under a stream of air.
5. The residues were redissolved in a small volume of hexane and plated on aluminum planchettes. The container was rinsed twice with hexane, and the rinses were added to the original extracts on the planchettes.

Determination of Radioactivity in Fatty Acids

Fatty acids extracted and plated as described above were counted using a thin-window flow counter. No correction was made for self-absorption.

Preparation of Protein

Protein was prepared according to the procedure described by Manchester and Young (114) for rat diaphragm:

1. About 100 mg. of heart ventricle was homogenized in 10 ml. of 10 percent TCA.
2. After centrifugation, the precipitate was resuspended in 10 ml. of 10 percent TCA and heated for 15 minutes at 90°C.

3. The insoluble material was extracted twice with 1.5 ml. of 0.4 M NaOH, and the insoluble residue was discarded.
4. The dissolved protein was re-precipitated with 5 ml. of 10 percent TCA.
5. The precipitate was washed twice with 2 ml. of ethanol-ether (1:1, by volume), then once with 2 ml. of ether.

Determination of Radioactivity in Protein

The procedure outlined above yielded 8 to 10 mg. of protein from about 100 mg. of heart. The protein was resuspended in a small volume of acetone and plated on tared and greased aluminum planchettes and counted. Corrections were made for self-absorption.

CHAPTER III

RESULTS

THE METABOLISM OF GLUCOSE-U-C^{14} BY THE ISOLATED PERFUSED GUINEA PIG HEART

INTRODUCTION

The normal mammalian heart can utilize glucose, pyruvate and lactate. Carbohydrate utilization by the heart is quite markedly affected by diet and by diabetes (54,58,64,66,68,69) and by a number of disease states (40,47,56,115,116,117). The specific effect of catecholamines on cardiac contractility is well known, but the problem of how this effect is brought about metabolically is not well understood.

The metabolism of glucose-U-C^{14} by the isolated guinea pig heart is described in this chapter. The effects of non-carbohydrate substrates, adrenaline and insulin, and of the dietary state of the animal on glucose metabolism are reported.

A. GLUCOSE-U-C^{14} METABOLISM WHEN ADDED AS THE SOLE SUBSTRATE

1. Oxidation of Glucose-U-C^{14} to $C^{14}O_2$

Glucose-U-C^{14}, when added at a concentration of 5 mM, is oxidized to $C^{14}O_2$, attaining a constant rate of about 10 μ moles of glucose/gm. wet weight of heart/hour (Figure III.1).

With the volume of perfusate and the concentration of glucose used, the concentration of glucose in the perfusing fluid is not reduced more than 20 percent after 1 hr.

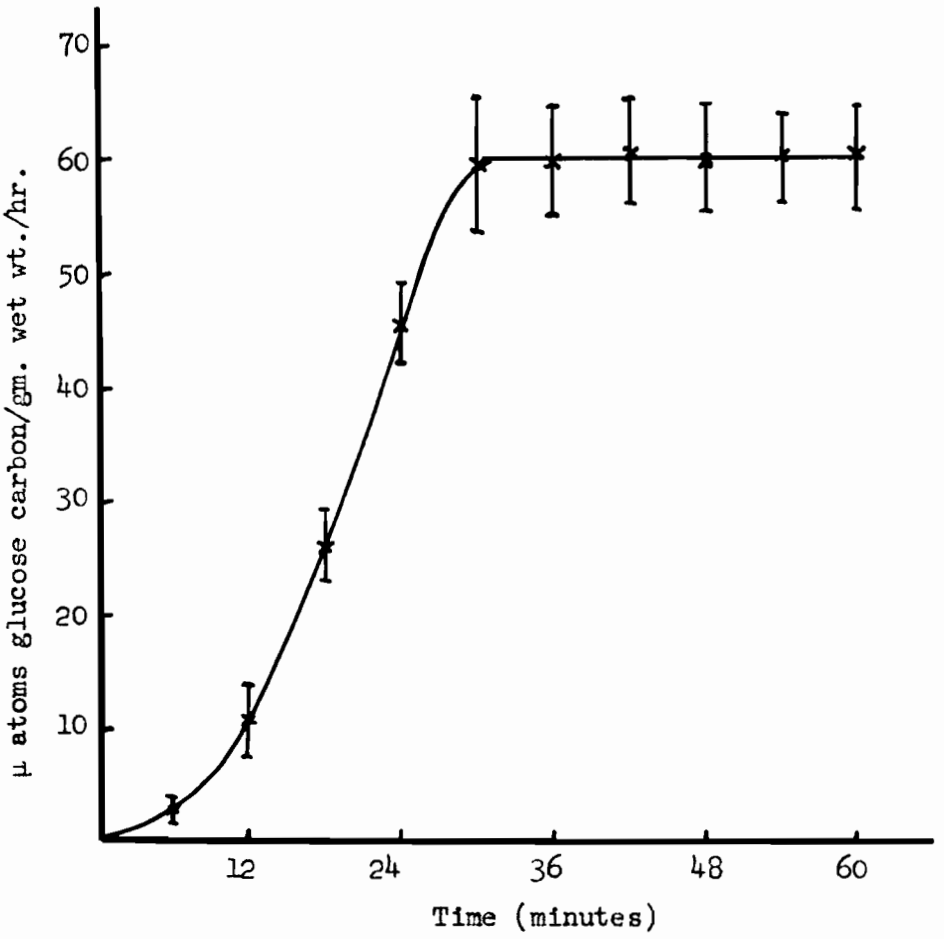
The rate of glucose oxidation is about 60 percent as high as the total glucose uptake reported by Williamson (64) for the rat heart.

2. Incorporation of Glucose-U-C^{14} into Amino Acids and Protein

After 60 minutes of perfusion in the presence of glucose-U-C^{14}, C^{14}-labelled glutamate, glutamine, alanine and aspartate were found. The amount of radioactivity incorporated into aspartate, compared with that in other amino acids listed, was quite small. Since the method used for estimation of radioactivity was not sufficiently sensitive to measure accurately the incorporation into aspartate, these data are not included. The results presented in Table III.2, column 1 show that there is relatively high incorporation of glucose carbon into glutamate, with somewhat lesser amounts in glutamine and alanine. The distribution of radioactivity obtained from C^{14}-glucose into amino acids is consistent with the assumption that glucose is oxidized primarily by way of the citric acid cycle. Radioactive labelling in

FIGURE III.1

$C^{14}O_2$ PRODUCTION FROM GLUCOSE-U-C^{14} BY THE PERFUSED GUINEA PIG HEART



Hearts were perfused as described in A.1 and in Methods.
Vertical bars represent one standard error from 10 experiments.
Glucose concentration: 5 mM.

TABLE III.2

THE INCORPORATION OF RADIOACTIVITY FROM GLUCOSE-U-C^{14} INTO AMINO ACIDS IN PERFUSED GUINEA PIG HEARTS

mμ atoms glucose carbon/mg.dry wt./hr. ± S.E.M.

A. Fed Animals

Additions	Glutamate	Glutamine	Alanine
1. None	30.2 ± 2.4 (6)	9.3 ± 1.7	8.4 ± 1.5
2. Acetate	0.5 (5)	0.5	15.4 ± 3.3
3. Propionate	10.1 ± 4.4 (3)	1.4 ± 0.5	12.0 ± 3.9
4. Butyrate	0.5 (2)	0.5	14.5 ± 2.7
5. Pentanoate	0.5 (1)	0.5	17.1
6. Insulin	56.0 ± 5.9 (4)	8.8 ± 3.6	22.0 ± 4.7
7. Adrenaline	17.6 ± 4.3 (3)	0.5	11.1 ± 1.3
8. Insulin and adrenaline	21.4 ± 5.5 (3)	4.0 ± 1.4	35.1 ± 9.8
9. Acetate and insulin	6.1 ± 2.0 (4)	0.7 ± 0.3	26.2 ± 4.7
10. Acetate and adrenaline	0.5 (6)	0.5	38.8 ± 7.7
11. Acetate, insulin and adrenaline	14.5 ± 3.8 (4)	2.4 ± 0.5	29.0 ± 6.4

TABLE III.2

THE INCORPORATION OF RADIOACTIVITY FROM GLUCOSE-U-C^{14} INTO AMINO ACIDS IN PERFUSED GUINEA PIG HEARTS

mμ atoms glucose carbon/mg.dry wt./hr. ± S.E.M.

B. Starved Animals

Additions	Glutamate	Glutamine	Alanine
1. None	0.5 (5)	0.5	9.8 ± 1.9
2. Insulin and adrenaline	6.6 ± 1.9 (2)	1.6 ± 0.5	22.4 ± 7.7

Conditions were as described in Methods.

Concentrations: acetate, 5 mM; propionate, 5 mM; butyrate, 2.5 mM; pentanoate, 5 mM; glucose, 5 mM; adrenaline, 0.25 μ gm./ml.; insulin, 10 mu/ml.

alanine, aspartate and glutamate can result from transamination reactions involving pyruvate, oxaloacetate and α-ketoglutarate respectively. Glutamine is formed subsequently by the glutamine synthetase reaction from glutamate in the presence of ATP. These reactions, as well as about 20 additional transaminase reactions, have been shown to occur in heart muscle homogenates (96). Hicks and Kerly (99) were able to demonstrate reversible transamination by the perfused rat heart involving glutamate and aspartate, but none between other pairs of amino- and keto- acids.

There is relatively low but significant incorporation of glucose carbon into heart protein (Table III.3), indicating that the rate of protein synthesis by the isolated heart is relatively low. The radioactivity incorporated in these experiments into protein from glucose-U-C^{14} is in the same range as that obtained by Wool and Manchester (118) from glycine-1-C^{14} into the protein of perfused rat hearts under very similar conditions.

3. Incorporation of Glucose-U-C^{14} into Cardiac Glycogen

Glucose is significantly incorporated into cardiac glycogen, the net incorporation after 1 hr. of perfusion in the presence of 5 mM glucose being 2.6 μ moles of glucose/gm. dry weight of heart (Table III.4). This result is in good agreement with the results of Cavert, Rhee, and Deal (119), who found that after 4 hours of perfusion with C^{14}-labelled glucose, up to 30 percent of the glycogen of perfused dog hearts was derived from exogenous glucose.

TABLE III.3

INCORPORATION OF RADIOACTIVITY FROM GLUCOSE-U-C^{14} INTO PROTEINS BY PERFUSED GUINEA PIG HEARTS

Hearts from Fed Animals

Additions	Counts/minute/mg. of protein	
None	28 ± 6	(7)
Insulin	39 ± 8	(4)
Acetate	< 3	(4)
Adrenaline	10 ± 4	(3)

Hearts from Starved Animals

None	< 4	(3)
Insulin	10 ± 3	(3)
Adrenaline	< 2	(3)

Hearts were perfused with 40 ml. of medium containing 200 μ moles of glucose with a specific activity of 1.25×10^3 counts/min./μ mole.

Concentrations: acetate, 5 mM; insulin, 10 mu/ml.; adrenaline, 0.25 μ gm./ml.

TABLE III.4

INCORPORATION OF GLUCOSE-U-C^{14} INTO CARDIAC GLYCOGEN

A. Fed Animals	μ moles/gm. dry wt./hr. ± S.E.M.	
Additions		
1. None	2.6 ± 0.5	(6)
2. Acetate	6.6 ± 1.6	(5)
3. Propionate	3.5 ± 1.2	(3)
4. Butyrate	7.2 ± 2.4	(2)
5. Pentanoate	9.1	(1)
6. Insulin	14.0 ± 2.7	(5)
7. Adrenaline	$8 \times 10^{-3} \pm 4 \times 10^{-3}$	(5)
8. Insulin and adrenaline	$16 \times 10^{-3} \pm 9 \times 10^{-3}$	(4)
9. Acetate and insulin	16.7 ± 4.0	(5)
10. Acetate and adrenaline	$12 \times 10^{-3} \pm 4 \times 10^{-3}$	(4)
11. Acetate, insulin and adrenaline	$25 \times 10^{-3} \pm 9 \times 10^{-3}$	(3)
B. Starved Animals		
Additions		
1. None	0.14 ± 0.06	(5)
2. Insulin and adrenaline	$5 \times 10^{-3} \pm 2 \times 10^{-3}$	(3)

Conditions were as described in Methods.

Concentrations: glucose, 5 mM; acetate, 5 mM; propionate, 5 mM; butyrate, 2.5 mM; pentanoate, 5 mM; insulin, 10 mu/ml.; adrenaline, 0.25 μ gm./ml.

4. Effect of Perfusion with Glucose on Total Cardiac Glycogen

In order to determine the level of glycogen in guinea pig hearts before perfusion, two groups of 5 animals were sacrificed and their hearts extracted and assayed for glycogen. The first group comprised animals fed a standard diet, and the second group was starved for 72 hours. The results are shown in Table III.5. Data obtained for cardiac glycogen from starved animals applies to results which will be presented later in this chapter.

By comparing the average glycogen levels in hearts before and after perfusion, it appears that there is a relatively small but significant amount of glycogenolysis during perfusion under these conditions (Table III.5 and III.6, column 1). It is not possible, however, to use these data as an exact measurement of glycogen changes during perfusion, since there is considerable individual variability in the amount of cardiac glycogen before perfusion. Most of the glycogen is retained on aerobic perfusion in the presence of glucose. This statement does not preclude an active turnover of cardiac glycogen, but suggests only that the level of glycogen is maintained under the stated conditions.

5. The Accumulation of Radioactive Lactate from Glucose-U-C^{14}

When glucose alone is added to the perfusing medium, relatively little lactate-C^{14} accumulates from exogenous glucose-U-C^{14} (Table III.7). It is assumed then that most of the glucose taken up is oxidized to CO_2. This assumption is probably valid in view of the fact that glycolytic intermediates fail to accumulate in the perfused rat heart when glucose alone is added to the perfusion medium (69, 76).

TABLE III.5

CARDIAC GLYCOGEN FROM FED AND STARVED GUINEA PIGS BEFORE PERFUSION

	Glycogen as μ moles of glucose/gm. dry wt. ± S.E.M.
Fed guinea pigs	48 ± 7 (5)
Starved guinea pigs (72 hours)	134 ± 12 (5)

Glycogen was extracted and estimated as described in Methods.

TABLE III.6

RESIDUAL CARDIAC GLYCOGEN AFTER PERFUSION IN THE PRESENCE OF GLUCOSE

	Glycogen as μ moles glucose/gm. dry wt. ± S.E.M.	
A. Fed Animals		
Additions		
1. None	37 ± 4	(4)
2. Acetate	51 ± 6	(4)
3. Insulin	70 ± 12	(3)
4. Acetate and insulin	86 ± 11	(5)
5. Adrenaline	5 ± 2	(5)
6. Insulin and adrenaline	7 ± 3	(3)
B. Starved Animals		
Additions		
1. None	41 ± 8	(5)
2. Insulin and adrenaline	12 ± 5	(3)

Glycogen was extracted after perfusion for 60 minutes at 37°C. with 40 ml. of perfusion fluid containing 5 mM glucose and the additions indicated. Acetate, insulin and adrenaline, when present, were added at initial concentrations of 5 mM, 10 mu/ml. and 0.25 μ gm.ml. respectively.

TABLE III.7

FORMATION OF RADIOACTIVE LACTATE FROM GLUCOSE-U-C^{14}

BY PERFUSED GUINEA PIG HEARTS

		μ moles lactate produced from Glucose-U-C^{14}/hr. ± S.E.M.	
A.	Fed Animals		
Additions			
1.	None	4 ± 2	(4)
2.	Acetate	49 ± 8	(7)
3.	Propionate	10 ± 3	(3)
4.	Butyrate	64	(1)
5.	Pentanoate	58	(1)
6.	Insulin	68 ± 7	(4)
7.	Insulin and acetate	102 ± 9	(3)
8.	Adrenaline	70 ± 10	(3)
9.	Adrenaline and acetate	145 ± 38	(4)
10.	Insulin and adrenaline	115 ± 16	(4)
11.	Acetate, insulin and adrenaline	130 ± 19	(3)
B.	Starved Animals		
1.	None	20 ± 5	(3)
2.	Insulin and adrenaline	75 ± 27	(2)

Hearts were perfused one hour at 37°C.

Concentrations: acetate, 5 mM; propionate, 5 mM; butyrate, 2.5 mM; pentanoate, 5 mM; adrenaline, 0.25 μ gm./ml.; insulin, 10 mu/ml.

Radioactive lactate was determined as described in Methods.

The present results indicate that nearly all of the glucose taken up by the cell is oxidized to CO_2, and that glucose entry is the major rate-limiting step in glucose utilization and its ultimate oxidation to CO_2.

6. Total Lactate Accumulation in the Presence of Glucose

Total lactate concentration in perfusates was determined enzymatically. Lactate not accounted for as radioactive lactate is presumed to have accumulated as the result of glycogen breakdown. When glucose is added to the perfusing medium the total lactate formed rarely reaches concentrations in excess of 1 mM. A significant portion of the total lactate is not radioactive, indicating that some of the lactate formed is derived from glycogen (Table III.8).

7. Lipid Synthesis from Glucose-U-C^{14}

Total triglyceride and long-chain free fatty acids were extracted from the heart. The total radioactivity from glucose-U-C^{14} found in the lipid extract was never more than 100 counts/min./gm. of tissue. This value accounts for only 5×10^{-3} μ atoms of glucose carbon incorporated into triglyceride and long-chain free fatty acids per gram of heart.

8. Summary

(a) Glucose-U-C^{14}, when present at a concentration of 5 mM, is oxidized at a rate of 10 μ moles/gm. wet wt./hr. by the perfused guinea pig heart.

(b) Radioactivity from glucose-U-C^{14} is incorporated into free glutamate, glutamine, alanine and aspartate.

(c) Incorporation of radioactivity from glucose-U-C^{14} into heart protein is relatively low but significant.

TABLE III.8

TOTAL LACTATE FORMATION BY GUINEA PIG HEARTS PERFUSED IN THE PRESENCE OF GLUCOSE

		μ moles lactate produced/hr. ± S.E.M.	
A.	Fed Animals		
	Additions		
1.	None	26 ± 9	(4)
2.	Acetate	84 ± 22	(4)
3.	Propionate	38 ± 14	(3)
4.	Butyrate	79	(1)
5.	Pentanoate	60	(1)
6.	Insulin	112 ± 23	(5)
7.	Insulin and acetate	185 ± 26	(3)
8.	Adrenaline	181 ± 32	(3)
9.	Adrenaline and acetate	223 ± 45	(4)
10.	Insulin and adrenaline	240 ± 73	(4)
11.	Acetate, insulin and adrenaline	150 ± 18	(2)
B.	Starved Animals		
	Additions		
1.	None	108 ± 29	(3)
2.	Insulin and adrenaline	260 ± 24	(2)

Hearts weighing 1.6 to 1.8 gms. were perfused for one hour with 40 ml. of perfusing medium containing an initial glucose concentration of 5 mM. Fatty acids, when present, were at 5 mM concentration except butyrate, which was 2.5 mM. Adrenaline and insulin were present at 0.25 μ gm./ml. and 10 mu/ml. respectively. Lactate was determined enzymatically as described in Methods.

(d) There is a high rate of incorporation of glucose-U-C^{14} into cardiac glycogen.

(e) There is a slight drop in cardiac glycogen on perfusion in the presence of glucose for 1 hour.

(f) Very little radioactive lactate is formed from glucose-U-C^{14}. The total lactate concentration never exceeds concentrations normally found in the blood of the intact animal.

(g) The perfused guinea pig heart does not synthesize appreciable amounts of long-chain free fatty acids or triglyceride from exogenous glucose.

B. THE EFFECTS OF INSULIN ON GLUCOSE-U-C^{14} METABOLISM

1. Oxidation of Glucose-U-C^{14} to $C^{14}O_2$

The present work was carried out to measure directly the effect of insulin on metabolites derived from glucose-U-C^{14}. The results shown in Table III.9 indicate that insulin has a small and possibly not significant effect on glucose oxidation by the perfused guinea pig heart. These data are taken from curves similar to the one shown in Figure III.1. The optimal rate of $C^{14}O_2$ production is determined by a line drawn through the last 5 points on the curve, i.e. after the rate of $C^{14}O_2$ production has become constant.

Williamson (64) recently reported that insulin effects a large increase (250 percent) in the oxidation of exogenous glucose to CO_2 by the perfused rat heart. Bleehen and Fisher (107), as well as the former author, found that the glucose taken up by the isolated rat heart is increased about four-fold by insulin. The conclusions of Williamson concerning the effect of insulin on glucose oxidation are based on calculations of glucose taken up which is not accounted for as glycogen or lactate.

2. The Effect of Insulin on the Incorporation of Glucose-U-C^{14} into Amino Acids and Proteins

As shown in Table III.2, insulin increases the incorporation of carbon from glucose-U-C^{14} into glutamate and alanine. The result with glutamate is difficult to explain in view of the fact that insulin has only a minimal effect on CO_2 formation from glucose, and

TABLE III.9

THE EFFECT OF INSULIN ON $C^{14}O_2$ PRODUCTION FROM GLUCOSE-U-C^{14} BY PERFUSED GUINEA PIG HEARTS

Additions	μ atoms of glucose carbon/gm. wet wt./hr. ± S.E.M.
None	61 ± 6 (10)
Insulin	77 ± 8 (5)

Initial concentrations of glucose and insulin were 5 mM and 10 mu/ml. respectively.

Conditions were as described in Methods.

presumably on the amount of glucose carbon passing through the citric acid cycle. The elevated incorporation into alanine may be explained as follows: lactate accumulates from exogenous glucose, as described later in this section, as a result of insulin administration. Concomitant with an elevated level of lactate, the level of pyruvate should also be increased since the two acids are in dynamic equilibrium. The extent of transamination involving pyruvate would increase, resulting in an increased amount of radioactivity in alanine.

Insulin also increases the incorporation of glucose-U-C^{14} into heart proteins (Table III.3), although this effect is not so clear cut as the effect on incorporation into free amino acids.

These results are in agreement with those presented by Manchester and Young (120, 121) and by Manchester and Krahl (122). These workers found that carbon-14 from C^{14}-labelled glucose, pyruvate and a number of citric acid cycle intermediates is incorporated into the proteins and free amino acids of rat diaphragm. The amino acids formed from glucose were aspartic and glutamic acids and alanine. Insulin stimulated the formation of these amino acids and their incorporation into proteins.

3. <u>The Effect of Insulin on the Incorporation of Glucose-U-C^{14} into Cardiac Glycogen</u>

Insulin increases the amount of radioactivity incorporated into cardiac glycogen from glucose-U-C^{14} about five-fold when glucose is present at a concentration of 5 mM (Table III.4). C^{14}-label accounts for about 35 percent of the original cardiac glycogen, and suggests

a net synthesis of glycogen in the presence of insulin. This result supports the widespread belief that insulin increases the rate of entry of glucose into the cell, making it available for glycogen synthesis as well as for glycolysis.

Morgan et al. (61), in studies of glucose utilization by the perfused rat heart, have shown that in the absence of added insulin, transport into the cell is the major limiting step for glucose uptake at physiological concentrations, while at higher concentrations phosphorylation becomes limiting. With the addition of insulin, glucose uptake is increased because of the increased transport, and phosphorylation becomes the limiting step even at low glucose concentrations.

4. The Effect of Perfusion in the Presence of Glucose and Insulin on Cardiac Glycogen

Insulin effects net synthesis of glycogen in the presence of glucose (Table III.6). Radioactivity in lactate produced during perfusion indicates that not all of the lactate formed in the presence of insulin is derived from C^{14}-labelled glucose. These results indicate that glycogen breakdown occurs in the presence of insulin, but that resynthesis from glucose is more rapid, resulting in net glycogen synthesis.

5. The Effect of Insulin on Lactate Accumulation

Large amounts of lactate accumulate from exogenous glucose in the presence of insulin (Table III.7). Newsholme et al. (76) have pointed out the fact that there is no accumulation of glycolytic intermediates

from glucose by the isolated rat heart in the absence of insulin. The present data support the idea that glycolysis is not limiting for glucose utilization when no insulin is present, and that the addition of insulin increases the rate of glycolysis by allowing a faster rate of entry of glucose into the cell.

6. Summary

(a) Insulin slightly increases the rate of oxidation of glucose-U-C^{14} to $C^{14}O_2$.

(b) The incorporation of radioactivity from glucose-U-C^{14} into glutamate and alanine, and into heart proteins is significantly increased by insulin.

(c) Insulin effects a five-fold increase in the net synthesis of glycogen from glucose-U-C^{14}. The total amount of cardiac glycogen increases significantly on perfusion in the presence of glucose and insulin.

(d) Lactate accumulates in the perfusing medium when both glucose and insulin are present. A large percentage of the total lactate formed is derived from exogenous glucose, but there is also a significant amount of lactate formed from cardiac glycogen.

C. THE EFFECT OF STARVATION ON GLUCOSE-U-C^{14} METABOLISM

Cardiac carbohydrate metabolism is impaired by starvation and diabetes, both in the intact animal (54, 58) and in the isolated hearts from starved or diabetic animals (64,66,68,69).

In this section the effects of starvation on the metabolic fate of exogenously added glucose-U-C^{14} are followed.

1. The Effect of Starvation on the Oxidation of Glucose-U-C^{14} to $C^{14}O_2$

The results presented in Figure III.10 show that CO_2 formation from exogenous glucose is almost completely abolished by starvation.

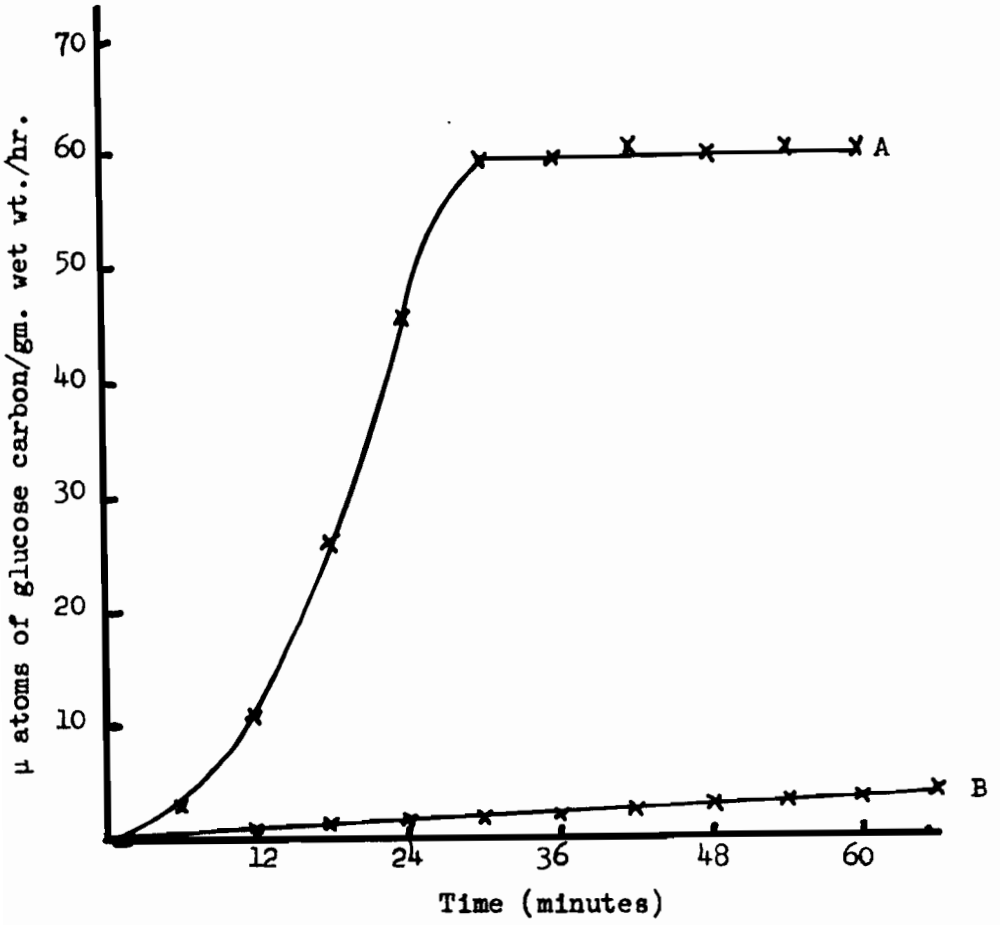
Newsholme et al. (76) reported that starvation reduces the glucose uptake of the isolated rat heart by about half. These authors assigned this decrease to an inhibition of the phosphofructokinase reaction, adjudged by the fact that there is an accumulation of F6P by the hearts of starved rats.

2. The Effect of Starvation on the Incorporation of Glucose-U-C^{14} into Amino Acids and Proteins

As shown in Table III.2, there is no detectible incorporation of radioactivity from glucose-U-C^{14} into glutamate or glutamine by hearts from starved guinea pigs, but the incorporation of label into alanine persists. These results substantiate the finding that very little glucose enters the citric acid cycle, and also suggest that glucose is being metabolized to pyruvate and lactate as evidenced by the incorporation of C^{14} from glucose into alanine.

FIGURE III.10

THE EFFECT OF STARVATION ON $C^{14}O_2$ PRODUCTION FROM GLUCOSE-U-C^{14} BY THE PERFUSED GUINEA PIG HEART



Conditions were described in C.1 and in Methods.

A - hearts from fed guinea pigs.
B - hearts from fasted guinea pigs.

Glucose concentration: 5 mM

It is further observed that the incorporation of glucose carbon into proteins is reduced to an almost undetectible level (Table III.3). This result is interpreted as being a reflection of the decreased incorporation of radioactivity into amino acids.

3. The Effect of Starvation on the Incorporation of Glucose-U-C^{14} into Cardiac Glycogen

Fasting markedly decreases the net incorporation of glucose-U-C^{14} into cardiac glycogen (Table III.4).

4. The Effect of Starvation on Cardiac Glycogen during Perfusion in the Presence of Glucose

The cardiac glycogen of guinea pigs is approximately tripled by 72 hrs. of fasting (Table III.5). Although hearts from starved guinea pigs have a higher initial glycogen content than those from fed guinea pigs, the former show a markedly greater rate of glycogenolysis during perfusion in the presence of glucose than the latter (Tables III.5 and III.6). This observation probably explains the decreased net synthesis of cardiac glycogen from labelled glucose as a result of starvation.

Cardiac glycogen increases with fasting (73) and with untreated diabetes (74), in contrast to the depletion of glycogen in liver and diaphragm (123).

5. The Effect of Starvation on Lactate Formation from Exogenous Glucose-U-C^{14}

Starvation increases the amount of lactate which accumulates from glucose-U-C^{14} (Table III.7). This result might be expected in view

of the greatly decreased oxidation of glucose to CO_2 by the hearts of starved guinea pigs. The increase in accumulation of labelled lactate does not account wholly, however, for the difference in the amount of $C^{14}O_2$ produced by these hearts as compared to hearts from fed animals. This may be explained by the fact that there is a much greater rate of glycogenolysis by hearts from animals in the starved condition than by hearts from fed animals. The glucose residues from glycogen entering into the glycolytic pathway would tend to saturate the glycolytic enzymes, thereby reducing the rate of glycolysis of exogenous glucose.

6. The Effect of Starvation on Total Lactate Accumulation

In addition to the increased lactate production from exogenous glucose in starvation, the total lactate produced is increased even more markedly (Table III.8). This result is not surprising in view of the fact that starvation greatly increases the rate of glycogenolysis by the perfused heart, while decreasing CO_2 formation from glucose.

7. Summary

(a) The oxidation of glucose-U-C^{14} to $C^{14}O_2$ by perfused guinea pig hearts is suppressed more than 90 percent by starving the animal for a period of 72 hours.

(b) The incorporation of radioactivity from glucose-U-C^{14} into glutamate and glutamine in the perfused heart is almost completely blocked by starvation, while the radioactive labelling in alanine is undiminished.

(c) The cardiac glycogen of guinea pigs is increased approximately three-fold by a 72-hour fast.

(d) Lactate production by the perfused heart, both from exogenous glucose and cardiac glycogen, is markedly increased by starvation.

D. THE EFFECT OF SHORT-CHAIN FATTY ACIDS ON THE METABOLISM OF GLUCOSE-U-C^{14} BY THE PERFUSED GUINEA PIG HEART

Previous work on intact animals (3,33,37,38) has suggested that fatty acids are important metabolites for the heart. Shipp et al. (72) showed that, in the perfused rat heart, palmitate is rapidly oxidized to CO_2 and that the utilization of glucose is suppressed by palmitate. Hall (89), and Williamson and Krebs (80) reported a similar effect on glucose utilization by acetoacetate in perfused rat hearts. These results infer that the decrease in the utilization of glucose by cardiac muscle from starved or diabetic animals occurs as a result of increased oxidation of fatty substances. The metabolic lesion affecting carbohydrate metabolism must be related to a metabolic step or steps common to all substances which suppress the utilization of carbohydrate.

The effects of several individual short-chain fatty acids on the utilization of glucose-U-C^{14} by perfused guinea pig hearts are described in this section.

1. The Effect of Short-Chain Fatty Acids on the Oxidation of Glucose-U-C^{14} to $C^{14}O_2$

The rate of oxidation of glucose-U-C^{14} to $C^{14}O_2$ by the perfused guinea pig heart is markedly reduced by the addition of all short-chain fatty acids which can give rise to acetyl-CoA (Table III.11). The rate of glucose oxidation in the presence of fatty acids is about the same as that obtained using hearts from starved animals in the absence of added fatty acids.

TABLE III.11

THE EFFECT OF SHORT-CHAIN FATTY ACIDS ON THE OXIDATION OF GLUCOSE-U-C^{14} TO $C^{14}O_2$ BY PERFUSED GUINEA PIG HEARTS

ADDITIONS	% OF CONTROL
None	100
5 mM acetate	8
5 mM propionate	62
5 mM butyrate	6
5 mM pentanoate	8
1 mM heptanoate	21

All perfusions were carried out at 37°C. with 40 ml. of perfusion fluid containing glucose at a concentration of 5 mM. Values are taken from the rates of $C^{14}O_2$ production attained during the second 30 minutes of perfusion and are averages of 3 or more separate experiments.

Propionate, which does not give rise to acetyl-CoA (124,125, 126,127), has a much smaller effect on glucose utilization than do any other fatty acids. These results show that all fatty substances which are metabolized via acetyl-CoA markedly suppress glucose oxidation, and suggest that this suppressed oxidation is effected by reducing the amount of glucose carbon which enters the citric acid cycle.

2. The Effect of Short-Chain Fatty Acids on the Incorporation of Glucose-U-C^{14} into Amino Acids and Protein

Table III.2 shows that the incorporation of C^{14} from glucose into glutamate and glutamine is reduced to almost undetectible levels by fatty acids, while the incorporation of C^{14} into alanine is increased. The amount of radioactivity incorporated into proteins is reduced, again reflecting the reduced C^{14}-labelling of amino acids from glucose-U-C^{14} (Table III.3).

There is a pronounced reduction in the incorporation of glucose carbon into glutamate in the presence of propionate. This effect is disproportionate to the reduction in $C^{14}O_2$ production from glucose-U-C^{14} effected by propionate. As shown in a later chapter, propionate is readily oxidized by the perfused guinea pig heart. It is likely, therefore, that propionate oxidation gives rise to increased levels of citric acid cycle intermediates, and that the decrease in amino acid labelling from C^{14}-glucose effected by propionate is the result of isotopic dilution of intermediates in the citric acid cycle which gives rise to amino acids.

3. The Effect of Short-Chain Fatty Acids on the Incorporation of Glucose-U-C^{14} into Cardiac Glycogen

The amount of glycogen synthesized from glucose-U-C^{14}, in the presence of acetate, butyrate or pentanoate is at least double that synthesized in their absence. Incorporation of glucose-U-C^{14} into cardiac glycogen is not significantly increased by propionate (Table III.4).

Shipp et al. (72) found that palmitate increases the amount of radioactive glucose incorporated into the glycogen of rat hearts. Williamson and Krebs (80) reported a slight increase in glycogen of rat hearts as a result of perfusion in the presence of glucose and acetoacetate or -hydroxybutyrate. Newsholme et al. (76) reported that rat heart glycogen is increased significantly by perfusion with glucose in the presence of ketone bodies or octanoate. In addition, the latter authors found that the concentrations of hexose monophosphates are increased and that of FDP is decreased by ketone bodies or octanoate. They conclude from these results that phosphofructokinase is inhibited by the oxidation of ketone bodies and fatty acids. It has been suggested that glycogenesis is stimulated by fatty acids in two ways: (a) accumulating glucose residues, which otherwise would be metabolized by glycolysis, may be diverted to glycogen synthesis; and (b) the higher concentration of G6P may activate the enzyme which transfers glucose residues from UDPG to glycogen (128). It must be concluded from the present results that propionate oxidation does not affect hexose phosphorylation in the same way as do other fatty acids.

4. The Effect of Short-Chain Fatty Acids on Glycogen Retention

Total cardiac glycogen was determined after perfusion with radioactive glucose and short-chain fatty acids. There apparently is no net glycogen synthesis, but there is more residual glycogen present after perfusion in the presence of fatty acids than with glucose alone (Table III.6).

5. The Effect of Short-Chain Fatty Acids on Lactate Accumulation from Glucose-U-C^{14}

Lactate derived from glucose-U-C^{14} accumulates extensively when either acetate, butyrate or pentanoate is present in the perfusing medium (Table III.7). Indeed, radioactive lactate accounts for nearly all of the difference in glucose-U-C^{14} oxidized to $C^{14}O_2$ in the presence of fatty acids compared with that obtained in their absence. This result strongly suggests that the most important block to glucose utilization by fatty acids is a reduction in the amount of glucose metabolites which enter into the citric acid cycle.

As in the data described earlier, propionate does not block glucose utilization as the other fatty acids do; i.e., the amount of radioactivity from glucose-U-C^{14} which accumulates as lactate is not greatly affected by propionate (Table III.7).

In view of these results, it appears that carbohydrate cannot compete favorably with acetyl-CoA formed from fatty acids for entry into the citric acid cycle, and that perhaps the decarboxylation of pyruvate is inhibited by fatty acids. It is unlikely that the reduction in glucose oxidation results from an unfavorable competition for CoA.

If this were true propionate would be expected to inhibit glucose oxidation to about the same extent as do other fatty acids.

6. The Effect of Short-Chain Fatty Acids on Total Lactate Production

Total lactate production is very markedly increased by the short-chain fatty acids which give rise to acetyl-CoA, but is not increased significantly by propionate (Table III.8). The amount of lactate which accumulates from glycogen, however, cannot be determined precisely, since this value can be obtained only by difference between the total lactate and lactate produced from radioactive glucose.

7. Summary

(a) When present at equimolar concentrations with glucose, short-chain fatty acids which give rise to acetyl-CoA suppress the oxidation of glucose-U-C^{14} to $C^{14}O_2$ by more than 90 percent. Propionate reduces glucose oxidation about 40 percent.

(b) Incorporation of radioactivity from glucose-U-C^{14} into glutamate, glutamine and protein is almost completely suppressed, and incorporation of glucose carbon into alanine is increased by fatty acids which give rise to acetyl-CoA. The effects of propionate on the labelling of amino acids from radioactive glucose are less pronounced than those of other short-chain fatty acids.

(c) Fatty acids, with the exception of propionate, increase the net incorporation of glucose-U-C^{14} into cardiac glycogen. There is less net glycogenolysis in the presence of fatty acids than in their absence.

(d) Lactate derived from glucose-U-C^{14} accumulates markedly in the presence of short-chain fatty acids. Propionate does not affect either total lactate production or lactate derived from glucose-U-C^{14}.

E. THE EFFECT OF ADRENALINE ON THE METABOLISM OF GLUCOSE-U-C^{14} BY THE PERFUSED GUINEA PIG HEART

1. The Effect of Adrenaline on the Oxidation of Glucose-U-C^{14} to $C^{14}O_2$

L-adrenaline at an initial concentration of 0.25 μ gm./ml. increases the rate of oxidation of glucose-U-C^{14} about 270 percent. Figure III.12 shows the average results from several individual perfusion experiments, comparing the rates of oxidation of glucose in the absence and presence of adrenaline. Adrenaline also increases the beat rate and the intensity of the beat as well as the rate of coronary perfusion (see Methods).

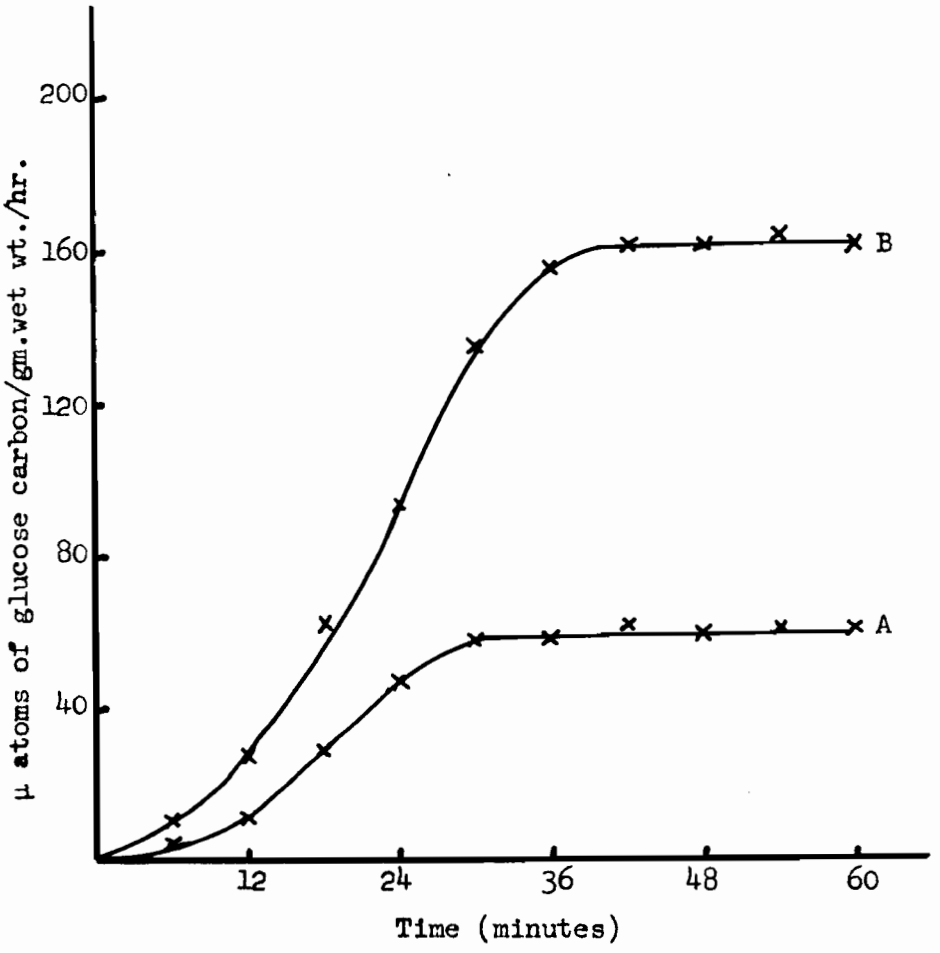
2. The Effect of Adrenaline on the Incorporation of Radioactivity from Glucose-U-C^{14} into Amino Acids and Protein

Adrenaline decreases the incorporation of C^{14} from glucose into glutamate about 40 percent and almost completely abolishes glutamine formation. The incorporation of radioactivity into alanine is increased (Table III.2). Incorporation of label into proteins is decreased by adrenaline to about the same extent as that into glutamate (Table III.3).

The increased incorporation of glucose carbon into alanine suggests a large accumulation of lactate. It might be expected that adrenaline would increase the amount of incorporation of C^{14} into glutamate and glutamine, since more glucose carbon passes through the citric acid cycle. The result obtained, however, is characteristic of the generalized catabolic action of adrenaline. The pronounced effect of adrenaline on glutamine formation is of particular interest. Glutamine

FIGURE III.12

THE EFFECT OF ADRENALINE ON $C^{14}O_2$ PRODUCTION FROM GLUCOSE-U-C^{14} BY PERFUSED GUINEA PIG HEARTS



A - Control, no additions.
B - - adrenaline (0.25 μ gm./ml.)

Conditions were as described in E.1 and in Methods.
Glucose concentration: 5 mM.

synthesis from glutamate requires ATP. It may be that more of the available ATP is used for contraction, resulting in decreased utilization of ATP for synthetic reactions.

3. The Effect of Adrenaline on Incorporation of Glucose-U-C^{14} into Cardiac Glycogen

The amount of radioactivity from glucose-U-C^{14} found in glycogen after perfusion in the presence of adrenaline is only 1 to 3 percent of that found when adrenaline is absent (Table III.4). This result does not necessarily indicate that the rate of glycogen synthesis from glucose is decreased by adrenaline, since the radioactivity in glycogen is the result of balance between synthetic and degradative reactions. The glycogenolytic action of adrenaline has been demonstrated in many tissues, and these results show that cardiac muscle is no exception.

4. The Effect of Adrenaline on Glycogen Retention

Only about 10 percent of the initial cardiac glycogen remains after 1 hr. of perfusion in the presence of adrenaline (Table III.6). A considerable portion of the glycogen is oxidized to CO_2, the remainder accumulating as lactate.

5. The Effect of Adrenaline on the Accumulation of Lactate Derived from Glucose-U-C^{14}

The amount of lactate which is produced from glucose-U-C^{14} is increased more than ten-fold by adrenaline (Table III.7). This result shows that the rate of glycolysis is greatly stimulated by adrenaline,

as judged by the fact that lactate from exogenous glucose accumulates simultaneously with a greatly accelerated oxidation of glucose. Although the complete oxidation of carbohydrate is stimulated by adrenaline, glycolysis is increased to a greater degree than the oxidative mechanism, and entry of pyruvate into the citric acid cycle becomes the limiting step. This effect may be explained by a limited availability of oxygen when the rate of oxygen extraction by the heart is stimulated by adrenaline, or by saturation of the enzymes or co-enzymes concerned with oxidative metabolism.

6. The Effect of Adrenaline on Total Lactate Accumulation

Adrenaline effects a very pronounced accumulation of lactate, derived both from exogenous glucose and from glycogen (Table III.8). Lactate accumulates, even though aerobic metabolism of carbohydrate is substantially increased. This result demonstrates the fact that neither entry of glucose nor its subsequent glycolysis is limiting for glucose utilization when adrenaline is present.

7. Summary

(a) Adrenaline, when present at an initial concentration of 0.25 μ gm./ml. of perfusing medium, stimulates the oxidation of glucose-U-C^{14} to $C^{14}O_2$ almost three-fold.

(b) Incorporation of radioactivity from glucose-U-C^{14} into glutamate, glutamine and protein is diminished by adrenaline. Incorporation of glucose carbon into alanine is increased.

(c) The net incorporation of glucose-U-C^{14} into cardiac glycogen is very low when adrenaline is present. Cardiac glycogen is almost completely depleted after 1 hour of perfusion in the presence of adrenaline.

(d) Lactate accumulates in the perfusing medium as a result of adrenaline action. The lactate which accumulates is derived from exogenous glucose and from glycogen.

F. THE METABOLISM OF GLUCOSE-U-C^{14} BY THE PERFUSED GUINEA PIG HEART IN THE PRESENCE OF INSULIN AND ADRENALINE

1. The Effect of Insulin and Adrenaline on the Oxidation of Glucose-U-C^{14} to $C^{14}O_2$

The rate of oxidation of glucose-U-C^{14} to $C^{14}O_2$ in the presence of insulin and adrenaline is not significantly different from that obtained when adrenaline alone is added (Table III.13). This result indicates that adrenaline is at least as effective as insulin in stimulating glucose uptake. This conclusion was previously indicated by the fact that lactate accumulates from glucose in the presence of adrenaline, in spite of the greatly accelerated oxidation of glucose.

2. The Effect of Insulin and Adrenaline on the Incorporation of Glucose-U-C^{14} into Amino Acids

In the presence of adrenaline insulin has little, if any, additional effect on incorporation of radioactivity into glutamate from glucose-U-C^{14} when compared to the effect of adrenaline alone. Under these conditions, however, there is detectible glutamine synthesis from labelled glucose and a considerable increase in the incorporation of glucose carbon into alanine. The latter result suggests that insulin further stimulates lactate formation from glucose, even in the presence of adrenaline.

3. The Effect of Insulin and Adrenaline on the Incorporation of Glucose-U-C^{14} into Cardiac Glycogen

The amount of radioactivity in cardiac glycogen formed from glucose-U-C^{14} was shown in the preceding section to be reduced by adrenaline to

TABLE III.13

THE EFFECT OF INSULIN AND ADRENALINE ON THE OXIDATION OF GLUCOSE-U-C^{14} TO $C^{14}O_2$ BY PERFUSED GUINEA PIG HEARTS

Additions	$C^{14}O_2$ Production μ atoms glucose carbon/gm.wet wt./hr. ± S.E.M.
None	61 ± 6 (10)
Adrenaline	160 ± 29 (5)
Adrenaline and insulin	149 ± 20 (4)

Conditions were as described in Methods.

Glucose was present at 5 mM initial concentration. Insulin and adrenaline were added to concentrations of 10 mu/ml. and 0.25 μ gm./ml. respectively. $C^{14}O_2$ production was determined from rates attained during the second 30 minutes of perfusion.

1 to 3 percent of that formed in the absence of adrenaline. When both insulin and adrenaline are added to the medium perfusing a heart the net incorporation of radioactivity into glycogen from glucose-U-C^{14} is not significantly different from that obtained when adrenaline alone is added (Table III.4). Thus, the myoglycostatic effect of insulin is reversed by adrenaline. Superficially, this result would be expected, due to the fact that glycogen is rapidly depleted by adrenaline. However, if the glycogen-synthesizing action of insulin persists in the presence of adrenaline, it would be expected that the amount of glucose-U-C^{14} found at any time in cardiac glycogen would be increased by insulin, even when there is a very high rate of glycogenolysis.

Morgan et al. (61) reported that, in the presence of glucose and insulin, both glucose and hexose phosphates accumulate in the perfused rat heart, indicating that phosphorylation steps become limiting when insulin is present. These authors suggested that insulin facilitates glycogen synthesis, indirectly as a result of increased glucose uptake and directly as a result of accumulation of glucose and phosphorylated hexose intermediates. If adrenaline accelerates phosphorylation adequately to prevent accumulation of glucose and hexose phosphates, according to this hypothesis insulin would be unable to promote glycogen synthesis in the presence of adrenaline.

4. The Effect of Insulin and Adrenaline on Glycogen Retention

Insulin does not have a significant effect on the amount of cardiac glycogen which remains after perfusion with glucose in the presence

of adrenaline (Table III.6). Therefore, insulin does not affect significantly the synthesis or breakdown of glycogen when adrenaline is present.

5. The Effect of Insulin and Adrenaline on the Accumulation of Lactate Derived from Glucose-U-C^{14}

Although adrenaline and insulin, when added singly, cause a large accumulation of radioactive lactate derived from glucose-U-C^{14}, when both insulin and adrenaline are present in the perfusing medium the lactate accumulation from labelled glucose is higher than when only one is present (Table III.7). This result substantiates the inference from the high incorporation of radioactivity into alanine, that lactate derived from exogenous glucose accumulates to an extremely high level when both adrenaline and insulin are present in the perfusate.

6. The Effect of Insulin and Adrenaline on Total Lactate Accumulation

Insulin increases the total amount of lactate which accumulates in the presence of adrenaline. This increase is accounted for by an increase in the amount of lactate formed from exogenous glucose, with the contribution to lactate from glycogen remaining unchanged.

7. Summary

(a) In the presence of adrenaline and insulin, the rate of oxidation of glucose-U-C^{14} to $C^{14}O_2$ is not significantly different from that obtained when adrenaline alone is present.

(b) In the presence of adrenaline, insulin increases the amount of radioactive labelling in alanine and glutamine from glucose-U-C^{14}

but does not affect radioactive labelling of glutamate.

(c) In the presence of adrenaline, insulin does not affect significantly the incorporation of radioactivity from glucose-U-C^{14} into cardiac glycogen.

(d) In the presence of adrenaline, insulin further increases the total lactate which accumulates. This increase is accounted for by an increase in the amount of exogenous glucose which accumulates as lactate.

G. THE EFFECT OF INSULIN ON THE INHIBITION BY ACETATE OF THE UTILIZATION OF GLUCOSE-U-C^{14} BY THE PERFUSED GUINEA PIG HEART

It was shown in an earlier section that short-chain fatty acids which give rise to acetyl-CoA reduce the rate of CO_2 formation from exogenous glucose by more than 90 percent. In the absence of added fatty acids, insulin increases the accumulation of lactate and slightly increases the amount of oxidation of exogenous glucose to CO_2.

The effect of insulin on glucose metabolism in the presence of fatty acids is described in this section.

1. The Effect of Insulin on Acetate Inhibition of the Oxidation of Glucose-U-C^{14} to $C^{14}O_2$

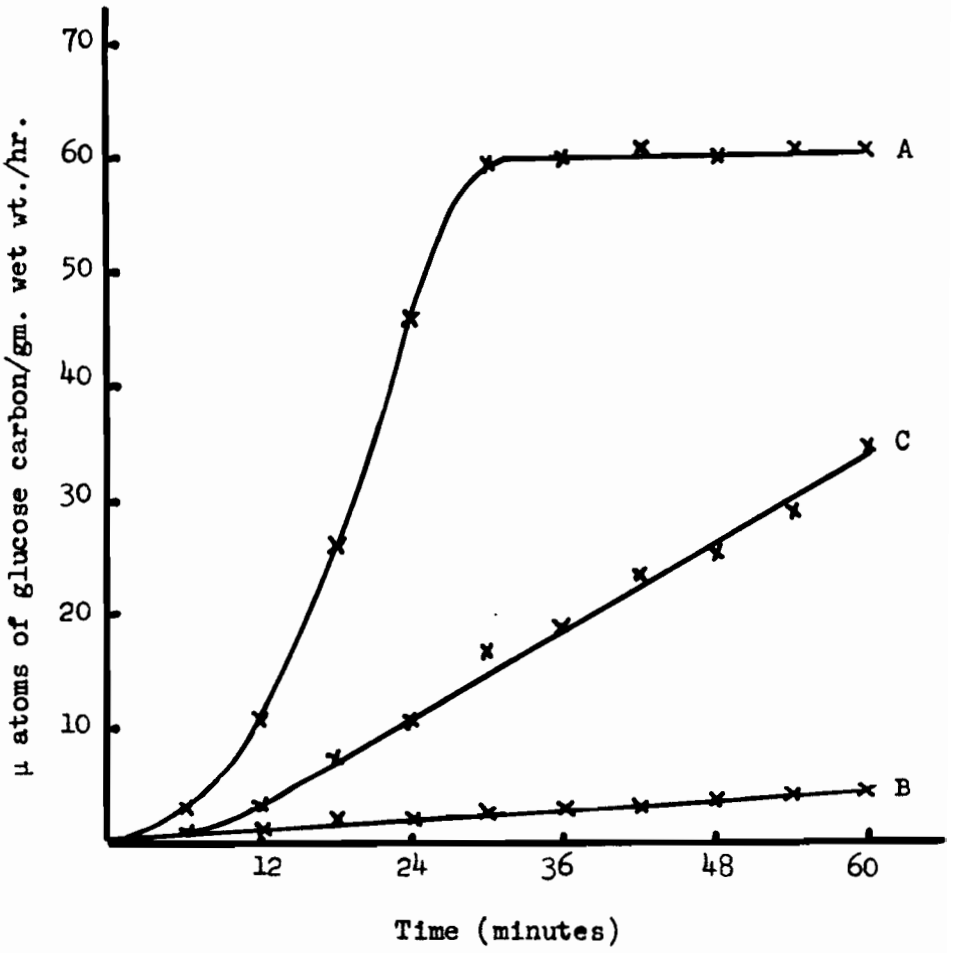
Insulin, when added at an initial concentration of 10 mu/ml. of perfusing medium, progressively increases $C^{14}O_2$ production from glucose-U-C^{14} (Figure III.14). These data indicate that insulin has an effect on carbohydrate utilization separate from its effect on the entry of glucose into the cell. The stimulation of glucose oxidation is not instantaneous, since the rate of $C^{14}O_2$ production from glucose-U-C^{14} is still increasing after one hour of perfusion in the presence of insulin. The final rate in the presence of acetate and insulin is about half that attained in the absence of acetate.

2. The Effect of Insulin on the Incorporation of Radioactivity from Glucose-U-C^{14} into Amino Acids in the Presence of Acetate

As described earlier, acetate reduces the incorporation of radioactivity into glutamate and glutamine to undetectible levels, while

FIGURE III.14

THE EFFECT OF INSULIN ON ACETATE INHIBITION OF THE OXIDATION OF GLUCOSE-U-C^{14} TO $C^{14}O_2$



A - control (no additions)
B - - acetate (5 mM)
C - - acetate and insulin (10 mu/ml.)

Conditions as described in G.1 and in Methods.

Glucose concentration: 5 mM

increasing the incorporation of radioactivity into alanine. When insulin is added in the presence of acetate, however, there is significant incorporation of C^{14} from glucose into glutamate and glutamine, amounting to about one-fifth that obtained in the absence of acetate (Table III.2). This result supports the previous evidence which demonstrates that insulin increases the amount of glucose carbon entering the citric acid cycle in the presence of acetate. Presumably, glutamate can become labelled only by way of a transamination reaction involving α-ketoglutarate.

3. The Effect of Insulin on the Incorporation of Glucose-U-C^{14} into Cardiac Glycogen in the Presence of Acetate

There is apparently a higher net incorporation of exogenous glucose into glycogen in the presence of insulin and acetate than in the presence of insulin alone. This effect is not well established, however, due to the fact that there is considerable variability in the results obtained between individual experiments, both in the presence and absence of insulin.

4. The Effect of Insulin on the Accumulation of Lactate Derived from Glucose-U-C^{14} in the Presence of Acetate

As shown in a previous section, both insulin and acetate effect an accumulation of lactate derived from exogenous glucose.

When both insulin and acetate are present in the perfusing medium there is an even greater accumulation of radioactive lactate from glucose-U-C^{14} than with either insulin or acetate alone (Table III.7). The effects of insulin and acetate on lactate accumulation are approximately additive, since insulin increases lactate formation by increasing

the amount of glucose available for glycolysis, while acetate causes lactate accumulation by reducing the rate of entry of pyruvate into the citric acid cycle.

5. Summary

(a) Insulin partially reverses the inhibitory effect of acetate on the oxidation of glucose-U-C^{14} to $C^{14}O_2$.

(b) Insulin partially reverses the inhibitory effect of acetate on the incorporation of radioactivity from glucose-U-C^{14} into glutamate and glutamine.

(c) Insulin increases the amount of glucose-U-C^{14} incorporated into cardiac glycogen in the presence of acetate.

(d) More lactate derived from glucose-U-C^{14} is formed when both insulin and acetate are present than with one alone.

H. THE EFFECT OF ADRENALINE ON THE METABOLISM OF GLUCOSE-U-C^{14} IN THE PRESENCE OF ACETATE

1. The Effect of Adrenaline on the Oxidation of Glucose-U-C^{14} to $C^{14}O_2$ in the Presence of Acetate

In the presence of acetate, adrenaline increases the rate of oxidation of glucose-U-C^{14} to $C^{14}O_2$ about eight-fold (Figure III.15).

2. The Effect of Adrenaline on the Incorporation of Radioactivity Derived from Glucose-U-C^{14} into Amino Acids in presence of Acetate

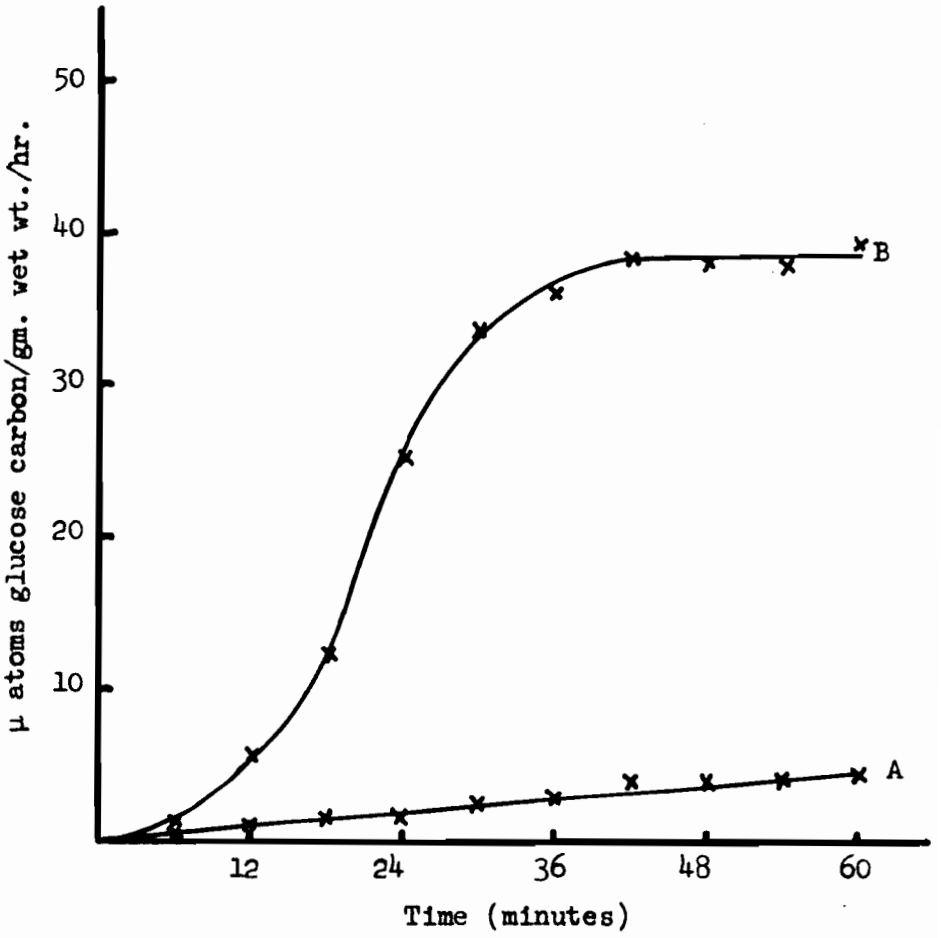
Neither glutamate nor glutamine is significantly labelled from glucose-U-C^{14} in the presence of both acetate and adrenaline. The incorporation of radioactivity into alanine, however, is increased by adrenaline (Table III.2). The lack of appreciable incorporation of radioactivity into glutamate and glutamine is probably due to isotopic dilution of citric acid cycle intermediates formed from glucose-U-C^{14} by intermediates derived from acetate, glycogen and other endogenous substrates.

3. The Effect of Adrenaline on Incorporation of Glucose-U-C^{14} into Cardiac Glycogen in the Presence of Acetate

The net amount of radioactivity incorporated into cardiac glycogen in the presence of acetate and adrenaline is very low, and not significantly different from that found when acetate is not present in the perfusing medium (Table III.4).

FIGURE III.15

THE EFFECT OF ADRENALINE ON THE OXIDATION OF GLUCOSE-U-C^{14} TO $C^{14}O_2$ IN THE PRESENCE OF ACETATE



Hearts were perfused with 40 ml. of perfusion fluid containing glucose and acetate at 5 mM initial concentrations. Adrenaline, when added, was present at 0.25 μ gm./ml.
Other conditions as described in H.1 and in Methods.

A - - acetate
B - - acetate and adrenaline

4. The Effect of Adrenaline on Glycogen Retention in the Presence of Acetate

Adrenaline effects a high rate of glycogenolysis, whether or not acetate is present. The amount of cardiac glycogen which remains after 1 hr. of perfusion is apparently slightly higher, however, when acetate is present (Table III.6).

5. The Accumulation of Radioactive Lactate from Glucose-U-C^{14} in the Presence of Adrenaline and Acetate

Lactate derived from glucose-U-C^{14} accumulates to a greater extent in the presence of adrenaline and acetate than when either one is added alone (Table III.7). It is concluded that acetate causes lactate accumulation by reducing the rate of entry of carbohydrate material into the citric acid cycle, and adrenaline further increases lactate formation by increasing the rate of glycolysis.

6. Summary

(a) In the presence of acetate, adrenaline, at an initial concentration of 0.25 μ gm./ml. of perfusing medium, increases the rate of oxidation of glucose-U-C^{14} to $C^{14}O_2$ about eight-fold.

(b) The amount of incorporation of radioactivity from glucose-U-C^{14} into glutamate and glutamine in the presence of acetate is not significantly increased by adrenaline, but the amount of radioactivity incorporated into alanine is increased.

(c) Adrenaline reduces the net incorporation of exogenous glucose into cardiac glycogen in the presence of acetate to extremely low levels. This result is probably due to an overriding glycogenolytic effect by adrenaline.

(d) Adrenaline further increases the amount of radioactive lactate produced in the presence of acetate.

J. THE COMBINED EFFECTS OF INSULIN AND ADRENALINE ON THE METABOLISM OF GLUCOSE-U-C^{14} IN THE PRESENCE OF ACETATE

1. The Effect of Insulin and Adrenaline on the Oxidation of Glucose-U-C^{14} to $C^{14}O_2$ in the Presence of Acetate

In the presence of insulin and adrenaline, acetate inhibits the oxidation of exogenous glucose to CO_2 only 40 percent (Figure III.16), whereas in their absence acetate inhibits 92 percent (Table III.11).

These results demonstrate the fact that the oxidation of glucose by cardiac muscle is greatly influenced by adrenaline and insulin, particularly when glucose must compete with fatty acids for oxidation.

2. The Effect of Adrenaline and Insulin on the Incorporation of Radioactivity from Glucose-U-C^{14} into Amino Acids in the Presence of Acetate

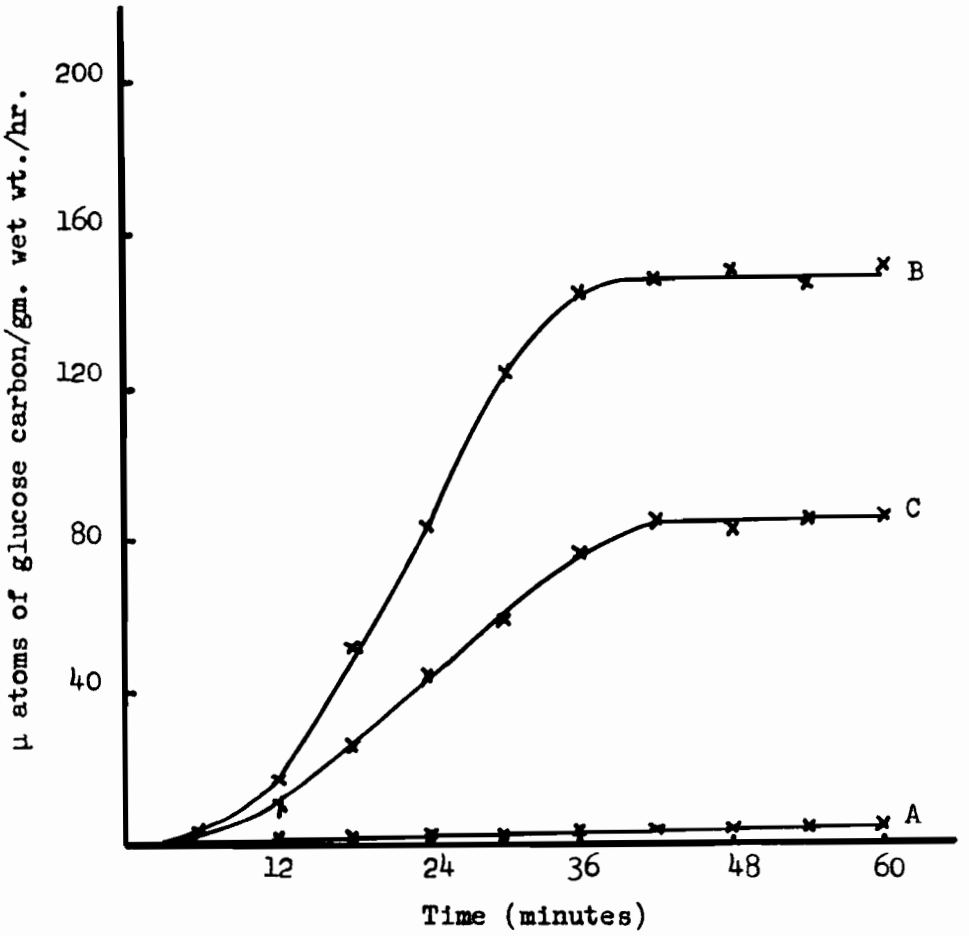
When acetate is present in the perfusing medium, the addition of insulin and adrenaline increases the amount of radioactivity incorporated into glutamate and glutamine from glucose-U-C^{14} to levels more than half those obtained in the absence of acetate. The incorporation into alanine is unchanged (Table III.2).

3. The Effect of Adrenaline and Insulin on the Incorporation of Glucose-U-C^{14} into Cardiac Glycogen in the Presence of Acetate

The decrease in the incorporation of glucose-U-C^{14} into cardiac glycogen in the presence of acetate exerted by adrenaline is not significantly affected by insulin (Table III.4).

FIGURE III.16

THE COMBINED EFFECT OF INSULIN AND ADRENALINE ON THE OXIDATION OF GLUCOSE-U-C^{14} TO $C^{14}O_2$ IN THE PRESENCE OF ACETATE



Conditions as described in J.1 and in Methods.

A - - acetate
B - - acetate, insulin and adrenaline
C - - insulin and adrenaline

Concentrations: glucose, 5 mM; acetate, 5 mM; insulin, 10 mu/ml.; adrenaline 0.25 μ gm./ml.

4. The Effect of Insulin and Adrenaline on Glycogen Retention in the Presence of Acetate

There is a very pronounced glycogenolytic effect by adrenaline, even in the presence of both insulin and acetate. More than 80 percent of the initial cardiac glycogen is lost by perfusing one hour with glucose in the presence of adrenaline, whether or not insulin and acetate, added singly or in combination, are present in the perfusion medium.

5. The Effect of Adrenaline and Insulin on the Accumulation of Radioactive Lactate Derived from Glucose U-C^{14} in the Presence of Acetate

The accumulation of lactate derived from exogenous glucose in the presence of acetate is further increased by insulin or adrenaline. When both insulin and adrenaline are added, however, the lactate accumulation from glucose is not significantly greater than when either one is added alone (Table III.7).

6. Summary

(a) When both insulin and adrenaline are present, the inhibitory effect of acetate on the oxidation of exogenous glucose-U-C^{14} to $C^{14}O_2$ is reversed to a large extent.

(b) A combination of insulin and adrenaline in the perfusing medium reverses the inhibitory effect of acetate on the incorporation of radioactivity into glutamate and glutamine.

(c) The net incorporation of glucose-U-C^{14} into cardiac glycogen in the presence of adrenaline is not significantly increased by the addition of insulin and acetate.

(d) The total accumulation of radioactive lactate derived from glucose-U-C^{14} in the presence of acetate is increased by the addition of either insulin or adrenaline, but is not further increased when both are present.

K. THE EFFECT OF INSULIN AND ADRENALINE ON THE METABOLISM OF GLUCOSE-U-C^{14} BY HEARTS FROM STARVED GUINEA PIGS

In preceding sections a number of similarities were demonstrated between the effects of starvation and of fatty acids on the utilization of glucose by the perfused guinea pig heart. In addition, it was shown that insulin and adrenaline, when added together to the perfusing medium, partly reverse the effects of fatty acids on the metabolism of glucose by hearts from fed animals.

The combined effects of insulin and adrenaline on the metabolism of glucose-U-C^{14} by the hearts from starved guinea pigs are described in this section.

1. The Effect of Insulin and Adrenaline on the Oxidation of Glucose-U-C^{14} to $C^{14}O_2$

The oxidation of glucose-U-C^{14} to $C^{14}O_2$ by hearts from guinea pigs which were starved for a period of 72 hours is increased more than ten-fold when both insulin and adrenaline are added (Table III.17). This result is similar to, although somewhat smaller than that produced by insulin and adrenaline on the oxidation of glucose in the presence of acetate.

2. The Combined Effect of Insulin and Adrenaline on the Incorporation of Radioactivity into Amino Acids from Glucose-U-C^{14}

As shown previously, there is no detectible incorporation of radioactivity from glucose-U-C^{14} into glutamate or glutamine when hearts from fasted guinea pigs are perfused with glucose. In the

TABLE III.17

THE COMBINED EFFECT OF INSULIN AND ADRENALINE ON THE OXIDATION OF GLUCOSE-U-C^{14} TO $C^{14}O_2$ BY HEARTS OF STARVED GUINEA PIGS

Additions	$C^{14}O_2$ Production μ atoms glucose carbon/gm.wet wt./hr. ± S.E.M.
None	4.2 ± 0.6 (5)
Insulin and adrenaline	48.4 ± 7.5 (2)

Animals were deprived of food 72 hours before sacrificing. Conditions of experiments were as described in Methods.

Concentrations: glucose, 5 mM; insulin, 10 mu/ml.; adrenaline, 0.25 μ gm./ml.

presence of insulin and adrenaline, however, radioactivity is found in both glutamate and glutamine. The incorporation of C^{14}-label into alanine is not changed appreciably (Table III.2).

3. The Combined Effect of Insulin and Adrenaline on the Incorporation of Glucose-U-C^{14} into Cardiac Glycogen

The amount of glucose-U-C^{14} incorporated into cardiac glycogen, in the presence of adrenaline and insulin, is considerably lower than in their absence (Table III.4).

4. The Combined Effect of Insulin and Adrenaline on Cardiac Glycogen Retention

Glycogen is almost completely lost on perfusion in the presence of both insulin and adrenaline (Table III.6). As pointed out earlier, the level of glycogen at the onset of perfusion is much higher in hearts from starved guinea pigs than in hearts from fed animals. This result shows that a considerably larger amount of glycogen in hearts from starved animals undergoes glycolysis than in hearts from fed animals. It would be expected that products of glycolysis derived from exogenous glucose and from glycogen would be oxidized at rates proportional to their respective concentrations. Ultimately, therefore, incorporation of radioactivity into glutamate or glutamine, and production of $C^{14}O_2$ from glucose-U-C^{14} would be reduced in hearts from starved animals. These considerations explain the fact that insulin and adrenaline are less effective in reversing the inhibition by starvation of glucose utilization than the inhibition by acetate.

5. The Combined Effect of Insulin and Adrenaline on the Accumulation of Lactate Derived from Glucose-U-C^{14}

The amount of lactate-C^{14} which accumulates from glucose-U-C^{14} is considerably increased by insulin and adrenaline, but this increase is smaller than that obtained in hearts from fed animals (Table III.7). The effect of insulin and adrenaline in hearts from starved guinea pigs is probably diminished as a result of the greater amount of carbohydrate intermediates formed from glycogen by these hearts.

6. Summary

(a) Insulin and adrenaline, in combination, greatly increase the amount of glucose-U-C^{14} oxidized to $C^{14}O_2$ by hearts from starved guinea pigs.

(b) Insulin and adrenaline elicit an increase in the incorporation of glucose-U-C^{14} into glutamate and glutamine in hearts from starved guinea pigs.

(c) The net amount of glucose-U-C^{14} incorporated into glycogen of hearts from starved guinea pigs, when perfused in the presence of insulin and adrenaline, is very low. More than 90 percent of the initial cardiac glycogen is lost in 1 hr. of perfusion in the presence of insulin and adrenaline.

(d) The amount of lactate formed from glucose-U-C^{14} by hearts from starved guinea pigs is increased when insulin and adrenaline are present.

CHAPTER IV

THE METABOLISM OF LACTATE-1-C^{14} AND LACTATE-2-C^{14} BY THE PERFUSED GUINEA PIG HEART

INTRODUCTION

There have been numerous reports that the mammalian heart *in vivo* extracts lactate from the blood. At physiological concentrations, glucose and lactate are used by the human heart in approximately equal amounts (3, 34). The lactate utilization of both the diabetic human heart and diabetic dog heart is significantly reduced. The extraction of lactate and pyruvate by the isolated rat heart is reduced by fatty acids and by starvation (64, 69).

The results presented in Chapter III demonstrate that the utilization of exogenous glucose by the perfused guinea pig heart is markedly affected by starvation, fatty acids, insulin and adrenaline. The data indicate that the pronounced reduction in the utilization of glucose caused by starvation or the addition of fatty acids is due primarily to a block in the entry of pyruvate into the citric acid cycle. Insulin and adrenaline, when added in combination, are seen to reverse the inhibition of glucose oxidation by fatty acids or starvation.

The experiments described in this chapter were designed to determine whether the conditions which regulate glucose utilization also affect the metabolism of lactate.

Perfusions were carried out using sodium DL-lactate-1-C^{14} and sodium DL-lactate-2-C^{14}. The initial concentration of lactate as the racemic mixture was 12 mM in all cases.

A. THE METABOLISM OF C^{14}-LABELLED LACTATE WHEN ADDED AS THE SOLE SUBSTRATE

1. The Oxidation of Lactate-1-C^{14} and Lactate-2-C^{14} to $C^{14}O_2$.

Lactate-1-C^{14} and lactate-2-C^{14}, when present at a concentration of 6 mM (of the L- isomer), are oxidized to $C^{14}O_2$ by the perfused guinea pig heart at the rate of about 60 μ moles of lactate/gm. wet weight of tissue/hr. (Table IV.1), assuming equal rates of oxidation of all three carbon atoms. The rates of $C^{14}O_2$ formation from lactate-1-C^{14} and lactate-2-C^{14} are not significantly different.

2. The Incorporation of Radioactivity from C^{14}-Lactate into Amino Acids

When guinea pig hearts are perfused in the presence of lactate-1-C^{14}, alanine is the only amino acid into which radioactivity is incorporated. This result shows that radioactive carbon from lactate enters into the citric acid cycle primarily after being decarboxylated through condensation with oxaloacetate.

When hearts are perfused with lactate-2-C^{14}, however, radioactivity is incorporated into glutamate, glutamine, alanine and aspartate. As observed with glucose-U-C^{14} as substrate, glutamate is heavily labelled, with lesser amounts of radioactivity in glutamine and alanine (Table IV.2). The values for incorporation into aspartate are not recorded, since they are very low.

TABLE IV.1

$C^{14}O_2$ PRODUCTION FROM RADIOACTIVE LACTATE

	μ atoms of labelled carbon/gm./hr.	
A. Fed Animals	Lactate-1-C^{14}	Lactate-2-C^{14}
Additions		
1. None	58 ± 5 (4)	61 ± 3 (2)
2. Acetate	7 ± 3 (4)	8 ± 2 (2)
3. Acetate and insulin	-	12 ± 3 (3)
4. Acetate, adrenaline and insulin	31 ± 6 (2)	34 ± 4 (2)
5. Adrenaline	76 ± 8 (3)	-
6. Insulin	55 ± 3 (2)	-
7. Glucose and insulin	54 (1)	-
B. Starved Animals		
Additions		
1. None	12 ± 4 (3)	-

Conditions were as described in Methods.

Concentrations: DL-lactate, 12 mM; acetate, 5 mM; glucose, 5 mM; insulin, 10 mu/ml.; adrenaline, 0.25 μ gm./ml.

TABLE IV.2

INCORPORATION OF RADIOACTIVITY FROM LACTATE-2-C^{14} INTO AMINO ACIDS

A. Fed Animals	mμ atoms lactate-2 carbon/mg. dry weight		
Additions	Glutamate	Glutamine	Alanine
1. None	28.4 ± 5.1 (4)	7.1 ± 2.2	2.7 ± 0.4
2. Insulin	31.0 ± 4.3 (2)	6.8 ± 1.7	3.3 ± 0.6
3. Acetate	2.1 ± 0.4 (3)	0.5	3.1 ± 0.3
4. Insulin and acetate	1.9 ± 0.5 (2)	0.5	3.3 ± 0.4
5. Acetate, insulin and adrenaline	2.2 ± 0.3 (2)	0.5	2.4 ± 0.5
B. Starved Animals			
Additions			
1. None	5.4 ± 0.7 (2)	1.2 ± 0.3	4.2 ± 0.4

Conditions were as described in Methods.

Concentrations: DL-lactate, 12 mM; acetate, 5 mM; insulin, 10 mu/ml.; adrenaline, 0.25 μ gm./ml.

3. The Incorporation of Radioactivity from C^{14}-Lactate into Cardiac Glycogen

There is definite incorporation of radioactivity from C^{14}-labelled lactate into cardiac glycogen, although it is less than one-percent of that incorporated from glucose-U-C^{14} (Table IV.3). This result shows that there is no appreciable reversal of glycolysis, even in the presence of very high concentrations of lactate. Lactate does not serve effectively as a precursor of cardiac glycogen.

4. The Effect of Perfusion with Lactate on Total Cardiac Glycogen

Most of the initial cardiac glycogen is retained after perfusion for one hour in the presence of 12 mM DL-lactate (Tables III.5 and IV.4). The average value obtained for residual glycogen after perfusion with lactate is not significantly different from that obtained when glucose is present. This result, however, suggests a lower rate of glycogenolysis in the presence of lactate since lactate does not contribute appreciably to glycogen resynthesis.

5. Lipid Synthesis from Lactate-2-C^{14}

The radioactivity from lactate-2-C^{14} found in total triglyceride and long-chain free fatty acids never exceeds 150 counts/min./gm. of tissue. There is therefore no appreciable long-chain fatty acid synthesis from lactate by the perfused guinea pig heart.

6. Summary

(a) DL-lactate-1-C^{14} and DL-lactate-2-C^{14}, when present at a concentration of 12 mM, are oxidized by the perfused guinea pig heart at a rate of 60 μ moles/gm. wet weight of tissue/hr.

(b) Radioactivity from lactate-1-C^{14} is incorporated into alanine. Radioactivity from lactate-2-C^{14} is incorporated into glutamate, glutamine, alanine and aspartate.

(c) There is low but significant incorporation of radioactivity from C^{14}-lactate into cardiac glycogen.

(d) There is a slight drop in cardiac glycogen on perfusion in the presence of lactate for one hour.

(e) The perfused guinea pig heart does not synthesize appreciable amounts of long-chain fatty acids or triglyceride from exogenous lactate.

TABLE IV.3

INCORPORATION OF RADIOACTIVITY FROM LACTATE-2-C^{14} INTO CARDIAC GLYCOGEN

	Radioactivity in Glycogen counts/gm.dry weight of heart ± S.E.M.
A. Fed Animals	
Additions	
1. None	650 ± 80 (4)
2. Acetate	830 ± 110 (4)
3. Adrenaline	0 (2)
4. Adrenaline, insulin and acetate	0 (4)
5. Insulin and acetate	840 ± 120 (2)
B. Starved Animals	
Additions	
1. None	1200 ± 350 (3)

Conditions were as described in Methods.

Concentrations: DL-lactate, 12 mM; acetate, 5 mM; insulin, 10 mu/ml.; adrenaline, 0.25 μ gm./ml.; DL-lactate-2-C^{14}, 5 μ curies.

Glycogen was extracted after 60 minutes of perfusion.

TABLE IV.4

RESIDUAL CARDIAC GLYCOGEN AFTER PERFUSION IN THE PRESENCE OF LACTATE

	Cardiac Glycogen μ moles of Glucose/gm.dry wt. ± S.E.M.	
A. Fed Animals		
Additions		
1. None	44 ± 5	(4)
2. Insulin	59 ± 8	(2)
3. Insulin and glucose	104	(1)
4. Adrenaline	2 ± 1	(3)
5. Acetate	38 ± 11	(4)
6. Acetate and insulin	40 ± 12	(2)
7. Acetate, insulin and adrenaline	2 ± 1	(4)
B. Starved Animals		
Additions		
1. None	61 ± 10	(3)

Glycogen was extracted after perfusion for one hour at 37°C. with 40 ml. of perfusion fluid containing 12 mM DL-lactate and the additions indicated.

Concentrations: acetate, 5 mM; glucose, 5 mM; insulin, 10 mu/ml.; adrenaline, 0.25 μ gm./ml.

B. THE EFFECT OF INSULIN ON THE METABOLISM OF EXOGENOUS LACTATE-C^{14}

Insulin does not significantly effect the metabolism of C^{14}-lactate (Tables IV.1, IV.3 and IV.4).

C. THE EFFECT OF ADRENALINE ON THE METABOLISM OF LACTATE-1-C^{14}

1. The Effect of Adrenaline on the Oxidation of Lactate-1-C^{14} to $C^{14}O_2$.

Adrenaline, at a concentration of 0.25 μ gm./ml., increases the rate of $C^{14}O_2$ production from lactate-1-C^{14} about 25 percent (Table IV.1). This effect is in marked contrast to the very large stimulation by adrenaline of the oxidation of exogenous glucose.

2. The Effect of Adrenaline on Incorporation of Radioactivity from Lactate-1-C^{14} into Cardiac Glycogen

In the presence of adrenaline there is no detectible incorporation of radioactivity from lactate-1-C^{14} into cardiac glycogen (Table IV.3).

3. The Effect of Adrenaline on Cardiac Glycogen Retention in the Presence of Lactate

Almost all of the initial cardiac glycogen is lost on perfusion for one hour in the presence of adrenaline and lactate (Table IV.4). This very pronounced glycogenolytic effect of adrenaline accounts for the inability to detect net incorporation of radioactivity into glycogen.

4. Summary

(a) Adrenaline, when present at a concentration of 0.25 μ gm./ml., increases the rate of $C^{14}O_2$ formation from lactate-1-C^{14} about 25 percent.

(b) Cardiac glycogen is almost completely lost on perfusion for one hour in the presence of adrenaline and lactate.

(c) There is no detectible labelling of cardiac glycogen from lactate-1-C^{14} in the presence of adrenaline.

D. THE EFFECT OF STARVATION ON THE METABOLISM OF LACTATE-1-C^{14}

1. The Effect of Starvation on the Formation of $C^{14}O_2$ from Lactate-1-C^{14}

The formation of $C^{14}O_2$ from lactate-1-C^{14} is suppressed about 80 percent by starvation of the guinea pig for a period of 72 hours (Table IV.1). The effect of starvation on the decarboxylation of lactate is almost as great as its effect on the oxidation of exogenous glucose. This result is in contrast to that reported by Williamson (64), who found that starvation reduces the amount of lactate utilization by the perfused rat heart only about 20 percent.

2. The Effect of Starvation on the Incorporation of Radioactivity from Lactate-2-C^{14} into Amino Acids

Corresponding to the marked decrease in the oxidation of lactate, starvation decreases the incorporation of radioactivity from lactate-2-C^{14} into glutamate and glutamine more than 80 percent, while the incorporation into alanine remains unchanged (Table IV.2).

3. The Effect of Starvation on the Incorporation of Radioactivity into Cardiac Glycogen

The amount of radioactivity incorporated into the glycogen of hearts from starved guinea pigs is somewhat higher than that incorporated into the glycogen of hearts from fed animals (Table IV.3). This result is in contrast to that obtained for glucose incorporation into glycogen, and is an anomaly in view of the increased glycogenolysis observed during perfusion of hearts from starved animals.

4. Glycogen Retention by Hearts from Starved Guinea Pigs in the Presence of Lactate

There is somewhat more residual glycogen in hearts from starved guinea pigs than in hearts from fed ones after perfusion with lactate (Table IV.4). There is, however, a considerably greater rate of glycogenolysis during perfusion of hearts from starved animals, since the initial glycogen levels are much higher (Table III.5). This result is similar to that obtained when hearts are perfused with glucose.

5. Summary

(a) The oxidation of lactate-1-C^{14} to $C^{14}O_2$ by perfused guinea pig hearts is suppressed more than 80 percent by starving the animal for 72 hours.

(b) The incorporation of radioactivity from lactate-2-C^{14} into glutamate and glutamine in the perfused heart is suppressed more than 80 percent by starvation, while the radioactive labelling in alanine is undiminished.

(c) The incorporation of radioactivity from lactate-2-C^{14} into cardiac glycogen is slightly increased by starvation.

(d) There is a high rate of glycogenolysis in hearts from starved guinea pigs on perfusion with lactate.

E. THE EFFECT OF ACETATE ON THE METABOLISM OF LACTATE-1-C^{14} AND LACTATE-2-C^{14}

In Chapter III the effects of short-chain fatty acids on glucose metabolism by the perfused guinea pig heart were shown to be very similar to the effects of starvation. These data suggest strongly that the most pronounced effect of fatty acids or starvation on the cardiac metabolism of glucose is a decreased entry of carbohydrate into the citric acid cycle, with a large accumulation of lactate from glucose.

It was therefore of interest to determine the effects of short-chain fatty acids on the utilization of C^{14}-labelled lactate.

1. The Effect of Acetate on the Oxidation of Lactate-1-C^{14} and Lactate-2-C^{14} to $C^{14}O_2$

Acetate, when added at a concentration of 5 mM, suppresses the formation of $C^{14}O_2$ from lactate-1-C^{14} and lactate-2-C^{14} almost 90 percent (Table IV.1). The effect of acetate on lactate oxidation is of about the same magnitude as that on glucose oxidation.

2. The Effect of Acetate on the Incorporation of Radioactivity from Lactate-2-C^{14} into Amino Acids

The amount of radioactivity incorporated into glutamate and glutamine from lactate-2-C^{14} is reduced more than 80 percent by acetate, while the incorporation of radioactivity into alanine is unchanged (Table IV.2).

These effects are very similar to the effects of acetate and other short-chain fatty acids on the incorporation of radioactivity from glucose-U-C^{14} into glutamate and glutamine. There is, however, one major difference, i.e. the radioactivity from glucose-U-C^{14} incorporated into alanine is considerably increased by acetate, while the incorporation into alanine from lactate-2-C^{14} is not significantly changed by acetate. In the former case, more alanine becomes labelled probably due to an accumulation of lactate, and hence of pyruvate, in the presence of acetate. In the latter case, since lactate is already present at a relatively high concentration, inhibition of lactate oxidation would not be expected to have a significant effect on incorporation of label from C^{14}-lactate into alanine.

3. <u>The Effect of Acetate on the Incorporation of Radioactivity Derived from Lactate-2-C^{14} into Cardiac Glycogen</u>

The amount of radioactivity derived from lactate-2-C^{14} which is incorporated into cardiac glycogen is not significantly changed in the presence of acetate.

4. <u>Cardiac Glycogen Retention on Perfusion in the Presence of Lactate and Acetate</u>

Acetate has no significant effect on the amount of glycogen retained by hearts perfused for one hour in the presence of lactate (Table IV.4).

5. Summary

(a) Acetate, when added in equimolar concentration with lactate, suppresses $C^{14}O_2$ formation from lactate-1-C^{14} or lactate-2-C^{14} almost 90 percent.

(b) Acetate reduces the incorporation of radioactivity derived from lactate-2-C^{14} into glutamate and glutamine more than 80 percent. The incorporation into alanine is unchanged.

(c) Acetate has no effect on the incorporation of radioactivity from C^{14}-lactate into cardiac glycogen or on the amount of glycogen which remains after perfusion in the presence of lactate.

F. THE EFFECT OF INSULIN ON THE METABOLISM OF LACTATE-2-C^{14} IN THE PRESENCE OF ACETATE

1. The Effect of Insulin on the Oxidation of Lactate-2-C^{14} to $C^{14}O_2$ in the Presence of Acetate

Insulin has a small effect on the rate of $C^{14}O_2$ formation from lactate-2-C^{14} in the presence of acetate (Table IV.1). It appears that, although insulin partially reverses the inhibition by acetate of glucose oxidation, it is relatively ineffectual in reversing the effect of acetate on lactate oxidation.

2. The Effect of Insulin on the Incorporation of Radioactivity Derived from Lactate-2-C^{14} into Amino Acids in the Presence of Acetate

In the presence of acetate, insulin does not have a significant effect on the incorporation of radioactivity from lactate-2-C^{14} into either glutamate, glutamine or alanine (Table IV.2).

3. The Effect of Insulin on Incorporation of Radioactivity Derived from Lactate-2-C^{14} into Cardiac Glycogen in the Presence of Acetate

Insulin does not affect the amount of radioactivity incorporated into glycogen from lactate-2-C^{14}, either in the presence or absence of acetate (Table IV.3).

4. Summary

(a) Insulin slightly increases the rate of formation of $C^{14}O_2$ from lactate-2-C^{14} in the presence of acetate.

(b) Insulin has no effect on the incorporation of radioactivity from lactate-2-C^{14} into glutamate, glutamine or alanine, either in the presence or absence of acetate.

(c) Insulin does not affect the amount of radioactivity derived from lactate-2-C^{14} incorporated into glycogen in the presence of acetate.

G. THE COMBINED EFFECT OF ADRENALINE AND INSULIN ON THE METABOLISM OF LACTATE-1-C^{14} AND LACTATE-2-C^{14} IN THE PRESENCE OF ACETATE

1. The Effect of Adrenaline and Insulin on the Oxidation of Lactate-1-C^{14} and Lactate-2-C^{14} in the Presence of Acetate

In the presence of insulin and adrenaline, acetate suppresses the oxidation of exogenous lactate-1-C^{14} and lactate-2-C^{14} only about 50 percent, whereas in their absence acetate inhibits about 90 percent (Table IV.1).

It was shown in an earlier section that insulin has little or no effect on lactate oxidation, either in the presence or absence of acetate. The large increase in the oxidation of lactate in the presence of acetate must therefore be due to the effect of adrenaline.

2. The Combined Effect of Insulin and Adrenaline on the Incorporation of Radioactivity from Lactate-2-C^{14} into Amino Acids in the Presence of Acetate

In the presence of acetate, the incorporation of label from radioactive lactate into glutamate, glutamine or alanine is not significantly changed by insulin and adrenaline (Table IV.2). This result is surprising in view of the fact that much more carbon from lactate presumably enters the citric acid cycle in the presence of both adrenaline and insulin. This finding is difficult to explain, since adrenaline and insulin markedly increase the incorporation of radioactivity into glutamate and glutamine from glucose-U-C^{14} in the presence of acetate (Chapter III).

3. The Combined Effect of Insulin and Adrenaline on Glycogen Retention and Incorporation of Radioactivity from Lactate-2-C^{14} into Cardiac Glycogen

With or without acetate, there is no detectible incorporation of radioactivity from lactate-2-C^{14} into cardiac glycogen when adrenaline and insulin are both present (Table IV.3). This lack of net incorporation is probably due to the fact that most of the initial glycogen is lost when adrenaline is present (Table IV.4).

4. Summary

(a) In the presence of acetate, insulin and adrenaline added together increase the rate of $C^{14}O_2$ production from lactate-1-C^{14} or lactate-2-C^{14} five- to six-fold.

(b) In the presence of acetate, insulin and adrenaline have no significant effect on the incorporation of radioactivity from lactate-2-C^{14} into glutamate, glutamine and alanine.

(c) With or without acetate, there is very little residual cardiac glycogen after perfusion with lactate in the presence of adrenaline and insulin. There is no detectible incorporation of label from radioactive lactate into glycogen.

CHAPTER V

THE METABOLISM OF SHORT-CHAIN FATTY ACIDS BY THE PERFUSED GUINEA PIG HEART

INTRODUCTION

Fatty acids are utilized by the heart *in situ*, by the isolated heart, and by tissue slices (3). Soluble preparations from heart muscle have been found to catalyze the oxidation of fatty acids (129). Heart muscle mitochondria can convert acetate to its active form, providing oxidation of any member of the citric acid cycle is taking place concurrently (130). Work on the heart-lung preparation also has demonstrated the utilization of non-carbohydrate material by the heart. For example, it was found that when animals are fed a diet consisting mainly of butter the respiratory quotient of the isolated heart is low (131), suggesting that as much as 80 percent of the total metabolism may be derived from non-carbohydrate (132).

In preceding chapters it has been shown that short-chain fatty acids have a very marked effect on the utilization of exogenous glucose and lactate by the perfused guinea pig heart. The metabolism of C^{14}-labelled acetate, propionate and butyrate is described in this chapter. The effects of adrenaline, starvation and glucose on fatty acid metabolism are also described.

A. THE METABOLISM OF SHORT-CHAIN FATTY ACIDS WHEN ADDED AS SOLE SUBSTRATE

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

(a) The Oxidation of Acetate-1-C^{14} to $C^{14}O_2$

Acetate-1-C^{14}, when added at a concentration of 5 mM, is oxidized to $C^{14}O_2$ at an average rate of 75 μ moles/gm. wet weight of heart/hour, assuming equal rates of oxidation of both carbon atoms (Table V.1). This rate of oxidation of acetate is about three times as great as that of glucose oxidation.

(b) The Incorporation of Radioactivity Derived from Acetate-1-C^{14} into Amino Acids

Radioactivity from acetate-1-C^{14} is incorporated into glutamate, glutamine and, to a much smaller extent, into aspartate. There is no detectible incorporation into alanine (Table V.2). The amount of radioactivity incorporated into glutamate and glutamine is about three times as great as that from C^{14}-labelled glucose. Incorporation of radioactivity into amino acids appears to be proportional to the amount of C^{14}-labelled substrate which is oxidized in the citric acid cycle.

The absence of incorporation of radioactivity into alanine suggests that there is no appreciable formation of pyruvate from acetate, by reversal of the pyruvic dehydrogenase reaction, by the oxaloacetic carboxylase reaction or via malate and the NADP-dependent malic dehydrogenase reaction.

TABLE V.1

OXIDATION OF ACETATE-1-C^{14} TO $C^{14}O_2$ BY PERFUSED GUINEA PIG HEARTS

	μ moles acetate/gm. wet weight 1 hr. ± S.E.M.	
A. Hearts from Fed Guinea Pigs		
Additions		
1. None	75 ± 7	(7)
2. Adrenaline	118 ± 16	(4)
3. Glucose	72 ± 5	(2)
4. Glucose and adrenaline	110 ± 8	(2)
5. Propionate	70 ± 3	(2)
B. Hearts from Starved Guinea Pigs		
Additions		
1. None	69 ± 6	(3)

Hearts were perfused for one hour at 37°C. with 40 ml. of perfusing medium.

Concentrations: acetate, 5 mM; glucose, 10 mM; propionate, 5 mM; adrenaline, 0.25 μ gm./ml. Oxidation rates are taken from average rates attained over the second 30 minutes of perfusion, assuming equal rates of oxidation of both acetate carbons.

TABLE V.2

INCORPORATION OF RADIOACTIVITY FROM ACETATE-1-C^{14} INTO AMINO ACIDS

Additions	mμ atoms acetate-1 carbon/mg. dry wt./hr. ± S.E.M.		
	Glutamate	Glutamine	Aspartate
1. None	17.1 ± 3.2 (8)	4.0 ± 1.4	0.4 ± 0.2
2. Glucose	17.6 ± 4.6 (2)	5.6 ± 2.0	0.3 ± 0.1
3. Adrenaline	6.5 ± 2.1 (4)	1.9 ± 0.7	0.2
4. Propionate	8.8 ± 1.6 (2)	2.8 ± 0.6	1.0 ± 0.3

Conditions were as described in Methods.

Concentrations: acetate, 5 mM; glucose, 10 mM; propionate, 5 mM; adrenaline, 0.25 μ gm./ml.

(c) The Incorporation of Radioactivity from Acetate-1-C^{14} into Cardiac Glycogen

Under no conditions is radioactivity from acetate-1-C^{14} incorporated into cardiac glycogen.

(d) The Effect of Perfusion in the Presence of Acetate on Cardiac Glycogen

When acetate is present as sole substrate, about half of the initial cardiac glycogen is lost during perfusion for one hour (Table V.3). The amount of glycogenolysis is quite variable, varying between about 30 and 70 percent of the total glycogen.

(e) The Formation of Radioactive Lactate from Acetate-1-C^{14}

No lactate-C^{14} is formed by the perfused heart from acetate-1-C^{14}.

(f) The Total Accumulation of Lactate in the Presence of Acetate

Lactate accumulates to an average concentration of about 1 mM when guinea pig hearts are perfused for one hour in the presence of acetate (Table V.4). The amount of lactate formed is variable, but it is proportional to the amount of glycogen lost during perfusion, indicating that glycogen is the major source of lactate in the absence of glucose.

(g) The Synthesis of Long-Chain Fatty Acids and Triglycerides from Acetate-1-C^{14}

The total amount of radioactivity derived from acetate-1-C^{14} incorporated into triglycerides and long-chain fatty acids is extremely small. When hearts are perfused with 200 μ moles of acetate

TABLE V.3

RESIDUAL CARDIAC GLYCOGEN AFTER PERFUSION IN THE PRESENCE OF SHORT-CHAIN FATTY ACIDS

	Glycogen as μ moles glucose/ gm.dry wt. ± S.E.M.	
A. Substrate:acetate		
Additions		
1. None	26 ± 14	(6)
2. Adrenaline	3 ± 2	(3)
3. Propionate	22 ± 9	(3)
4. Glucose	84 ± 6	(2)
B. Substrate: propionate		
Additions		
1. None	30 ± 10	(3)

Glycogen was extracted after one hour of perfusion.

Concentrations: acetate, 5 mM; propionate, 5 mM; glucose, 10 mM; adrenaline, 0.25 μ gm./ml.

with a specific activity of 12,500 counts/minute/μ mole, an average of 430 counts/gm. of heart is found in the lipid fraction.

(h) Summary

(1) When present at an initial concentration of 5 mM, acetate-1-C^{14} is oxidized to $C^{14}O_2$ by perfused guinea pig hearts at a rate of 75 μ moles/gm. wet weight/hr.

(2) Radioactivity from acetate-1-C^{14} is incorporated into glutamate, glutamine and aspartate. No detectible radioactivity is found in alanine.

(3) Radioactivity derived from acetate-1-C^{14} is not incorporated into cardiac glycogen.

(4) About 50 percent of the initial cardiac glycogen is lost on perfusion for one hour in the presence of 5 mM acetate.

(5) Lactate is not formed from exogenous acetate-1-C^{14}.

(6) When acetate alone is present in the perfusing medium, a considerable amount of lactate accumulates. The lactate is derived primarily from cardiac glycogen.

(7) There is little, but significant synthesis of long-chain fatty acids from acetate-1-C^{14} by the perfused guinea pig heart.

TABLE V.4

TOTAL LACTATE FORMATION BY GUINEA PIG HEARTS PERFUSED IN THE PRESENCE OF SHORT-CHAIN FATTY ACIDS

	μ moles lactate produced ± S.E.M.
A. Substrate: acetate	
Additions	
1. None	51 ± 11 (7)
2. Adrenaline	107 ± 16 (3)
3. Propionate	49 ± 8 (3)
4. Glucose	126 ± 19 (2)
B. Substrate: propionate	
Additions	
1. None	19 ± 6 (3)

Perfusions were carried out for one hour at 37°C.

Concentrations: acetate, 5 mM; propionate, 5 mM; glucose, 10 mM; adrenaline, 0.25 μ gm/ml.

2. The Metabolism of Butyrate-1-C^{14}

(a) The Oxidation of Butyrate-1-C^{14} to $C^{14}O_2$

When present at an initial concentration of 2.5 mM, butyrate-1-C^{14} is oxidized to $C^{14}O_2$ at a rate of 35 μ moles/gm. wet weight/hr. (Table V.5). Considering the number of carbon atoms oxidized, butyrate is oxidized to CO_2 at about the same rate as is acetate.

(b) Incorporation of Radioactivity Derived from Butyrate-1-C^{14} into Amino Acids

Radioactivity from butyrate-1-C^{14} is incorporated into glutamate, glutamine and aspartate, but not into alanine (Table V.6). The amount of label from butyrate-1-C^{14} incorporated into amino acids is about half that from acetate-1-C^{14}. This result indicates that equal quantities of carbon are incorporated into amino acids from butyrate and acetate.

(c) The Incorporation of Radioactivity Derived from Butyrate-1-C^{14} into Cardiac Glycogen

Radioactivity from butyrate-1-C^{14} is not incorporated into glycogen of perfused guinea pig hearts.

(d) Summary

(1) Butyrate-1-C^{14}, when present at an initial concentration of 2.5 mM, is oxidized to $C^{14}O_2$ by the perfused guinea pig heart at a rate of 35 μ moles/gm. wet wt./hr.

(2) Radioactivity from butyrate-1-C^{14} is incorporated into glutamate, glutamine and aspartate.

(3) Radioactivity from butyrate-1-C^{14} is not incorporated into cardiac glycogen.

TABLE V.5

OXIDATION OF BUTYRATE-1-C^{14} TO $C^{14}O_2$ BY PERFUSED GUINEA PIG HEARTS

Substrate	μ moles/gm. wet weight/hr. ± S.E.M.
Butyrate-1-C^{14} 2.5 mM	35 ± 4 (3)

Conditions were as described in Methods.

Oxidation rates were taken from the averages of the second 30 minutes of perfusion, assuming that all butyrate carbons are oxidized at equal rates.

TABLE V.6

THE INCORPORATION OF RADIOACTIVITY FROM BUTYRATE-1-C^{14} INTO AMINO ACIDS

	mμ atoms of butyrate-1 carbon/mg. dry wt./hr. ± S.E.M.	
Glutamate	Glutamine	Aspartate
7.6 ± 1.0 (3)	1.5 ± 0.5 (3)	0.3 ± 0.2 (3)

Conditions were as described in Methods.

Concentration of butyrate: 2.5 mM.

3. The Metabolism of Propionate

Propionate is known to be oxidized in a number of animal tissues by a quite different mechanism than are fatty acids with an even number of carbon atoms (124,125,126,127). This sequence is as follows:

$$\text{propionate} + \text{ATP} + \text{CoA} \longrightarrow \text{Propionyl-CoA}$$
$$\text{propionyl CoA} + \text{ATP} + CO_2 \longrightarrow \text{methylmalonyl-CoA}$$
$$\text{methylmalonyl CoA} \longrightarrow \text{succinyl-CoA}$$

This pathway for propionate metabolism has been shown to exist in pig heart extracts (124,125).

The results described in Chapter III have shown that propionate does not affect glucose utilization by the perfused guinea pig heart to a very large extent, while all other fatty acids studied markedly affect glucose metabolism. Experiments reported in this section were carried out to determine how well propionate is utilized by the intact heart, and whether propionate is metabolized by the intact heart according to the pathway described above.

(a) Oxidation of Propionate-1-C^{14} to $C^{14}O_2$

When present at an initial concentration of 5 mM, propionate-1-C^{14} is oxidized to $C^{14}O_2$ by the perfused guinea pig heart at a rate of about 35 μ moles/gm. wet weight/hr. (Table V.7). The lag time for attaining an optimal rate of oxidation is relatively short, compared to that for glucose and for other fatty acids. It can be seen from Figure V.8 that during the first six minutes of perfusion a considerable amount of $C^{14}O_2$ is produced from propionate-1-C^{14}.

TABLE V.7

OXIDATION OF PROPIONATE-1-C^{14} TO $C^{14}O_2$ BY PERFUSED GUINEA PIG HEARTS

Additions	μ moles/gm. wet wt./hr. ± S.E.M.
None	34 ± 3 (4)
Acetate	33 ± 5 (2)

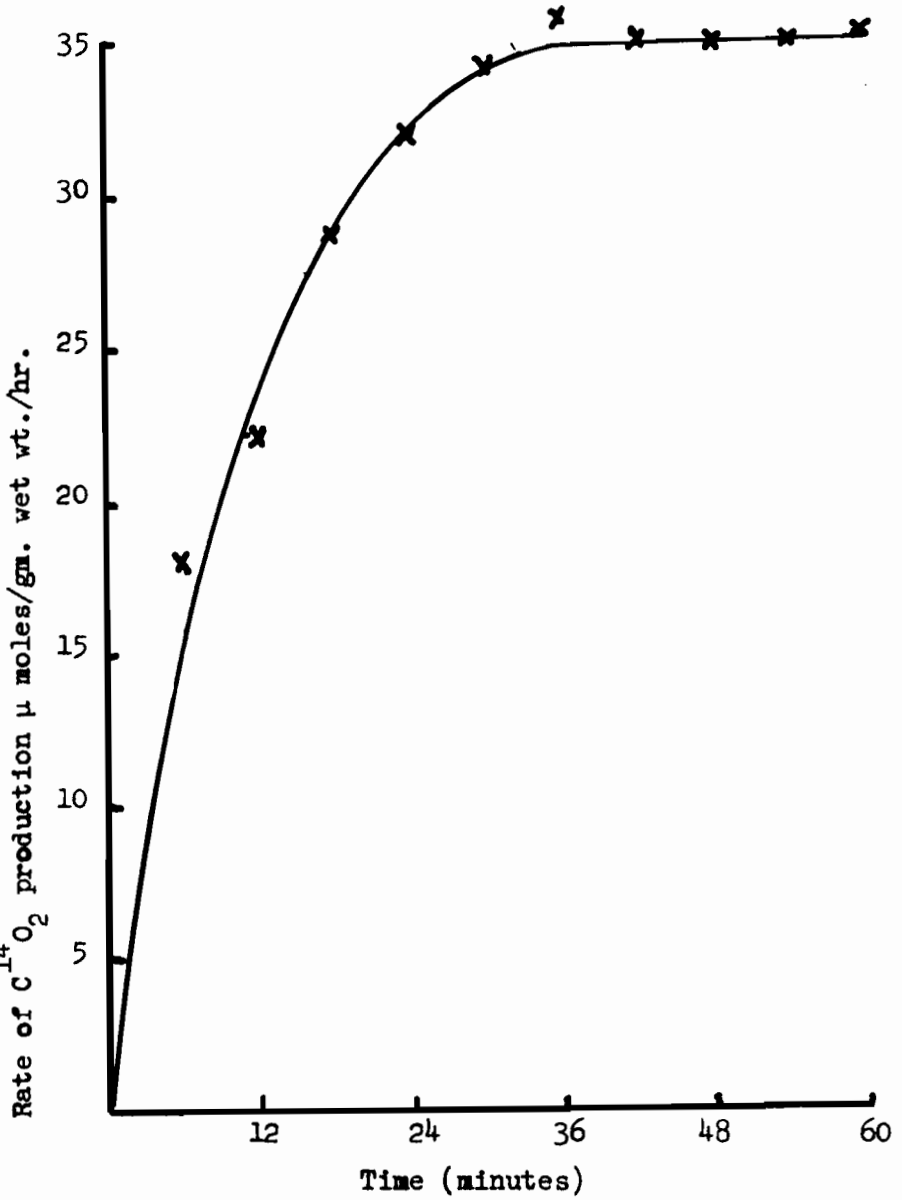
Conditions were as described in Methods.

Concentrations: propionate, 5 mM; acetate, 5 mM.

Oxidation rates are taken from average rates attained during the second 30 minutes of perfusion, assuming equal rates of utilization of all propionate carbons.

FIGURE V.8

$C^{14}O_2$ PRODUCTION FROM PROPIONATE-1-C^{14} BY THE PERFUSED GUINEA PIG HEART



Conditions were as described in Methods.

Concentration of propionate: 5 mM.
Each point is the average of two determinations.

This result suggests that propionate metabolism by the perfused heart is almost instantaneous, and that there are no effective barriers to the uptake or metabolism of propionate. The rate obtained for propionate oxidation shows that propionate is utilized by cardiac muscle to about the same extent as is acetate or butyrate.

(b) The Incorporation of Radioactivity Derived from Propionate-1-C^{14} into Amino Acids

Radioactivity from propionate-1-C^{14} is incorporated into glutamate, glutamine and aspartate (Table V.9). The relative amounts of radioactivity incorporated into glutamate and aspartate are quite different from those obtained from acetate-1-C^{14}, butyrate-1-C^{14} or from glucose-U-C^{14}; i.e. aspartate is more highly labelled, and glutamate is less highly labelled from propionate-1-C^{14}. If propionate enters into the citric acid cycle via CO_2-fixation and the subsequent formation of succinyl-CoA as described by Flavin et al. (125), propionate oxidation gives rise to an increased concentration of oxaloacetate and presumably to an increase in the amount of aspartate which is formed by transamination with oxaloacetate. Since succinate is a symmetrical molecule, radioactivity from propionate-1-C^{14} is incorporated into both carboxyl-carbons of succinate, and half of the radioactivity incorporated into succinate is lost before formation of α-ketoglutarate. These results therefore show that propionate is readily utilized by the perfused guinea pig heart, and strongly suggest that propionate is oxidized according to the pathway described above.

TABLE V.9

INCORPORATION OF RADIOACTIVITY FROM PROPIONATE-1-C^{14} INTO AMINO ACIDS

	mμ atoms propionate-1 carbon/mg. dry wt. ± S.E.M.	
Glutamate	Glutamine	Aspartate
2.3 ± 0.5 (4)	0.5 ± 0.2 (4)	4.4 ± 0.7 (4)

Conditions were as described in Methods.

Concentration of propionate was 5 mM.

(c) Incorporation of Radioactivity from Propionate-1-C^{14} into Cardiac Glycogen

There is no significant incorporation of radioactivity from propionate-1-C^{14} into glycogen of the perfused guinea pig heart.

(d) Accumulation of Lactate on Perfusion with Propionate

A small amount of lactate is found in the perfusing medium after perfusing for one hour in the presence of propionate (Table V.4). Less lactate accumulates in the presence of propionate than in the presence of acetate, indicating that a higher percentage of carbohydrate derived from cardiac glycogen is oxidized to CO_2 in the presence of propionate than in the presence of acetate.

(e) The Incorporation of Radioactivity from Propionate-1-C^{14} into Long-Chain Fatty Acids and Triglycerides

When guinea pig hearts are perfused for one hour in the presence of 200 μ moles of propionate-1-C^{14} with a specific activity of 12,500 counts/μ mole, an average of 270 counts/gm. of heart was found in the fraction containing long-chain fatty acids and triglycerides. This value is not significantly different from the value obtained for lipid synthesis from acetate-1-C^{14}, and further indicates that there is very little lipid synthesis by the perfused heart.

(f) Summary

(1) Propionate-1-C^{14}, when present at an initial concentration of 5 mM, is oxidized to $C^{14}O_2$ by the perfused guinea pig heart at a rate of about 35 μ moles/gm. wet weight/hr.

(2) Radioactivity derived from propionate-1-C^{14} is incorporated into aspartate, glutamate and glutamine with the highest activity found in aspartate.

(3) Propionate-1-C^{14} is not incorporated into glycogen of the perfused guinea pig heart.

(4) Little lactate is formed when propionate alone is present in the perfusing medium.

(5) There is little but significant synthesis of long-chain fatty acids from propionate-1-C^{14} by the perfused guinea pig heart.

B. THE EFFECT OF ADRENALINE ON THE METABOLISM OF ACETATE-1-C^{14}

1. The Effect of Adrenaline on the Oxidation of Acetate-1-C^{14} to $C^{14}O_2$

Adrenaline, at an initial concentration of 0.25 μ gm./ml., increases the rate of oxidation of acetate-1-C^{14} to $C^{14}O_2$ about 60 percent (Table V.1).

2. The Effect of Adrenaline on Incorporation of Radioactivity from Acetate-1-C^{14} into Amino Acids

Adrenaline decreases the amount of radioactivity from acetate-1-C^{14} incorporated into glutamate, glutamine and aspartate. The amount of radioactivity incorporated into aspartate in the absence of adrenaline is very low, but there is no detectible label in this amino acid when adrenaline is present (Table V.2). This effect may be due to isotopic dilution as a result of stimulated oxidation of endogenous substrates, or may result from a decreased concentration of citric acid cycle intermediates when adrenaline is present.

3. The Effect of Adrenaline on Cardiac Glycogen Retention during Perfusion in the Presence of Acetate

After one hour of perfusion in the presence of acetate and adrenaline almost no glycogen remains (Table V.3).

4. The Effect of Adrenaline on Total Lactate Accumulation in the Presence of Acetate

Adrenaline increases the amount of lactate which accumulates when hearts are perfused in the presence of acetate. The lactate which accumulates is accounted for largely by the increase in glycogen loss due to adrenaline (Table V.4).

5. Summary

(a) Adrenaline, when present at an initial concentration of 0.25 μ gm./ml., increases the rate of oxidation of acetate-1-C^{14} to $C^{14}O_2$ by the perfused guinea pig heart about 60 percent.

(b) Adrenaline decreases the incorporation of radioactivity from acetate-1-C^{14} into glutamate, glutamine and aspartate.

(c) Adrenaline effects an almost complete loss of cardiac glycogen when guinea pig hearts are perfused in the presence of acetate.

(d) Adrenaline increases the amount of lactate which accumulates in the presence of acetate.

C. THE EFFECT OF CONCENTRATION ON THE OXIDATION OF ACETATE-1-C^{14} TO $C^{14}O_2$

Hearts were perfused with 40 ml. of perfusing medium containing 1, 5, and 20 mM acetate to which was added acetate-1-C^{14} and adrenaline. As shown in Figure V.10, the utilization of acetate by the perfused guinea pig heart is essentially independent of acetate concentration, even at concentrations as low as 1 mM. When acetate is present at an initial concentration of 1 mM, the rate of $C^{14}O_2$ production in the first few minutes of perfusion is almost the same as that obtained when present at higher concentrations. The rate of oxidation of acetate starts to decrease only after a large percentage of the total acetate has been oxidized. More than 90 percent of the total acetate (when added at an initial concentration of 1 mM) is recovered as $C^{14}O_2$ during one hour of perfusion.

D. THE EFFECT OF GLUCOSE ON THE METABOLISM OF ACETATE-1-C^{14}

In Chapter III acetate, as well as other short-chain fatty acids, was shown to have a very marked effect on glucose utilization. The results indicate that glucose is unable to compete effectively with fatty acids for oxidation by the perfused guinea pig heart.

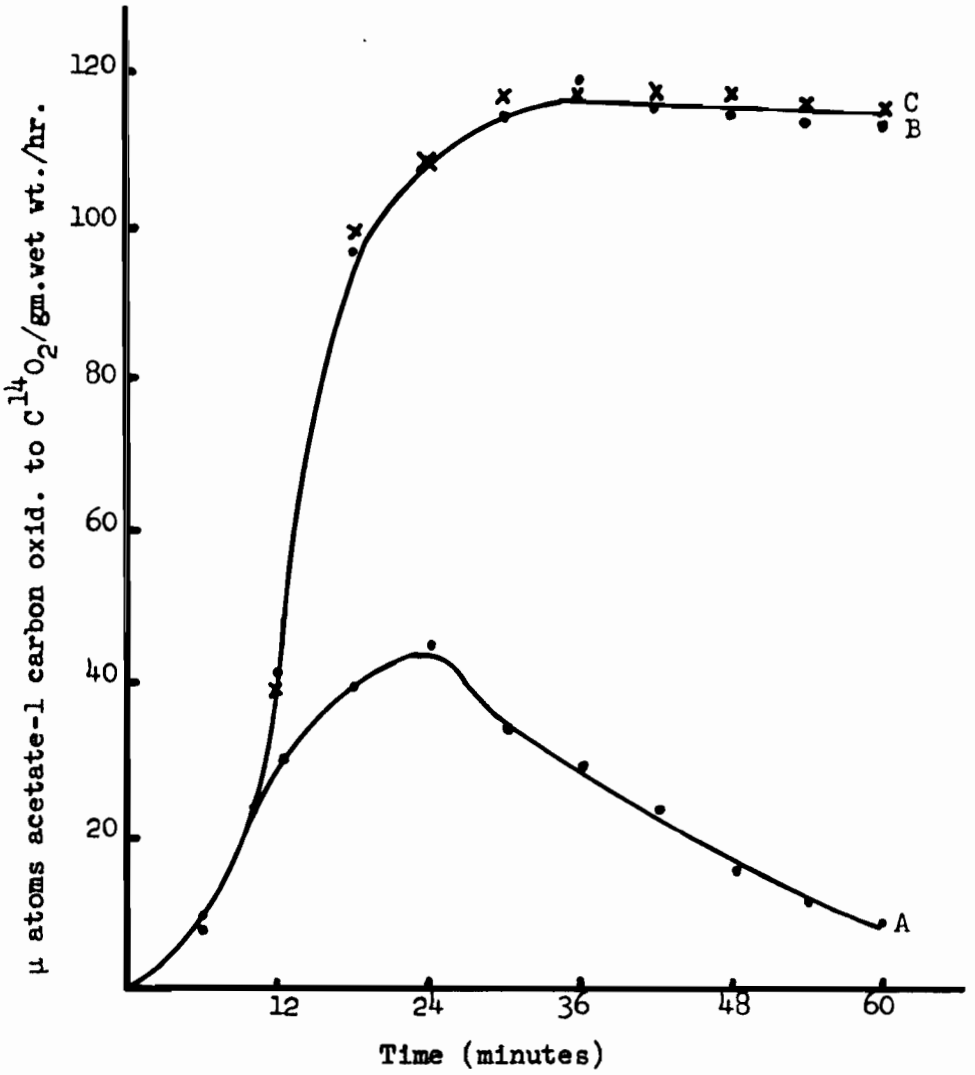
The effect of high concentrations of glucose on acetate metabolism is described in this section.

1. The Effect of Glucose on the Oxidation of Acetate-1-C^{14} to $C^{14}O_2$

The oxidation of acetate-1-C^{14} to $C^{14}O_2$ is not significantly affected by glucose, even when glucose is present at twice the concentration of acetate (Table V.1).

FIGURE V.10

THE EFFECT OF CONCENTRATION ON THE RATE OF OXIDATION OF ACETATE-1-C^{14} TO $C^{14}O_2$ BY THE PERFUSED GUINEA PIG HEART



Perfusions were carried out at 37°C. with 40 ml. of perfusion medium. Each point is the average of two or more experiments. Adrenaline was present at an initial concentration of 0.25 μ gm./ml. Acetate concentrations:

A - 1 mM
B - 5 mM
C - 20 mM

2. The Effect of Glucose on the Incorporation of Radioactivity from Acetate-1-C^{14} into Amino Acids

Glucose, when present at a concentration of 10 mM, does not significantly affect the amount of radioactivity from acetate-1-C^{14} which is incorporated into amino acids. This result suggests that little glucose enters the citric acid cycle in the presence of acetate, even when glucose is present at twice normal blood concentration.

3. The Effect of Glucose on Cardiac Glycogen Retention on Perfusion in the Presence of Acetate

Glucose, when present at a concentration of 10 mM, confers net glycogen synthesis by the guinea pig heart perfused in the presence of acetate (Table V.3). This result is quite similar to that of Williamson and Krebs (80), who found that the glycogen content was increased when rat hearts were perfused in the presence of glucose and acetoacetate. Shipp et al. (72) found that when rat hearts were perfused in the presence of glucose and palmitate there was more residual glycogen than when they were perfused with glucose alone. It appears, therefore, that the oxidation of fatty acids and ketone bodies not only decreases the oxidation of glucose, but also causes a much higher rate of glycogen synthesis from exogenous glucose. It is unlikely that the myoglycostatic effect of fatty substances is related to the suppression by these substances of pyruvate oxidation since there is very little incorporation of exogenous lactate into cardiac glycogen. Garland et al. (69) have ascribed the glycogen

conserving effect of fatty substances to an inhibition of the phosphofructokinase reaction as a result of their oxidation.

4. The Effect of Glucose on Total Lactate Accumulation by Hearts Perfused in the Presence of Acetate

When glucose is present at a concentration of 10 mM, there is a relatively high accumulation of lactate in the perfusate when hearts are perfused in the presence of acetate (Table V.4). The main portion of the lactate which accumulates under these conditions must come from exogenous glucose, since there is net glycogen synthesis in the presence of 10 mM glucose.

5. Summary

(a) Glucose does not have a significant effect on the oxidation of acetate-1-C^{14} to $C^{14}O_2$, even when it is present at twice the concentration of acetate.

(b) Glucose, at a concentration of 10 mM, does not affect the incorporation of radioactivity derived from acetate-1-C^{14} into amino acids.

(c) When guinea pig hearts are perfused with acetate and high concentrations of glucose, there is net glycogen synthesis.

(d) High concentrations of glucose elicit an accumulation of lactate when acetate is present in the perfusing medium.

E. THE EFFECT OF PROPIONATE ON THE METABOLISM OF ACETATE-1-C^{14}

1. The Effect of Propionate on the Oxidation of Acetate-1-C^{14} to $C^{14}O_2$

Propionate, when present at equimolar concentrations with acetate, does not significantly affect the rate of $C^{14}O_2$ production from acetate-1-C^{14} (Table V.1).

2. The Effect of Propionate on Incorporation of Radioactivity from Acetate-1-C^{14} into Amino Acids

Propionate, when present at equimolar concentrations with acetate, reduces the incorporation of radioactivity from acetate-1-C^{14} into glutamate, glutamine and aspartate (Table V.2). In view of the fact that propionate does not significantly affect acetate oxidation, the decrease in the incorporation of label into amino acids is probably brought about by isotopic dilution of intermediates in the citric acid cycle.

3. The Effect of Propionate on Cardiac Glycogen Retention during Perfusion with Acetate

The amount of cardiac glycogen which remains after perfusion for one hour in the presence of acetate is not significantly affected by propionate (Table V.3).

4. The Effect of Propionate on Lactate Accumulation in the Presence of Acetate

The total lactate which accumulates in the perfusing medium in the presence of acetate is not affected by propionate (Table V.4).

In the presence or absence of propionate, the lactate which accumulates can be accounted for by the glycogen lost during perfusion. It is unlikely, in view of the effects of acetate on glucose oxidation (see Chapter III), that an appreciable percentage of the glycogen which is removed is oxidized to CO_2. Rather, it is likely that most of the degradative products of glycogen accumulate as lactate.

5. Summary

(a) Propionate does not significantly affect the rate of oxidation of acetate-1-C^{14} to $C^{14}O_2$.

(b) Propionate decreases the amount of radioactivity incorporated into glutamate, glutamine and aspartate from acetate-1-C^{14}.

(c) Propionate has no effect on the amount of cardiac glycogen which remains after perfusion in the presence of acetate.

(d) Propionate does not affect lactate accumulation in the presence of acetate.

F. THE EFFECT OF STARVATION ON THE OXIDATION OF ACETATE-1-C^{14} TO $C^{14}O_2$

Acetate-1-C^{14} is oxidized to $C^{14}O_2$ at about the same rate by hearts from guinea pigs which are starved 72 hours as by hearts from fed animals (Table V.1).

In Chapter III, starvation was shown to have a very marked effect on cardiac metabolism of carbohydrates. The present data suggest that cardiac fatty acid metabolism is unaffected by starvation. These results do not, however, preclude the possibility that the total utilization of fatty acids is increased by starvation. Indeed, it seems likely that lipid contributes more to the total respiration of hearts in starved animals than in fed ones. If this is true, however, there must be an increase in the metabolism of endogenous lipid by hearts from starved animals.

G. THE EFFECT OF ACETATE ON THE OXIDATION OF PROPIONATE-1-C^{14} TO $C^{14}O_2$

Acetate, when present at equimolar concentrations with propionate, does not significantly affect the rate of oxidation of propionate-1-C^{14} to $C^{14}O_2$ by the perfused guinea pig heart (Table V.7). The fact that neither acetate nor propionate affects the rate of oxidation of the other suggests that CoA is not limiting for fatty acid metabolism by the intact heart. This result further supports the previous conclusions that acetate and other fatty acids which give

rise to acetyl-CoA inhibit the oxidation of glucose either by providing unfavorable competition for entry into the citric acid cycle, or by a direct inhibition of the decarboxylation of pyruvate. If the former is true, there is very rapid activation of acetate to acetyl-CoA, providing a large pool of active acetate for entry into the oxidative cycle.

GENERAL DISCUSSION

The purpose of this investigation has been to study the fate of substrates utilized by the intact mammalian heart *in vitro*, and to determine the inter-relationships between the metabolism of carbohydrates and fatty acids, as well as the effects of insulin and adrenaline on the utilization of these substrates.

In the present investigation, glucose is shown to be well utilized by the isolated guinea pig heart. Radioactivity from exogenous glucose-U-C^{14} is incorporated into free glutamate, alanine, glutamine and aspartate. There is significant incorporation of radioactivity into proteins, and a large amount of incorporation of labelled glucose into cardiac glycogen. The $C^{14}O_2$ produced from exogenous glucose-U-C^{14}, when present as the sole substrate, accounts for only about 15 percent of the total oxidative metabolism of the perfused guinea pig heart. These results show that the aerobic metabolism of glucose, when present alone, can account for only a small percentage of the total oxygen consumption of the perfused heart. The present results further indicate that entry of carbohydrate into the citric acid cycle is not limiting to oxidation. Since very little lactate accumulates from exogenous glucose, and since there is a small net loss of glycogen, it is probable that, in the absence of insulin, glucose oxidation is limited by glucose availability

and that nearly all of the glucose removed from the medium is oxidized to CO_2. The fact that a large amount of exogenous glucose becomes incorporated into cardiac glycogen, even though net loss of glycogen occurs during perfusion, is an indication of rapid turnover of cardiac glycogen. The only amino acids synthesized from glucose are alanine, glutamate, glutamine and aspartate. It is unlikely, therefore, that transaminases other than glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase have a very important role in the intact heart. Cammarata et al. (96) demonstrated the presence of more than twenty transaminases in heart muscle extracts. The inability to show a larger number of these activities in the intact heart may be due to the absence of a very large pool of the corresponding amino acids in the cell.

Diabetes, starvation and the administration of fatty acids or ketone bodies all decrease the capacity of the heart to metabolize carbohydrate (3,33,53,64,66,68,69). It is likely that the lesion affecting carbohydrate metabolism is common to all of these conditions. The reduction in cardiac carbohydrate utilization in diabetic or starved animals may be due to an elevated level of fatty acids and ketone bodies in the blood. The data presented in the present investigation show that the oxidation of exogenous glucose or lactate to CO_2 by the perfused guinea pig heart is suppressed almost completely by short-chain fatty acids or by prolonged starvation. Under these conditions, there is a large accumulation of

lactate from exogenous glucose, indicating that neither the transport of glucose nor glycolysis is affected to an extent that would make these steps limiting for glucose utilization. The fact that the incorporation of glucose carbon into glutamate and glutamine is reduced to about the same extent as is the oxidation of glucose to CO_2, while the incorporation into alanine is increased, supports the thesis that the oxidation of glucose is inhibited primarily because of a reduction in the amount of carbohydrate which enters the citric acid cycle. Since fatty acids or starvation almost completely block CO_2 formation from glucose, while effecting a large accumulation of lactate from exogenous glucose, it is unlikely that the pentose shunt is very active. It therefore seems likely that glucose is oxidized in the heart primarily in the citric acid cycle, and that fatty substances reduce the rate of entry of carbohydrate into the citric acid cycle.

The fact that propionate has only a small effect on glucose utilization makes it unlikely that fatty acids and ketone bodies suppress carbohydrate oxidation by competition for CoA, especially in view of the fact that propionate and acetate have little effect on the oxidation of each other. Propionate, which must be activated to propionyl-CoA (124,125), is oxidized at about the same rate as acetate and butyrate. It is probable that all fatty substances which give rise to acetyl-CoA are readily activated by heart muscle, forming a very large pool of active acetate and in turn an unfavorable competition for the entry of carbohydrate into the citric acid cycle.

In some tissues, short-chain fatty acids have little effect on the oxidation of glucose. Straight-chain fatty acids of chain length from 2 to 8 carbon atoms have little effect on glucose oxidation by ascites tumors (133). The oxidation of glucose in brain in vitro is little affected by fatty acids (134). The large effect of fatty acids on glucose oxidation in the heart may occur as a result of the high activities of the fatty acid oxidases and of the acetate-activating enzyme. Another consideration is the fact that the pentose shunt is operative in tumors (135,136), while the present data suggest that this is not a major pathway for the oxidation of glucose in heart muscle.

The results presented in this thesis show that short-chain fatty acids increase the amount of glucose incorporated into glycogen of guinea pig hearts, and an increase in the amount of glycogen which remains after perfusion. Similar effects of ketone bodies (65) and of long-chain fatty acids (76) have been demonstrated in the perfused rat heart. It appears unlikely that this effect is brought about because of the decreased entry of carbohydrate into the citric acid cycle in the presence of fatty acids, since the present results have shown that there is no effect by fatty acids on the amount of incorporation of lactate carbon into glycogen, indicating little reversal of glycolysis. In the heart, starvation and diabetes cause a marked increase in glycogen, while they cause glycogen depletion in other tissues (72,123). Newsholme et al. (76) have proposed a mechanism for the myoglycostatic effect

of fatty acids and ketone bodies. They showed that in the rat heart the oxidation of ketone bodies and fatty acids is associated with an inhibition of the phosphofructokinase reaction. Inhibition of the phosphofructokinase reaction was considered to contribute to the increase in glycogen content in two ways. First, accumulating glycolytic intermediates, which would otherwise be converted to lactic acid, may be diverted to glycogen synthesis; and second, the higher concentration of G6P may activate UDPG-glycogen transglycosidase. Bowman (75) also found that, in the perfused rat heart, long-chain fatty acids increase the intracellular concentration of glucose and decrease glucose uptake. In both cases cited, however, studies were carried out in the presence of added insulin. In the absence of insulin, however, inhibition of phosphofructokinase is of doubtful importance, since phosphorylation is not rate-limiting in the absence of insulin (61). Recent work by Evans (137) has shown that in rat hearts perfused with glucose and palmitate, as much as 40 percent of the total glucose uptake is diverted to glycogen when the perfusion is carried out at around 30°C. This result lends further support to the importance of a block by fatty substances to glycolysis, and an explanation for the increase in cardiac glycogen effected by conditions <u>in vivo</u> which give rise to increased blood levels of fatty acids and ketone bodies.

Insulin was found, in the present work, to have the following effects on glucose metabolism by the perfused guinea pig heart:

(a) an increased transport of glucose, resulting in an accumulation of lactate; (b) increased glycogen synthesis from glucose; (c) a small increase in the amount of glucose oxidized to CO_2; and (d) increased incorporation of carbohydrate into amino acids and proteins. With the exception of (d), these effects can be explained by the action of insulin on the cell membrane, resulting in an increased uptake of glucose. The effect of insulin on glycogen synthesis is probably due to an increased concentration of of hexose phosphates inside the cell. Morgan et al. (61) have shown that in the absence of insulin, glucose transport is rate limiting for glucose utilization, but that in the presence of insulin, phosphorylation becomes limiting. It is reasonable to expect, therefore, that insulin elicits an increase in glycogen synthesis due to an accumulation of hexose phosphates inside the cell. There is some difference of opinion of whether or not glucose oxidation is increased by insulin. In rat diaphragm, Villie and Hastings (138) obtained a large increase in $C^{14}O_2$ production from glucose-U-C^{14} by insulin. Chain (139) found that glucose oxidation is not changed by insulin. Williamson and Krebs (80) reported that insulin increases glucose oxidation by the perfused rat heart more than 200 percent. The results of the present experiments with perfused guinea pig hearts show a small increase in glucose oxidation by insulin. The results can be explained by the stimulatory action of insulin on transport. In order for insulin to increase the rate of

oxidation to CO_2, entry of carbohydrate into the citric acid cycle must be accelerated. If the rate of transport is slower than that of entry into the citric acid cycle, an increase in the rate of transport would result in an increase in the amount of glucose oxidized. On the other hand, if the relative rates are reversed, an increase in transport would have no effect on the oxidation rate.

The increase by insulin on incorporation of glucose carbon into amino acids and proteins is difficult to explain on the basis of a greater availability of glucose inside the cell, particularly in view of the fact that insulin has only a small effect on the rate of oxidation of glucose. Wool and Krahl (140) found that insulin stimulates the incorporation of carbon from amino acids, glucose, fatty acids, and acids of the citric acid cycle into protein by rat diaphragm. This experimental evidence excludes the possibility that the stimulating effect of insulin on amino acid or glucose incorporation into protein is due to an increased rate of transport or increased concentration of metabolites.

Insulin stimulates fat synthesis in vivo (141). In vitro stimulation of fat synthesis by insulin was observed in several tissues of the rat (142), in white adipose tissue (143) and in brown adipose tissue (144). In adipose tissue and in the lactating mammary gland (143), insulin stimulates fat synthesis from glucose as well as acetate, stimulating it from the latter only in the presence of glucose. Glucose is therefore required to demonstrate an insulin effect

on fat synthesis in adipose tissue. Apparently, there are no reports of stimulated fat synthesis in muscle by insulin. The present results show that there is little fat synthesis from glucose or short-chain fatty acids by the surviving guinea pig heart, either in the presence or absence of insulin. In cardiac muscle, glucose has little effect on fatty acid oxidation, while fatty acids have a very pronounced effect on glucose utilization. If glucose is more readily oxidized in adipose tissue than in muscle in the presence of fatty acids, more fatty acid residues which would otherwise be oxidized might be diverted to fat synthesis. This reasoning would explain the stimulation by insulin of fat synthesis in adipose tissue and the lack of any significant effect on fat synthesis by insulin in muscle, solely as a result of increased availability of glucose.

Insulin causes a large increase in the amount of glucose oxidized by hearts perfused in the presence of fatty acids and by hearts from starved animals. The effect by insulin is shown to be an increase in the amount of carbohydrate which enters the citric acid cycle, since there is also an increase in the amount of radioactivity incorporated into glutamate and glutamine from C^{14}-labelled glucose. It is unlikely that this effect is due to an increased availability of glucose residues to the cell, since in the absence of insulin, considerable lactate derived from exogenous glucose accumulates when fatty acids are present. Insulin has no effect on the oxidation of fatty acids or on the amount of fat synthesized

from short-chain fatty acids. It therefore appears that insulin has a direct effect on the entry of carbohydrate into the citric acid cycle when fatty acids are present.

Adrenaline causes a large increase in the total oxygen consumption by whole animals (145). High concentrations of adrenaline double the oxygen uptake by surviving rat hearts in vitro (66). Glucose and lactate concentrations in the blood are increased by adrenaline (146). Adrenaline induces an increase in the release of fatty acids from adipose tissue, resulting presumably in an increase in the fatty acid content of the blood (147). The hyperglycemia produced by adrenaline has been assigned, at least in part, to the pronounced glycogenolytic effect by adrenaline. Muscle glycogen, in particular, is susceptible to this effect by adrenaline (148).

The present results have shown that adrenaline almost triples the amount of exogenous glucose oxidized by the perfused guinea pig heart. This large increase takes place simultaneously with very rapid glycogenolysis. Considering the amounts of exogenous glucose and glucose residues derived from glycogen which are oxidized, adrenaline produces as much as a five- to six-fold increase in carbohydrate oxidation. The utilization of glucose is stimulated at several points: in the absence of adrenaline, little lactate accumulates; insulin produces an accumulation of lactate without changing extensively the rate of glucose oxidation. In the presence of adrenaline, however, the oxidation of glucose is markedly stimulated, and lactate, derived

both from glycogen and from exogenous glucose, accumulates. In the presence of adrenaline, therefore, transport of glucose into the cell, glycolysis, and CO_2 production from glucose are all greatly stimulated. Oxidation via the citric acid cycle, although stimulated, becomes rate-limiting as evidenced by a large accumulation of lactate.

Fatty acid oxidation is increased by adrenaline, but to a much smaller extent than is glucose oxidation. This conclusion is valid only if one assumes that adrenaline has no effect on the rate of utilization of endogenous fat. It seems unlikely, however, that endogenous fatty acid oxidation is increased appreciably by adrenaline, since the increase in total oxygen consumption produced by adrenaline (66) is mainly accounted for, in the present study, by the increase in carbohydrate oxidation. The balance of effects by adrenaline on carbohydrate and fat metabolism in vivo, however, might be quite different. Fatty acids are liberated into the blood by adrenaline from adipose tissue (147), giving rise to a greater availability of lipid material for oxidative metabolism. In view of the pronounced effect by fatty acids on glucose oxidation, and the lack of any significant effect by glucose on fat utilization, the stimulation in vivo by adrenaline of carbohydrate oxidation by the heart may be much smaller than that obtained in vitro.

Glucose competes favorably with fatty acids for oxidation in the presence of either insulin or adrenaline. In their absence,

glucose oxidation is suppressed more than 90 percent by equimolar concentrations of short-chain fatty acids, but when both insulin and adrenaline are present glucose is oxidized in the presence of fatty acids about 60 percent as rapidly as in the absence of fatty acids. The oxidation of lactate in the presence of fatty acids is increased to about the same extent by insulin and adrenaline as is the oxidation of glucose.

The results presented have shown that the cardiac utilization of glucose is susceptible to a number of controls and, being a highly aerobic organ, the heart utilizes fatty acids more effectively than glucose. The contribution of carbohydrate to the total respiration is increased by insulin and by adrenaline.

SUMMARY

1. Glucose-U-C^{14}, when present at a concentration of 5 mM, is oxidized to $C^{14}O_2$ by the perfused guinea pig heart at a rate of 10 μ moles/gm. wet wt./hr. Radioactivity is incorporated into free glutamate, glutamine, alanine and aspartate, and into proteins. There is a slight drop in cardiac glycogen on perfusion for one hour in the presence of glucose, and a high rate of incorporation of glucose-U-C^{14} into glycogen. There is no appreciable synthesis of long-chain fatty acids or triglycerides from exogenous glucose. Very little radioactive lactate is formed from glucose-U-C^{14}.

2. Insulin slightly increases the rate of oxidation of glucose-U-C^{14} to $C^{14}O_2$. The incorporation of radioactivity into glutamate and alanine, and into proteins is increased by insulin. Insulin effects net glycogen synthesis and a five-fold increase in the amount of glucose-U-C^{14} incorporated into cardiac glycogen. Lactate, derived from both exogenous glucose and glycogen, accumulates in the perfusing medium when insulin is present.

3. The oxidation of glucose-U-C^{14} to $C^{14}O_2$ by perfused guinea pig hearts is suppressed more than 90 percent by starving the animals for a period of 72 hours. The incorporation of radioactivity from glucose-U-C^{14} into glutamate and glutamine is almost completely blocked by starvation, while the radioactive labelling in alanine is undiminished. The cardiac glycogen of guinea pigs is increased

approximately three-fold by a 72-hour fast. Lactate production, from exogenous glucose and cardiac glycogen, is markedly increased by starvation.

4. When present at equimolar concentration with glucose, short-chain fatty acids which give rise to acetyl-CoA suppress the oxidation of glucose-U-C^{14} to $C^{14}O_2$ more than 90 percent. Incorporation of radioactivity from glucose-U-C^{14} into glutamate, glutamine and proteins is almost completely suppressed, while incorporation of glucose carbon into alanine is increased. Short-chain fatty acids which give rise to acetyl-CoA increase the net incorporation of glucose-U-C^{14} into cardiac glycogen, and there is less net glycogenolysis in their presence than in their absence. Lactate derived from glucose-U-C^{14} accumulates in the presence of short-chain fatty acids.

Propionate reduces glucose oxidation about 40 percent, and has a much less pronounced effect on incorporation of glucose carbon into glutamate and glutamine than do fatty acids which give rise to acetyl-CoA. Propionate has little effect on incorporation of exogenous glucose into cardiac glycogen, or on the amount of lactate derived from exogenous glucose which accumulates in the perfusing medium.

5. Adrenaline stimulates the oxidation of glucose-U-C^{14} to $C^{14}O_2$ almost three-fold. Incorporation of radioactivity from glucose-U-C^{14} into glutamate, glutamine and proteins is diminished by adrenaline, while the incorporation into alanine is increased. Cardiac glycogen is almost completely depleted after one hour of perfusion in the

presence of glucose and adrenaline. Lactate accumulates in the perfusing medium as a result of adrenaline action. The lactate which accumulates is derived from exogenous glucose and from glycogen.

6. In the presence of insulin and adrenaline, the rate of oxidation of glucose-U-C^{14} to $C^{14}O_2$ is not significantly different from that obtained when adrenaline alone is present. In the presence of adrenaline, insulin increases the amount of radioactivity found in alanine and glutamine from glucose-U-C^{14} but does not affect the radioactivity of glutamate. In the presence of adrenaline, insulin does not affect the incorporation of radioactivity from glucose-U-C^{14} into cardiac glycogen. Insulin further increases the total lactate which accumulates in the presence of adrenaline. This increase is accounted for by an increase in the amount of exogenous glucose which accumulates as lactate.

7. Insulin partially reverses the inhibitory effects of acetate on the oxidation of glucose-U-C^{14} to $C^{14}O_2$ and on the incorporation of radioactivity into glutamate and glutamine. Insulin increases the amount of glucose-U-C^{14} which is incorporated into cardiac glycogen in the presence of acetate. More lactate derived from glucose-U-C^{14} is formed when both insulin and acetate are present than with either one alone.

8. In the presence of acetate, adrenaline increases the rate of oxidation of glucose-U-C^{14} to $C^{14}O_2$ about eight-fold. The amount of incorporation of radioactivity from glucose-U-C^{14} into glutamate and

glutamine in the presence of acetate is not increased by adrenaline, but the amount of radioactivity incorporated into alanine is increased. Adrenaline further increases the amount of radioactive lactate produced in the presence of acetate.

9. When both insulin and adrenaline are present, the inhibitory effects of acetate on the oxidation of glucose-U-C^{14} to $C^{14}O_2$ and on the incorporation of radioactivity into glutamate and glutamine are reversed to a large extent. The total accumulation of radioactive lactate derived from glucose-U-C^{14} in the presence of acetate is increased by the addition of either insulin or adrenaline, but is not further increased when both are present.

10. Insulin and adrenaline, in combination, greatly increase the amount of glucose-U-C^{14} oxidized to $C^{14}O_2$ by hearts from starved guinea pigs. Insulin and adrenaline elicit an increase in the incorporation of glucose-U-C^{14} into glutamate and glutamine in hearts from starved guinea pigs. The net amount of glucose-U-C^{14} incorporated into glycogen of hearts from starved guinea pigs, when perfused in the presence of insulin and adrenaline, is very low. More than 90 percent of the initial cardiac glycogen is lost in one hour of perfusion in the presence of insulin and adrenaline. The amount of lactate formed from glucose-U-C^{14} is increased when insulin and adrenaline are both present.

11. DL-lactate-1-C^{14} and DL-lactate-2-C^{14}, when present at a concentration of 12 mM, are oxidized by the perfused guinea pig heart at

a rate of 60 μ moles/gm. wet wt. of tissue/hr. Radioactivity from lactate-1-C^{14} is incorporated into alanine. Radioactivity from lactate-2-C^{14} is incorporated into glutamate, glutamine and, to a small extent, into aspartate. There is low but significant incorporation of radioactivity from C^{14}-lactate into cardiac glycogen. There is a slight drop in cardiac glycogen on perfusion in the presence of lactate for one hour. The perfused guinea pig heart does not synthesize appreciable amounts of long-chain fatty acids or triglycerides from exogenous lactate.

12. Insulin does not significantly affect the metabolism of C^{14}-lactate, when present as the sole substrate.

13. Adrenaline increases the rate of $C^{14}O_2$ formation from lactate-1-C^{14} about 25 percent. Cardiac glycogen is almost completely lost on perfusion for one hour in the presence of adrenaline and lactate. There is no detectible labelling of cardiac glycogen from lactate-1-C^{14} in the presence of adrenaline.

14. The oxidation of lactate-1-C^{14} to $C^{14}O_2$ by perfused guinea pig hearts is suppressed more than 80 percent by starving the animal for 72 hours. The incorporation of radioactivity from lactate-2-C^{14} into glutamate and glutamine in the perfused heart is suppressed more than 80 percent by starvation, while the radioactive labelling in alanine is undiminished. The incorporation of radioactivity from lactate-2-C^{14} into cardiac glycogen is slightly increased by starvation. There is a high rate of glycogenolysis in hearts from starved guinea pigs on perfusion with lactate.

15. When added in equimolar concentration with lactate, acetate suppresses $C^{14}O_2$ formation from lactate-1-C^{14} and lactate-2-C^{14} almost 90 percent. Acetate reduces the incorporation of radioactivity derived from lactate-2-C^{14} into glutamate and glutamine more than 80 percent. The incorporation into alanine is unchanged. Acetate has no effect on the incorporation of radioactivity from C^{14}-lactate into cardiac glycogen or on the amount of glycogen which remains after perfusion in the presence of lactate.

16. In the presence of acetate, insulin slightly increases the rate of formation of $C^{14}O_2$ from lactate-2-C^{14}, but has no effect on the incorporation of radioactivity from lactate-2-C^{14} into glutamate, glutamine, alanine, or into glycogen.

17. In the presence of acetate, insulin and adrenaline added together increase the rate of $C^{14}O_2$ production from lactate-1-C^{14} and lactate-2-C^{14} five- to six-fold, but do not affect the incorporation of radioactivity from lactate-2-C^{14} into glutamate, glutamine and alanine. With or without acetate, there is very little residual cardiac glycogen after perfusion for one hour with lactate in the presence of adrenaline and insulin. There is no detectible incorporation of label from radioactive lactate into glycogen.

18. When present at a concentration of 5 mM, acetate-1-C^{14} is oxidized to $C^{14}O_2$ by the perfused guinea pig heart at a rate of 75 μ moles/gm. wet wt./hr. Radioactivity from acetate-1-C^{14} is incorporated into

glutamate, glutamine and to a small extent into aspartate. No detectible radioactivity is found in alanine. Radioactivity derived from acetate-1-C^{14} is not incorporated into cardiac glycogen or into lactate. About 50 percent of the initial cardiac glycogen is lost by perfusing one hour in the presence of 5 mM acetate. Lactate, derived primarily from glycogen, accumulates when acetate alone is added to the perfusing medium. There is little but significant synthesis of long-chain fatty acids from acetate-1-C^{14}.

19. Butyrate-1-C^{14}, when present at a concentration of 2.5 mM, is oxidized to $C^{14}O_2$ by the perfused guinea pig heart at a rate of 35 μ moles/gm. wet wt./hr. Radioactivity from butyrate-1-C^{14} is incorporated into glutamate, glutamine and to a small extent into aspartate. Radioactivity from butyrate-1-C^{14} is not incorporated into cardiac glycogen.

20. Propionate-1-C^{14}, at a concentration of 5 mM, is oxidized to $C^{14}O_2$ by the perfused guinea pig heart at a rate of about 35 μ moles/gm. wet wt./hr. Radioactivity is incorporated into aspartate, and to a much smaller extent into glutamate and glutamine. Propionate-1-C^{14} is not incorporated into cardiac glycogen. Very little lactate is formed when propionate alone is added to the perfusing medium.

21. Adrenaline increases by 60 percent the rate of oxidation of acetate-1-C^{14} to $C^{14}O_2$ by the perfused guinea pig heart, while it decreases the incorporation of radioactivity into glutamate, glutamine

and aspartate. In its presence there is almost complete loss of glycogen, and a marked increase in the amount of lactate which accumulates in the presence of acetate.

22. The oxidation of acetate-1-C^{14} to $C^{14}O_2$ by the perfused guinea pig heart is independent of concentration at concentrations between 1 and 20 mM.

23. Glucose, even at a concentration of 10 mM, does not significantly affect the metabolism of 5 mM acetate. When hearts are perfused with acetate and high concentrations of glucose, there is net glycogen synthesis and a large accumulation of lactate in the perfusing medium.

24. When present at equimolar concentration with acetate, propionate does not affect the rate of oxidation of acetate-1-C^{14} to $C^{14}O_2$, but decreases the amount of carbon-14 incorporated into amino acids from acetate-1-C^{14}.

25. Hearts from starved guinea pigs oxidize acetate-1-C^{14} to $C^{14}O_2$ at about the same rate as hearts from fed animals.

26. Acetate, at equimolar concentration with propionate, does not significantly affect the rate of oxidation of propionate-1-C^{14} to $C^{14}O_2$ by perfused guinea pig hearts.

CLAIMS TO ORIGINAL RESEARCH

1. An apparatus for perfusing guinea pig hearts was devised which provides a high degree of oxygenation, a constant pressure approximating normal arterial blood pressure, and the ability to monitor the rate of $C^{14}O_2$ production from C^{14}-labelled substrates.

2. Radioactivity from glucose-U-C^{14} is incorporated by perfused guinea pig hearts into free glutamate, glutamine, alanine, aspartate, proteins, cardiac glycogen and lactate.

3. Acetate, butyrate, pentanoate and heptanoate almost completely suppress the incorporation of radioactivity from glucose-U-C^{14} by perfused guinea pig hearts into CO_2, glutamate, glutamine, and proteins, while the incorporation of radioactivity into alanine, lactate and cardiac glycogen is markedly increased.

4. The inhibitory effects of propionate on the conversion of glucose carbon to CO_2, glutamate, glutamine, alanine, lactate and cardiac glycogen are much less pronounced than the effects of other short-chain fatty acids.

5. Starvation markedly decreases the incorporation of radioactivity from glucose-U-C^{14} by perfused guinea pig hearts into $C^{14}O_2$, glutamate, glutamine, proteins and cardiac glycogen, and effects pronounced increases in the amount of incorporation into lactate.

6. Hearts from fed guinea pigs oxidize DL-lactate-1-C^{14} and DL-lactate-2-C^{14}, when present at a concentration of 12 mM, at a rate of about 60 μ moles of lactate/gm. wet wt. of tissue/hr. Radioactivity from lactate-1-C^{14} is incorporated into alanine, and radioactivity from lactate-2-C^{14} is incorporated into alanine, glutamate, glutamine, aspartate and, to a small degree, into cardiac glycogen.

7. Acetate, when present at equimolar concentration with lactate, suppresses more than 90 percent the oxidation by perfused guinea pig hearts of lactate carbon to CO_2 and the incorporation of radioactivity from lactate-2-C^{14} into glutamate and glutamine.

8. Starvation inhibits about 80 percent the incorporation of radioactivity from lactate-1-C^{14} into $C^{14}O_2$ and the incorporation of radioactivity from lactate-2-C^{14} into free glutamate and glutamine.

9. High concentrations of adrenaline increase the rate of oxidation of glucose-U-C^{14} to $C^{14}O_2$ by perfused hearts almost three-fold. Adrenaline effects a decrease in the amount of radioactivity from glucose-U-C^{14} incorporated into glutamate, glutamine and proteins, and increases the incorporation into alanine. In the presence of adrenaline, lactate derived from exogenous glucose and from cardiac glycogen accumulates in the perfusing medium.

When acetate is also present in the perfusing medium, adrenaline increases the oxidation of glucose-U-C^{14} to $C^{14}O_2$ almost ten-fold, without increasing appreciably the incorporation of radioactivity into glutamate and glutamine, but markedly increases the incorporation into alanine and lactate.

10. In the presence of insulin, the rate of oxidation of glucose-U-C^{14} to $C^{14}O_2$ by perfused guinea pig hearts is only slightly increased, and the radioactivity incorporated into glutamate, glutamine and proteins is doubled. Insulin effects a five- to six-fold increase in the amount of glucose-U-C^{14} incorporated into cardiac glycogen and large increases in the amount of lactate formation from exogenous glucose and in the amount of glucose carbon incorporated into alanine. There is an increase in cardiac glycogen during perfusion in the presence of glucose and insulin.

When acetate is present, insulin markedly increases the oxidation of glucose-U-C^{14} to $C^{14}O_2$. Insulin also increases the amount of glucose carbon incorporated into lactate, alanine, glutamate and glutamine in the presence of acetate.

11. The inhibitory effects of acetate and of starvation on the incorporation of glucose carbon into CO_2, glutamate and glutamine are largely reversed when hearts are perfused in the presence of both insulin and adrenaline.

Similarly, the inhibitory effects of acetate on lactate utilization are reversed by insulin and adrenaline.

12. Guinea pig hearts oxidize acetate-1-C^{14}, when present at a concentration of 5 mM, at a rate of about 75 μ moles/gm. wet wt. of tissue/hr. Radioactivity from acetate-1-C^{14} is incorporated

into glutamate, glutamine and, to a small extent, into aspartate. Lactate accumulates in the presence of acetate. Glucose does not significantly affect the metabolism of acetate.

13. Butyrate-1-C^{14}, when present at a concentration of 2.5 mM, is oxidized to $C^{14}O_2$ by perfused guinea pig hearts at a rate of about 35 μ moles of butyrate/gm. wet wt./hr. Radioactivity is incorporated into glutamate, glutamine and, to a small extent, into aspartate.

14. Propionate-1-C^{14}, when present at 5 mM concentration, is oxidized to $C^{14}O_2$ by perfused guinea pig hearts at a rate of about 35 μ moles/gm. wet wt. of tissue/hr. Radioactivity from propionate-1-C^{14} is incorporated into aspartate, and to a small extent, into glutamate and glutamine.

15. The rate of oxidation of propionate-1-C^{14} to $C^{14}O_2$ is not significantly affected by acetate, nor is the oxidation of acetate affected by propionate.

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