STUDIES ON INTERACTIONS BETWEEN THE METABOLISM OF FAT AND CARBOHYDRATE

A thesis submitted for the degree of

DOCTOR OF PHILOSOPHY

by

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January, 1964.

STATEMENT

The experiments described in this thesis were done entirely by myself.

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PREFACE

A characteristic of the metabolism of multicellular organisms is their utilization of complex organic molecules as sources of energy for the various activities they manifest. The physiology of the processes associated with the extraction of energy from fat, carbohydrate and protein is still not understood completely. Molecules of each of these classes of foodstuff are present in the environment of the cells of all tissues, and it is from this metabolic mixture of molecules that the enzyme systems of the cells must make their selection.

A great deal of experimental work has been carried out to try to decide the metabolic interrelationships of the various foodstuffs. In particular, many experiments have been done to show that the oxidation of glucose occurs preferentially over the oxidation of fat. As yet however, no precise estimate has been made of the rate at which fatty acids are oxidized in the body. In most of the experiments ^{14}C-labelled fatty acids have been given intravenously as single intravenous injections. It is difficult to assess the rate of oxidation of fatty acids given in this way in terms of their contribution to the total energy needs of the body. Similarly when single injections of labelled fatty acids are given together with glucose, it is not possible to determine with any degree of precision, the extent of competition

between the two substrates.

The work in this thesis describes experiments which were done to measure the extent of long chain fatty acid metabolism in unanaesthetized rats. Various ^{14}C-labelled long chain fatty acids incorporated into chylomicrons have been given by continuous intravenous infusion so as to provide for a large proportion of the rats' immediate energy requirements. The effect of glucose on the oxidation of the chylomicron fatty acids has been studied by continuously infusing these two substrates simultaneously in amounts commensurate with their normal rates of entry from the gut. Finally the metabolism of chylomicron fatty acids has been studied in an isolated autoperfused rat heart-lung preparation to demonstrate the utilization of particulate fat by these organs.

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CHAPTER ONE

INTRODUCTION

The realization that heat is produced in the animal body as a result of oxidative processes first led to experimental attempts to relate energy metabolism to heat production (Lavoisier & Laplace, 1780; Dulong, 1823; Despretz, 1824). These early experiments were designed to relate heat production to the amounts of carbon and hydrogen oxidized. Liebig (1842) stated that carbon and hydrogen were not burned in the body as such, but were oxidized in so far as they were constituents of protein, carbohydrate or fat. Liebig believed that oxygen caused the breakdown of carbohydrate and fat, while the breakdown of protein was due to muscular work. He suggested that the nitrogen of the urine might be used as a measure of the protein destroyed in the body. Subsequently, Bidder & Schmidt (1852) and Voit (1857) established that the nitrogen released by the breakdown of protein is excreted in the urine as urea.

Some of the first experiments involving the measurements of CO_2 production and O_2 consumption were carried out by Regnault & Reiset (1849, 1850). Although they did not attempt to determine from what foodstuff the respiratory CO_2 was derived, they noted that the ratio of

the amount of CO_2 produced to the amount of O_2 consumed varied appreciably, and this ratio seemed to be related to the type of food the animal ate. This ratio was subsequently termed the respiratory quotient (R.Q.) by Pflüger (cf. Lusk, 1931). As the composition of the various foodstuffs became known, it was possible to calculate how much CO_2 would be produced and how much O_2 would be consumed by their oxidation. Bischoff & Voit (1857) determined how much of a starving dog's flesh must have been burned to produce the amount of nitrogen excreted in its urine, and the quantities of CO_2 and O_2 consumed in this process. From the data Regnault & Reiset had obtained for the CO_2 production and O_2 consumption of a dog of comparable size, Bischoff & Voit estimated that the remaining gaseous exchange, after allowing for the breakdown of protein, must have been due entirely to the combustion of fat. Pettenkofer & Voit (1866) measured the CO_2 production, the O_2 consumption and the nitrogenous excretion of a fasting man and concluded that his metabolism was furnished by the combustion of protein and fat.

Rubner (1885a, b) determined the calorific values of various foodstuffs containing fat carbohydrate and protein when burned in the body, and he found that within limits, the various foodstuffs may replace each other in a maintenance diet according to their heat producing values.

Rubner also found that the heat production of animals increased following the ingestion of food, particularly protein. In 1894 Rubner determined indirectly the foodstuffs being oxidized by a dog by measuring its gaseous metabolism and nitrogenous excretion. At the same time he measured directly the heat liberated by the dog. The heat production which Rubner actually measured agreed closely with the heat produced by the combustion of the foodstuffs which he calculated had been burned in the dog's body. Thus the law of conservation of energy as applied to energy metabolism in animals, had been verified finally by experiment.

These basic techniques have been modified and refined by many subsequent workers, and extensive data have been obtained about metabolism in various conditions such as during fasting, muscular exercise, and in many disease processes. The results of many of these experiments were anticipated by Voit (1901) in summarizing the results of his work with Pettenkofer to that time. "We found that in starvation protein and fat alone were burned, that during work more fat was burned, and that less fat was consumed during rest, especially during sleep; that the carniverous dog could maintain himself on an exclusive protein diet, and if to such a diet fat were added, the fat was almost entirely deposited in the body; that carbohydrates, on the contrary were burned no matter how

much was given, and that they, like the fat of the food protected the body from fat loss, although more carbohydrates than fat had to be givento effect this purpose; that the metabolism in the body was not proportional to the combustibility of the substances outside the body, but that protein, which burns with great difficulty outside, metabolizes with the greatest ease, then carbohydrates, while fat, which burns readily outside, is the most difficultly combustible in the organism." (cf. Lusk, 1931).

From experimental results such as these, it was concluded that fat was a relatively inert substance in the body which was deposited in times of caloric excess and was utilized only when other more readily metabolizable substances were not available.

Many workers were critical of these experimental findings, and they questioned the use of the R.Q. for determining what substances were actually being oxidized in the body. As early as 1865, Bernard, who himself had done respiration experiments possibly before those of Regnault & Reiset (cf. Olmsted, 1939), had said that these types of studies were "like trying to tell what happens inside a house by watching what goes in by the door and comes out by the chimney". (Bernard, 1865). Cathcart & Markowitz (1927) pointed out that the components of the R.Q. must be extremely complex. Thus they said thata

R.Q. of 1, which was regarded traditionally as indicating the combustion of carbohydrates exclusively, would be produced by the conversion of glucose to fat, with the simultaneous oxidation of similar quantity of fat to CO_2 and H_2O. Under these circumstances it was possible that with a R.Q. of 1, some tissues could be deriving their energy requirements from the oxidation of fat. That this could in fact be the case was shown by Schoenheimer & Rittenberg (1936) who fed mice on a high carbohydrate diet and at the same time enriched the body water with deuterium. From the rate at which deuterium was incorporated into the fatty acids of the depot fats, they concluded that the fatty acids of the adipose tissue were being turned over rapidly. The quantitative significance of this process, and the role it plays in normal carbohydrate metabolism was demonstrated by Stetten & Boxer (1944a, b). These workers showed that rats which were maintained on a high carbohydrate diet metabolized only about 3% of their dietary carbohydrate by way of glycogen and that at least 10 times this amount of glucose was synthesized into fatty acids.

The mechanisms by which energy is produced and utilized in the living organism. The energy required by living matter is provided almost universally by the pyrophosphate bonds of adenosine triphosphate (ATP). In animals these energy rich bonds are synthesized by

reactions which are coupled to the oxidation of the various foodstuffs or stored energy substrates. Krebs (1959) pointed out that even in higher forms of life in which many aspects of metabolism are under precise hormonal and nervous control, the need for ATP in any individual cell might be regarded as the most primitive mechanism controlling the metabolism of the cell.

The production of energy in the living body from the combustion of the foodstuffs and stored energy substrates can be divided into three phases (Krebs, 1954b). In the first phase, the complex molecules are broken down into their constituent units; the proteins are broken down into the amino acids, the carbohydrates into the hexoses, and the fats into glycerol and fatty acids. Only small amounts of energy are released by these processes which take place in the gut during digestion, or in the tissues when stored energy reserves are mobilized. The large number of compounds which are formed in this first phase (about 20 amino acids, 3 or more hexoses, glycerol and several fatty acids) are burned in the second stage of oxidation to yield four end-products, CO_2, acetyl Coenzyme A (acetyl-CoA), α-ketoglutarate, and oxaloacetate. These three latter products are closely interrelated in that two of them, α-ketoglutarate and oxaloacetate, are intermediates in the tricarboxylic acid cycle. In this cycle of reactions the acetyl-CoA is

oxidized completely to CO_2 and H_2O. Of these compounds acetyl-CoA is the most important quantitatively. Thus up to 2/3rds of the carbon of glycerol and the hexoses, all of the carbon of the fatty acids, and about $\frac{1}{2}$ of the carbon of amino acids can give rise directly to acetyl-CoA (Krebs, 1954b). The acetyl-CoA must combine with oxaloacetic acid to be completely oxidized to CO_2 and H_2O (Stern, Coon & del Campillo, 1953). The citric acid formed by this combination passes through the reactions of the tricarboxylic acid cycle during which CO_2 and H_2O, equivalent to the acetate, are liberated and the oxaloacetic acid is regenerated. Acetyl-CoA cannot form oxaloacetate (Weinman, Strisower & Chaikoff, 1957), and so the oxidation of fatty acids which gives rise to acetyl-CoA, requires the concomitant metabolism of compounds which can lead to the formation of the small amounts of oxaloacetate required for the formation of citrate from acetyl-CoA. As approximately 2/3rds of the energy released during the oxidation of the various foodstuffs is released by the complete oxidation of acetyl-CoA, it is essential that this oxidation proceeds efficiently.

The energy which is released by these oxidative reactions is used for the synthesis of ATP, but in only a few such reactions is the formation of the ATP coupled directly to the oxidation. Most of the oxidations are

accomplished by the removal of hydrogen atoms and the ATP is synthesized in coupled reactions in which the hydrogen is oxidized to water. Many of the individual reactions use the same coupled side-reactions for the ultimate synthesis of ATP.

The oxidation of the constituents of the three apparently dissimilar types of foodstuff, the proteins, the carbohydrates and the fats, are thus intimately interrelated, not only in that they give rise to common intermediates which then traverse identical pathways, but the hydrogen which is removed in many of the individual reactions is oxidized by identical pathways.

The interconversion of the various foodstuffs within the body. Acetyl-CoA is formed from glycerol and the hexoses by the oxidative decarboxylation of a common intermediate, pyruvic acid. Pyruvic acid can also be carboxylated to form oxaloacetate directly or indirectly by way of malic acid (Krebs, 1954a). Thus the metabolism of the hexoses and their intermediates, and the amino acids can give rise to the compounds of the tricarboxylic acid cycle. If the metabolism of carbohydrates or proteins proceeds at such a rate that amounts of tricarboxylic acid cycle compounds will be formed in excess of those required for the formation of oxaloacetate, the excess amounts of intermediates can then be used for synthetic purposes (Weinman, Strisower & Chaikoff, 1957). Thus

oxaloacetate and α-ketoglutarate can be aminated to form amino acids, or oxaloacetate can be converted to phosphopyruvate (Krebs, 1954a). The phosphopyruvate can be converted to glucose by a reversal of the oxidative pathways, or it can be converted to pyruvate. The pyruvic acid can be aminated to form amino acids, or it can be decarboxylated oxidatively to form acetyl-CoA. By the reactions just discussed, a net synthesis of carbohydrate from amino acids can occur, but there can be no net synthesis of amino acids as the amino groups used in the aminations must be provided by other amino acids. These reactions provide a mechanism by which relative deficiencies of some amino acids can be corrected by relative excesses of others (cf. Soskin & Levine, 1952).

It is well known that higher animals can only store limited amounts of protein and carbohydrates; when no more of these substances can be stored, additional amounts of amino acids or hexoses which are absorbed, are metabolized obligatorily (cf. Soskin & Levine, 1952). From what has been said above, this obligatory metabolism must lead to the formation of acetyl-CoA. If the production of acetyl-CoA exceeds that necessary for energy requirements, the acetyl-CoA must be diverted to synthetic pathways, the most important of which, quantitatively, is the synthesis of fatty acids. As the

oxidation of fatty acids can give rise in the first place to acetyl-CoA only, it might be expected that the formation of large amounts of acetyl-CoA from other sources could restrict the breakdown of fatty acids.

In higher animals, the metabolism of any given type of cell is governed by the spectrum of enzymes which it contains. When a cell is presented with a variety of oxidizable substrates in excess of its immediate energy requirements, several possibilities arise as to which of the substances will be oxidized. If a cell possesses the necessary enzymes, it might metabolize any one or all of the substrates depending on their relative availability. The obligatory metabolism of one or more of the substances might restrict necessarily the metabolism of the others. Some metabolites might be oxidized preferentially over others so that their mere presence in adequate amounts might specifically reduce or inhibit the oxidation of others.

The oxidation of fatty acids. The principle of β-oxidation of fatty acids was first postulated by Knoop in 1904. The reactions of this scheme for the β-oxidation of fatty acids have been worked out in some detail in recent years and have been reviewed by Green (1963). Before a fatty acid can be oxidized it must be activated with Coenzyme A to form an acyl-CoA derivative. This activation requires the expenditure of energy which is

provided by ATP. The oxidation of the fatty acid then proceeds in a cyclic fashion leading to the formation of acetyl-CoA. In the first reaction of the cycle, the acyl-CoA is dehydrogenated in the α-β position to form an unsaturated derivative which is then hydrated to form the corresponding β-hydroxyacyl-CoA derivative. This is then oxidized to the corresponding β-ketoacyl-CoA which is thiolyzed by free CoA to form a new acyl-CoA derivative, 2 carbon atoms shorter than the previous one, and a molecule of acetyl-CoA. This sequence is repeated and straight chain fatty acids with an even number of carbon atoms, which are the predominant naturally occurring fatty acids, are completely oxidized to acetyl-CoA. All of the enzymes associated with these processes are integral components of mitochondria and it is possible that they are spatially located as a functional unit. Different enzymes have been isolated that react optimally with derivatives of different chain length so that as the chain length of the fatty acid is shortened progressively during the oxidation, the derivatives of the oxidation are acted on by different enzymes. Two hydrogen acceptors are required for the two oxidative steps in each turn of the cycle, a flavoprotein and diphosphopyridine nucleotide (DPN). The efficient oxidation of fatty acids is therefore dependent on the availability of CoA, ATP for the initial activation, and the continuous oxidation of

the reduced hydrogen acceptors. Any other processes which lead to the formation of acetyl-CoA could be in competition with the fatty acid oxidation system for the available supply of CoA. The availability of DPN could also be a limiting factor in this system when other systems are reducing DPN simultaneously.

The formation of ketone bodies. In some circumstances due to a relative deficiency of oxaloacetic acid, the formation of acetyl-CoA exceeds the rate at which it can be oxidized to CO_2 and H_2O (Lynen, 1954). Under these conditions two molecules of acetyl-CoA might condense to form acetoacetyl-CoA which can be hydrolyzed to form free acetoacetate. Acetoacetic acid can be reduced to form β-hydroxybutyric acid and these two substances are usually in equilibrium with each other. Acetoacetate can be decarboxylated spontaneously to form acetone. These three substances are referred to as ketone bodies. Before free acetoacetic acid can be utilized it must be activated with CoA and this can be accomplished readily in most tissues except in the liver. The liver has a low concentration of the enzymes necessary for the activation of the acetoacetate (cf. Green, 1963).

The relationship between the formation of ketone bodies by an animal and its nutritional status has been appreciated for a long time (cf. Woodyatt, 1948). Thus the production of ketone bodies is increased in conditions

in which an animal derives a large proportion of its energy requirements from the oxidation of fat. Early experiments with isolated perfused organs established that the perfused liver produces large amounts of ketone bodies when the perfusate contains fatty acids (cf. Soskin & Levine, 1952). Chaikoff & Soskin (1928) showed that the liver is the site of formation of ketone bodies *in vivo* but that the extra hepatic tissues are able to utilize the ketone bodies.

The synthesis of long chain fatty acids in animal tissues. It was shown that fatty acids are synthesized from 2 carbon units (Rittenberg & Bloch, 1945) before the central role of acetyl-CoA in both the oxidation and synthesis of fatty acids was appreciated. Two separate systems for the synthesis of long chain fatty acids have been discovered in animal tissues. These pathways which appear to have separate functions, have been reviewed in some detail (Wakil, 1963; Masoro, 1962; Gibson, 1963), and will be considered only in so far as they show some of the possible interactions between the metabolism of glucose and fatty acids. One of these pathways occurs completely outside the mitochondria and results in the synthesis of palmitate from acetyl-CoA *de novo*. The other pathway functions chiefly to increase the number of carbon atoms in naturally occurring fatty acids of intermediate chain length, and utilizes several of the

reverse reactions of the β-oxidation of fatty acids. The synthesis of fatty acids de novo, and the elongation of existing fatty acids, in contrast to their oxidation, requires reduced triphosphopyridine nucleotide (TPNH) as the hydrogen donor for some of the reductive steps. One of the most important pathways for the synthesis of TPNH is the oxidation of glucose by the direct oxidative pathway (hexose monophosphate shunt) (Krebs, 1954a). It has been shown that the oxidation of glucose by this pathway is enhanced during the synthesis of fatty acids in the liver (Felts, Doell & Chaikoff, 1956), in the mammary gland (Abraham & Chaikoff, 1959), and in adipose tissue (Winegrad & Renold, 1958). A number of workers have questioned that the availability of TPNH is a limiting factor in fatty acid synthesis (cf. Masoro, 1962), and there is some doubt as to whether the oxidation of glucose by the hexose monophosphate shunt stimulates fatty acid synthesis by providing TPNH, or whether fatty acid synthesis stimulates the oxidation of glucose by this pathway by oxidizing TPNH. Nevertheless, it appears that the metabolism of glucose is closely related to the synthesis of fatty acids, not only in that glucose provides the acetyl-CoA from which fatty acids are synthesized, but also in that glucose provides the hydrogen necessary for some of the reductive steps in the synthesis of fatty acids from acetyl-CoA.

The esterification of fatty acids. Fatty acids cannot be stored unless they are esterified, and triglycerides are the most important storage form of fatty acids. In the synthesis of triglycerides or phospholipids, fatty acid-CoA esters combine with α-glycerophosphate to form phosphatidic acid. The phosphate group is then removed by hydrolysis to form an α,β-diglyceride. The diglyceride can then react with another fatty acid-CoA ester to form a triglyceride, or with various cytidine diphosphate compounds to form phospholipids (Kennedy, 1958). In many tissues α-glycerophosphate can only be formed by the reduction of dihydroxyacetone phosphate which is an intermediary in the metabolism of the hexoses. Glycerol which is released during the hydrolysis of triglycerides cannot be utilized in tissues other than the liver, kidney and heart (cf. Weiss, Kennedy & Kiyasu, 1960).

Most enzymes which catalyze the synthesis of glycerides are specific for the naturally occurring long chain fatty acids (Kornberg & Pricer, 1953), and the short chain fatty acids can only be incorporated into triglycerides in any significant quantities in the lactating mammary gland (Hirsch, Lossow & Chaikoff, 1956). Thus the short chain fatty acids which cannot be esterified in most tissues and cannot be lengthened to

produce the naturally occuring long chain fatty acids which can be stored, must be metabolized if they are not to accumulate in the tissues.

The sites of synthesis of fatty acids in animals.

Chernick, Masoro & Chaikoff (1950) demonstrated that the rate of lipogenesis in liver slices from rats fed carbohydrate was 10 times as rapid as the rate of lipogenesis in slices of muscle or kidney, and they concluded that the liver was the principal site of lipogenesis in intact animals. The demonstration that adipose tissue is a potent site of fatty acid synthesis and the relatively large amount of this tissue in the body has suggested that this tissue might be responsible for most of the fatty acid synthesis in the animal body (cf. Fritz, 1961). In lactating animals, the mammary gland can synthesize large amounts of fatty acids, both long chain and short chain.

Transport of fatty acids in the body. The naturally occurring long chain fatty acids and their esters are characteristically insoluble in water and special mechanisms are employed for their transport in the blood and tissue fluids. In many of the early experiments in which the metabolism of fatty acids was studied, short chain, water soluble fatty acids were used. The metabolism of these short chain fatty acids differs in several respects from that of the naturally occurring long chain fatty acids.

McKibbin, Ferry & Stare (1946) showed that emulsions of coconut oil given intravenously to dogs were oxidized to provide some of the animal's energy requirements. Subsequently, ^{14}C-labelled long chain fatty acids have been incorporated into artificial emulsions and given intravenously to animals by several groups of workers in order to study various aspects of fat metabolism. As information has become available about the physio-chemical forms in which fat is normally transported in the body, it has been possible to study the metabolism of fatty acids under physiological conditions. The results of these later experiments have indicated that differences exist in the metabolism of fatty acids and these differences are related to the form in which the fat is given. It is necessary to consider therefore the forms in which the exogenous fatty acids from the diet and the endogenous fatty acids mobilized from the fat depots are transported in the body. This subject has been reviewed by Fredrickson & Gordon (1958a), Olson & Vester (1960) and Dole & Hamlin (1962).

Exogenous fatty acids. Long chain fatty acids are ingested primarily as triglycerides which are partially hydrolyzed during digestion to form a mixture of partial glycerides, soaps and unchanged triglycerides. (Blomstrand, Borgström & Dahlbach, 1959). This mixture

of substances, together with the bile salts, form micelles which are small enough to be absorbed into the intestinal mucosal cells. The hydrolyzed products are reesterified within the intestinal mucosal cells (Borgström, 1952c) and the absorbed fat is transported from the gut by way of the intestinal lymphatics and thoracic duct to the systemic circulation as particulate fat, the chylomicrons. The chylomicrons which form a very stable emulsion, consist mainly of triglycerides with smaller amounts of cholesterol esters and phospholipids and a small proportion of protein (Laurell, 1953). It is believed that the chylomicrons are stabilized in the blood by a surface layer of phospholipids (Frazer, 1946; Robinson, 1955).

Radioactive chylomicrons can be prepared by feeding ^{14}C-labelled fatty acid to animals with fistulae in their intestinal lymphatic ducts or in their thoracic ducts and collecting the lymph during the period of fat absorption. Labelled chylomicrons collected in this way have been used to study the removal of chylomicrons from the blood stream of dogs and rats and their subsequent oxidation (Havel & Fredrickson, 1956; French & Morris, 1957; French & Morris, 1958; Morris, 1958). The results of these experiments have indicated that the fractional rates at which the injected chylomicrons are removed from the circulation and their

fatty acids oxidized to CO_2 are inversely related to the total amount of chylomicron fat injected.

Chylomicrons are relatively large particles which scatter light and consequently cause a turbidity in the plasma during the absorption of a fat meal. It has not been demonstrated conclusively whether chylomicrons are removed from the circulation as intact units to any great extent or whether their removal requires that they be broken down into smaller products which may be removed more readily from the circulation. When heparin is injected intravenously into animals an enzyme is released into the blood which causes the rapid hydrolysis of chylomicrons to free fatty acids and glycerol. This hydrolysis leads to a clearing of the plasma of animals absorbing fat (Hahn, 1943; Swank & Wilmot, 1951). The removal of chylomicrons from the circulation can be delayed by the injection of heparin antagonists such as protamine (French & Morris, 1957). However most workers have concluded that the intravascular hydrolysis of chylomicrons, analagous to the post-heparin clearing response, is not of quantitative importance in the normal removal of chylomicrons from the circulation (cf. Dole & Hamlin, 1962).

When chylomicrons which have been labelled with a ^{14}C-fatty acid, are injected intravenously, a large proportion of the chylomicron fatty acids which are

cleared from the circulation can be recovered in the liver (French & Morris, 1958). Similar observations by other workers have led to the proposal that the liver plays a central role in the removal of chylomicrons from the circulation (Bragdon & Gordon, 1958; Borgstrom & Jordan, 1959; Olivecrona, 1962b). Dole & Hamlin (1962) in their review article, conclude that the liver, reticuloendothelial cells and adipose tissue are the most likely sites of removal of chylomicrons from the circulation, and they state "No direct evidence suggests that other tissues can remove significant quantities of particulate fat". The liver appears to be able to take up intact chylomicrons from the circulation by virtue of the open structure of the endothelium lining the hepatic sinusoids (Fawcett, 1955). Although the particles of some artificial fat emulsions are removed from the circulation by phagocytosis, the reticuloendothelial system does not appear to be of quantitative importance in the normal removal of chylomicrons from the circulation (cf. French, Morris & Robinson, 1958). An enzyme with properties similar to those of the post-heparin clearing factor has been extracted from heart and adipose tissue (Korn, 1958) and it has been suggested that this enzyme might be responsible for the uptake of chylomicrons by adipose tissue. However in many experiments the uptake of chylomicrons by adipose tissue has been slight. Similarly the small

proportion of an injection of chylomicron fatty acids which can be recovered from other extrahepatic tissues has led to the conclusion that these tissues are not important in the removal of chylomicrons from the circulation.

Endogenous fatty acids. Endogenous fatty acids are transported in the blood both as esterified fatty acids and unesterified fatty acids; these form soluble complexes with the plasma proteins. The main esters of fatty acids are cholesterol esters, triglycerides, and phospholipids and these are transported in association with the α and β globulins in the form of lipoproteins. Different classes of lipoproteins have been separated in terms of their densities and electrophoretic mobilities. Bragdon, Havel & Boyle (1956) showed that as the density of lipoproteins decreased, the proportion of triglycerides in the lipoproteins increased. The distinction between very low density lipoproteins with a high triglyceride content and chylomicrons of alimentary origin is quite arbitrary (Dole & Hamlin, 1962). There is evidence that in some conditions considerable amounts of endogenous fatty acids are transported as particulate lipid. Thus Morris & French (1958) concluded that the hyperlipaemia which develops in experimental nephrosis in rats, reflects an increased mobilization of endogenous fat as particulate lipid. Hatch, Abel & Kendall (1955) found

that a proportion of human subjects eating a low fat, high carbohydrate diet developed a hyperlipaemia associated with an increase in the very low density lipoproteins. Similar hyperlipaemic conditions have been reported in pregnancy and diabetes mellitus and following the intravenous injection of various detergents (cf. Dole & Hamlin, 1962). As these lipaemias occur in starved animals the particulate fat must be endogenous in origin. In experiments with the isolated perfused rat liver, Kay & Entenman (1961) found that the liver was able to add 'chylomicron-like bodies' to the perfusate. It is possible therefore that triglyceride in lipoproteins might be an important form in which fatty acids are transported in the body.

Unesterified fatty acids are transported in the main, bound to plasma albumin (Fredrickson & Gordon, 1958a). By injecting ^{14}C-labelled fatty acids which have been complexed with plasma albumin <u>in vitro</u> into experimental animals, it has been shown that the labelled fatty acids are removed rapidly from the circulation (Havel & Fredrickson, 1956; Fredrickson & Gordon, 1958b; Laurell, 1957) and are oxidized readily to CO_2 (Fredrickson & Gordon, 1958b). From a consideration of the circulating half-life of unesterified fatty acids and arterio-venous differences across such organs as the heart and liver it has been concluded that unesterified fatty acids are a

major form by which fat is transported in the body and they can provide for a large proportion of a fasting animal's energy requirements (Gordon & Cherkes, 1956; Gordon, 1957). The experiments of Gordon (1957) indicated that the unesterified fatty acids originated from a peripheral tissue. It was demonstrated subsequently that adipose tissue incubated *in vitro* releases fatty acids into the incubation medium (Gordon & Cherkes, 1958) and this tissue is thought to be the main source of plasma UFA.

The sites of oxidation of fatty acids in the animal body. Until comparatively recent years, it was believed that the extrahepatic tissues took part in fatty acid oxidation only in so far as they oxidized ketone bodies formed from the partial oxidation of fatty acids in the liver (cf. Gemmill, 1942). With the use of radioactive fatty acids, the direct oxidation of long chain fatty acids to CO_2 has been shown to occur in most tissues of the body, and many tissues are thought to derive a large proportion of their energy needs from the oxidation of fat under normal conditions (Geyer, Matthews & Stare, 1949; Allen, Friedman & Weinhouse, 1955; Volk, Millington & Weinhouse, 1952). In the studies of Allen *et al*. the oxidation of ^{14}C-palmitic acid was shown to occur in various tissues *in vitro* after they were removed from rats which had been fed ^{14}C-palmitic acid. Amongst the tissues studied, the heart was shown to oxidize fatty

acids which had been absorbed from the gut, but it was not possible to conclude whether the heart had taken up the fatty acids directly after they had been absorbed. On the basis of arterio-venous differences, the myocardium has been shown to extract both esterified and unesterified fatty acids from the blood (Gordon, 1957; Ballard, Danforth, Naegle & Bing, 1960). Although only a small proportion of the fatty acids of a single injection of chylomicrons which had been cleared from the circulation, could be recovered from the heart muscle (Bragdon & Gordon, 1958), the demonstration that the heart contains an enzyme similar to the post-heparin clearing factor (Korn, 1958) suggests that this organ might be able to utilize chylomicron fatty acids directly.

The effect of glucose on the metabolism of fatty acids.

The effect of glucose on the metabolism of fatty acids will be considered in respect to its effect on the absorption of fat from the gut, the transport of fatty acids in the circulation and the oxidation of fatty acids to CO_2.

The effect of glucose on the absorption of fat from the gut. There is some evidence to suggest that the presence of glucose in the gut delays the absorption of fat from the gut. Lewis, Allen & Weinhouse (1959) found that fat was absorbed more slowly from the gut of rats when glucose was fed simultaneously. Tilden & Shipley (1960) also found that fat was removed from the gut of dogs more

slowly when glucose was fed than when no glucose was fed. They based their finding on radiological studies, the rate of appearance of fat in the blood and the appearance of a blood clotting accelerator factor in the blood which is associated with the absorption of fat.

The effect of glucose on the transport of fatty acids in the blood. The concentration of unesterified fatty acids in the plasma has been shown to be related to the nutritional status of the animal. In fasting animals the concentration of unesterified fatty acids in the blood is higher than in fed animals, and these levels are reduced by feeding mixed meals or carbohydrate but not by fat (Dole, 1956). Gordon (1957) also found that the circulating levels of unesterified fatty acids were reduced by the administration of glucose and at the same time arterio-venous differences across the heart and splanchnic viscera were abolished. The release of fatty acids from adipose tissue in vitro was also reduced by the combined action of glucose and insulin (Gordon & Cherkes, 1958).

Bragdon, Havel & Gordon (1957) found that the feeding of glucose to rats did not influence the rate at which chylomicrons were removed from the blood. However Bragdon & Gordon (1958) showed that when chylomicrons labelled with ^{14}C-palmitic acid were injected intravenously,

the tissue distribution of the ^{14}C-palmitic acid was influenced by the administration of glucose. Thus more of the palmitic acid was recovered in the adipose tissue and less in the liver of rats fed large amounts of glucose than was the case in starved rats.

The effect of glucose on the oxidation of long chain fatty acids. From the results of some of the earliest studies in animal energy metabolism, it was concluded that carbohydrate which is converted to glucose in the body is oxidized in preference to fat. It was also realized in very early studies that the production of ketone bodies was associated with the metabolism of large amounts of fat, and in some of these ketoses, the excretion of ketone bodies in the urine could be reduced or abolished specifically by the administration of carbohydrate (cf. Woodyatt, 1948). It has been repeatedly observed that ^{14}C-labelled fatty acids are oxidized more rapidly to CO_2 by fasted animals than by fed animals. A similar situation holds for metabolic studies conducted on isolated tissues from fed and starved animals (cf. Fritz, 1961).

Lossow & Chaikoff (1955) carried out experiments in which they gave single intravenous injections of ^{14}C-labelled fatty acids to fasted rats and to rats which had been fed glucose. They found when either

^{14}C-palmitic acid or ^{14}C-tripalmitic was injected, the fasted animals excreted about 8 times as much $^{14}CO_2$ in the 6 hr following the injection than did the rats fed glucose. The oxidation of myristic acid was reduced to about 1/3rd by feeding glucose and the oxidation of lauric acid to about 2/3rds. The oxidation of short chain fatty acids was affected only slightly by feeding glucose. Lossow & Chaikoff postulated that when glucose was present in adequate amounts, the oxidation of the long chain fatty acids was specifically inhibited. The effect of the glucose was to restrict the initial breakdown of the fatty acids. The extent to which the oxidation of the shorter chain fatty acids was reduced was related to the ease with which they were converted directly to the long chain fatty acids in the body. The long chain fatty acids used in these experiments were given in the form of artificial emulsions while the short chain fatty acids were given as their sodium salts. Similar results to those of Lossow & Chaikoff (1955) were obtained when more physiological forms of lipid substrates were used. McCalla, Gates & Gordon (1957) and Bragdon (1958) carried out experiments with ^{14}C-palmitic acid complexed to serum albumin and ^{14}C-palmitic acid incorporated into homologous chylo-microns. They showed that although the oxidation of the palmitic acid proceeded more rapidly when injected into

rats than did the artificial emulsions used by Lossow & Chaikoff (1955), the feeding of glucose to the rats reduced the oxidation of the palmitate to about 1/8th of the rate observed in fasting rats. These experiments have been interpreted as demonstrating a specific inhibition of fatty acid oxidation by glucose, and have led to the general use of the expression 'the fatty acid sparing effect of glucose'. The amounts of glucose fed to the rats in order to demonstrate this effect were very large and doubt was expressed by some workers as to the physiological significance of the results in relation to the conditions of normal nutrition. Morris (1958) injected chylomicrons labelled with ^{14}C-palmitic acid into fasted rats and found that the administration of isocaloric amounts of glucose orally or intravenously did not reduce the oxidation of the chylomicrons to anything like the same extent as reported by other authors. He concluded that the degree to which the oxidation of the fatty acids was spared was no greater than could be accounted for by the relative availability of the two substrates, and he further demonstrated that the oxidation of labelled chylomicrons was spared by unlabelled chylomicrons to the same extent as it was by glucose. Fredrickson & Gordon (1958b) found that the oxidation of long chain fatty acids complexed to serum albumin by humansubjects was decreased only by about 50%

when glucose was ingested. Lewis, Allen & Weinhouse (1959) fed fat and glucose to rats either alone or together. They found that the oxidation of ^{14}C-palmitic acid given by mouth was delayed by the simultaneous feeding of glucose, whilst fat had no such effect on the oxidation of fed ^{14}C-glucose. These authors found that the delay in the oxidation of the ^{14}C-palmitic acid when glucose was fed was due to a delay in the absorption of the fat from the gut. Following this period, they showed that the rate of oxidation of the ^{14}C-palmitic acid was increased. These authors also showed that the oxidation of fed ^{14}C-palmitic acid was not affected by the presence of adequate stores of glycogen in the animals' tissues, but the oxidation of previously assimilated ^{14}C-palmitic acid was reduced by the feeding of glucose. Thus it appears that the pronounced sparing of fatty acid oxidation which occurred when large amounts of glucose were fed could not be demonstrated with amounts of glucose which resembled more closely normal levels of intake.

The marked sparing of fatty acids in some of the *in vivo* experiments is also difficult to reconcile with the results of many *in vitro* experiments. Allen, Friedman & Weinhouse (1955) found that both endogenous and exogenous long chain fatty acids were oxidized *in vitro* by liver, heart, kidney and brain preparations

from fasted rats. This oxidation proceeded as readily in the presence of glucose as when no glucose was added, and these authors concluded that the glucose and fatty acids were not in competition for the available oxygen. Lossow, Brown & Chaikoff (1955) when trying to reconcile their results *in vivo* with those of Allen, Friedman & Weinhouse *in vitro*, found that the oxidation of endogenous ^{14}C-palmitic acid by liver preparations was not reduced by the addition of glucose to the incubation medium, but if the animals from which the livers were taken were fed with glucose before the liver was removed, the oxidation of the palmitic acid was reduced. They found that the addition of fructose to the incubation medium reduced the oxidation of the palmitic acid by the liver preparations by about 50% and concluded that the lack of effect of glucose when added to the incubation medium was due to the inability of the livers from fasted rats to utilize glucose.

Fritz & Kaplan (1960), Fritz (1960) and Fritz & Kaplan (1961) found that the oxidation of ^{14}C-palmitic acid, bound to albumin, by isolated muscle preparations was reduced by the addition of glucose to the incubation medium and this effect was enhanced by the simultaneous addition of insulin. The decreased rates of oxidation of ^{14}C-palmitic acid were not due to decreased rates of uptake of the palmitate by the muscle but were associated

with increased rates of esterification of the palmitate. The reduced oxidation of palmitic acid in these experiments was not as significant as that reported by Lossow & Chaikoff (1955).

Fritz (1961) when reviewing factors controlling the rates of oxidation of long chain fatty acids accepts the results of Lossow & Chaikoff (1955) as demonstrating a phenomenon of physiological importance. He apparently regards any evidence for the reduction of fatty acid oxidation by glucose, no matter how small, as confirming this phenomenon. Thus he cites the work of Morris (1958), Fredrickson & Gordon (1958b), Lewis, Allen & Weinhouse (1959) and his own experiments on the oxidation of palmitic acid by muscle, none of which could be interpreted as demonstrating a specific inhibition of fatty acid oxidation by glucose, as demonstrating the 'fatty acid sparing effect of glucose'. Fritz (1961) also dismisses those <u>in vitro</u> experiments which failed to demonstrate a sparing effect of glucose as 'violating what is known from other sources concerning the glucose-sparing action of long chain fatty acids <u>in vivo</u>'.

It thus appears that there is no agreement on the question of the relative significance of fat and carbohydrate oxidation in energy metabolism or on the circumstances which determine their metabolic inter-relationships.

Summary. Animals can oxidize large amounts of long chain fatty acids, and most tissues are able to participate in this oxidation. However fat is always oxidized together with other foodstuffs and the metabolism of the principal constituents of the food are closely interrelated. The inability of the body to store large amounts of carbohydrate leads to the prompt metabolism of this substance when it is ingested in large amounts. The main metabolic product from this excess carbohydrate is fat. In the tissues in which fat is synthesized, this synthesis may lead to a reduction in the oxidation of fatty acids. In terms of the overall metabolism of the body as measured in respiration studies, this obligatory metabolism of carbohydrate has lead to the conclusion that this substance is metabolized in preference to fat. The synthesis of fatty acids occurs principally in the liver and adipose tissue and the reduction in the oxidation of fatty acids due to the synthesis of large amounts of fat would be quantitatively most important in these tissues. However, when lipid is being synthesized, increased amounts of glucose are available to the tissues generally. The reduction in the oxidation of long chain fatty acids in tissues which are not concerned significantly in fatty acid synthesis would require that the glucose is available in greater amounts than the fatty acids, or the glucose is oxidized in preference to the fatty acids. The preferential oxidation of glucose over fatty acids has not been demonstrated conclusively.

CHAPTER TWO

MATERIALS AND METHODS

Experimental animals. Male rats of an albino strain bred in the laboratory colony, were used for all of the experiments described. Water and commercial 'rat nuts' were fed to the rats *ad libitum* until they were used in an experiment. The composition of the 'rat nuts' was as follows: protein 20%, carbohydrate 54% and fat 9%, (manufacturer's analysis). The rats that were used in the metabolism experiments were all carefully matched for age and weight. They weighed 220±5 g when taken from their food and were 10 weeks of age. The rats used for thoracic duct cannulation weighed between 200 and 250 g and those used for the isolated heart-lung preparations weighed between 250 and 280 g and were 12-13 weeks of age.

Anaesthesia. Ether, administered by open inhalation, was used as the anaesthetic for most operative procedures. In some experiments, pentobarbitone (Nembutal, Abbott) was used. At the end of the metabolism experiments, the animals were anaesthetized with pentobarbitone before being bled out from the abdominal aorta.

Antibiotics. Those used were crystalline penicillin G (Commonwealth Serum Laboratories) and streptomycin sulphate (Glaxo-Allenburys).

Anticoagulants. Sterile solutions of heparin (Pularin, Evans Medical Ltd., 1000 units/ml.) or dried purified extract of lung (Boots Pure Drug Co. Ltd., 63.8 units/mg.) were used.

Chemicals. All chemicals used were of either analytical or laboratory reagent grade.

Electrolyte solutions. Physiological saline contained 0.85 g NaCl/100 ml. of solution.

Kreb's bicarbonate Ringer solution. The composition of this solution was as follows: NaCl 705 mg, KCl 36 mg, $CaCl_2$ 14 mg, $MgSO_4:7H_2O$ 15 mg, KH_2PO_4 17 mg and $NaHCO_3$ 214 mg per 100 ml. of solution. The concentrations of calcium and magnesium were half those originally recommended by Krebs and Henseleit (1932) to allow for the proportion of these ions normally bound by plasma proteins (Greene & Power, 1931; McClean & Hastings, 1935). Concentrated stock solutions of the individual electrolytes were diluted and mixed immediately before use, and the solution equilibrated against 5% CO_2 in O_2 for 1 hr. Penicillin, 500,000 units/litre was added to the solution.

Radioactive carbon labelled compounds. $NaH^{14}CO_3$ (1 millicurie/m-mole), generally labelled ^{14}C-glucose (10 millicurie/m-mole), 1-^{14}C-palmitic acid (2 millicurie/m-mole) and 1-^{14}C-linoleic acid (2 millicurie/m-mole) were obtained from the Radiochemical Centre, Amersham.

1-^{14}C-oleic acid (2 millicurie/m-mole) was obtained from the BioRad Corporation, California. These compounds were stated to be more than 98% radiopure. A stock solution of $NaH^{14}CO_3$ (50μc/ml.) was kept frozen. The ^{14}C-glucose, which was bought as a freeze-dried powder, was dissolved in physiological saline to give a concentration of 5μc/ml. and frozen. The labelled fatty acids were dissolved in benzene and stored in the refrigerator. Before using the fatty acids the benzene was blown off with a stream of nitrogen and 25-50μc of the fatty acids was dissolved in each ml. of fat to be fed.

General methods

The oxidation of the glucose and fatty acids was studied in intact animals by continuously infusing the labelled substrates into the animals' blood stream and recording the excretion of $^{14}CO_2$ in their expired breath. The infusions were given through indwelling venous cannulae which had been inserted at least 12 hr before the experiment so as to ensure that the rats had recovered completely from any effects of the anaesthetic.

The intravenous infusions were given with a slow injection apparatus (C.F. Palmer, London) which drove interchangeable tuberculin syringes. The apparatus was modified so that two syringes could be used for simultaneous infusions. The syringes were calibrated both by measurement and by infusing fluid into a burette.

Collection of blood samples. In some experiments, repeated blood samples were obtained from rats through indwelling venous cannulae. The blood was collected either into syringes moistened with heparin solution and then transferred into haematocrit tubes or directly into lengths of capillary tubing which had been dusted with heparin powder. These tubes were sealed at one end with plasticine and then used as haematocrit tubes. In these experiments, defibrinated blood was injected into the rats after each sample to replace the blood removed. This defibrinated blood was usually obtained from a litter mate of the experimental animal. The blood was defibrinated by collecting it on to glass beads. It was then filtered to remove the fibrin clots and used within a few hours of collection. Blood plasma was separated from the red cells by centrifuging at 3000 r.p.m. for 30 min. When necessary the blood was centrifuged at 4°C.

Freeze drying of tissues. Tissues were removed from experimental animals and frozen initially in liquid air and then freeze-dried.

Histological methods. Tissues were fixed in formol-saline. Paraffin sections were stained with haematoxylin and eosin and frozen sections with Fett Rot (Ciba 7B) to demonstrate the presence of fat.

Statistical methods. Standard statistical procedures as described by Mather (1951) were used for

analyzing the experimental data. The various statistics calculated are defined as follows:

The mean $\bar{x}$ of a series of observations x_1, x_2, x_3 .... x_n is given by $\bar{x} = \frac{S(x)}{n}$ where $S(x)$ is the sum of all the values of x and n is the number of observations.

The variance of a single observation V_x is given by

$$V_x = \frac{S(x-\bar{x})^2}{n-1} \qquad (d.f. = n-1)$$

The standard deviation of a single observation s_x is given by $s_x = \sqrt{V_x}$ $(d.f. = n-1)$

The variance of the mean $V_{\bar{x}}$ is given by

$$V_{\bar{x}} = \frac{V_x}{n} \qquad (d.f. = n-1)$$

The standard deviation of the mean (standard error) $s_{\bar{x}}$ is given by $s_{\bar{x}} = \sqrt{V_{\bar{x}}}$ $(d.f. = n-1)$

The variance of the difference between two means V_d $(d = \bar{x}_1 - \bar{x}_2)$ when $\bar{x}_1$ and $\bar{x}_2$ were calculated from independent observations is given by

$$V_d = V_{\bar{x}_1} + V_{\bar{x}_2} \qquad (d.f. = (n_1-1)+(n_2-1))$$

and the standard error of the difference between two means is given by $s_d = \sqrt{V_d}$ $(d.f. = (n_1-1)+(n_2-1))$

Linear regression analysis. When n pairs of paired observations x_1y_1, x_2y_2, x_3y_3 . . . . . x_ny_n were made, the relationship between x and y was calculated by finding the values of a and b in the equation

$$Y = a + b(x-\bar{x})$$

such that the variation of y about the line represented by this equation is minimal. x is considered the independent variate and y the dependent variate, and b is the coefficient of linear regression of y on x. The values of a and b were calculated by the method of least squares.

$$a = \bar{y} \qquad (d.f. = n-2)$$

$$b = \frac{Sy(x-\bar{x})}{S(x-\bar{x})^2} \qquad (d.f. = n-2)$$

The variance of y V_y is given by

$$V_y = \frac{1}{n-2}\left[Sy^2 - \frac{S^2y}{n} - \frac{S^2[y(x-\bar{x})]}{S(x-\bar{x})^2}\right] \qquad (d.f. = n-2)$$

The variances of a and b V_a and V_b are given by

$$V_a = V_{\bar{y}} = \frac{V_y}{n} \qquad (d.f. = n-2)$$

$$V_b = \frac{V_y}{S(x-\bar{x})^2} \qquad (d.f. = n-2)$$

The variance of the difference between two regression coefficients V_d $(d = b_1 - b_2)$ is given by

$$V_d = V_{b_1} - V_{b_2} \qquad (d.f. = (n_1-2)+(n_2-2))$$

The standard errors of a, b and d were calculated as the square roots of their respective variances.

Tests of significance. The significance of the various statistics calculated from experimental data was tested by the null hypothesis using a 't' test. The probability

of finding a 't' at least as large by chance for the number of degrees of freedom from which it was calculated was determined from standard tables of 't'. The value of the 't' was calculated for each statistic as the ratio of the statistic to its standard error.

Analysis of variance. When the total sum of squared deviations of a series of observations from their mean could be partitioned into independent sums of squares attributable to items of interest, the significance of an item was tested by comparing the mean square of the item to that of the residual or error mean square. The mean square of an item was calculated by dividing the sum of squares attributable to the item, by its number of degrees of freedom. The significance of the item was tested by the null hypothesis to determine if the degrees of freedom removed by the item removed a proportionately greater amount of variation from the total than the degrees of freedom attributable to error variation, within the limits of random sampling.

Detailed methods

Cannulation of the right external jugular vein.

This operation was carried out at least 12 hr before an experiment. The rat was anaesthetized with ether and the right external jugular vein was exposed. Two silk threads were placed around the vein. The cranial thread was used to tie the vein off while the caudal thread was

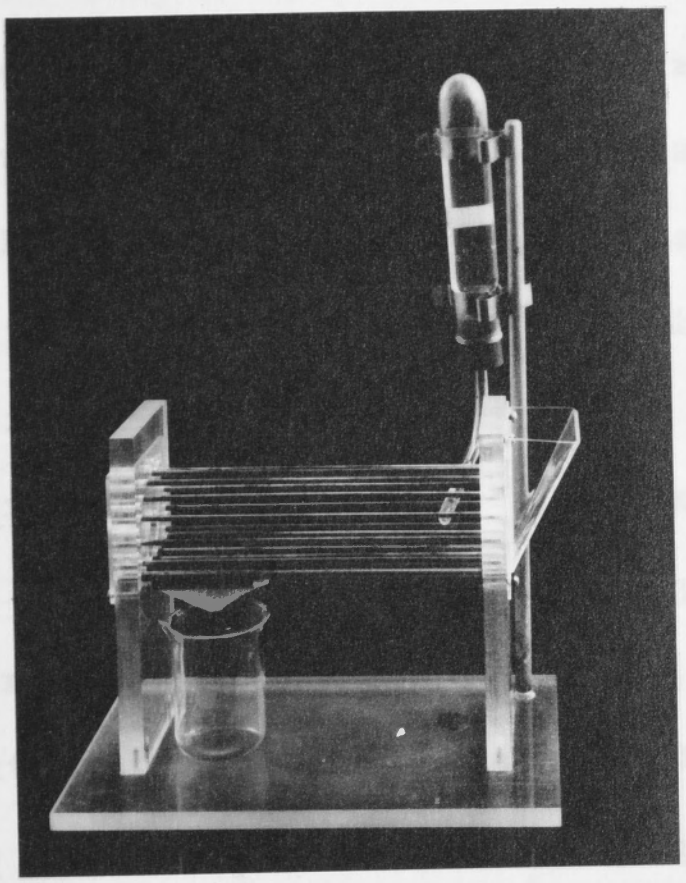


FIGURE 1.

The type of restraining cage used to accustom rats to conditions during an experiment. These cages were also used to confine rats whose thoracic ducts had been cannulated.

used to occlude the vein when it was cut. A length of Intramedic PE10 polyethylene tubing (Clay Adams, N.Y.), filled with physiological saline, was inserted into the vein and passed through the caudal tie towards the heart. Blood was drawn back into the cannula to test its patency and then the cannula was tied in place with the caudal tie and led out subcutaneously through a small stab incision in the dorsal surface of the neck. The wound was dusted with penicillin powder and then sutured. The cannula was tied along the back and the animal returned to a restraining cage. The end of the cannula was sealed by heating. In experiments in which repeated blood samples were required during the course of an infusion, or when simultaneous infusions were to be given, two cannulae were inserted into the one vein. The cannula used for obtaining blood samples was inserted into the heart.

Cannulation of the thoracic duct. Rat's thoracic ducts were cannulated by a method modified from that described by Bollman, Cain & Grindlay (1948). Under ether anaesthesia the peritoneal cavity was opened on the left side by an incision immediately behind the last rib extending along its length. The liver and stomach were packed off with swabs and the thoracic duct was exposed as it lay dorsal to the aorta. The duct was tied off as far cranially as possible between the crura

of the diaphragm. A tie was placed around the duct a little behind the first tie. The surface of the duct between these two ties was cleaned and a No. 1 polythene (Glaxo-Allenburys) cannula which had been bent through 180° in the shape of a 'U', was inserted into the duct and tied in place. Care was taken not to occlude a large branch of the intestinal lymphatic by passing the cannula too far caudally. The cannula was led to the exterior through a stab incision in the back behind the left kidney. A No. 2 polythene (Glaxo-Allenburys) tube, the end of which was protected with a short length of rubber tubing, was inserted into the stomach and tied in place with a purse-string suture. The gastrostomy tube was taken to the exterior through the abdominal incision. The peritoneal cavity was dusted with penicillin and streptomycin and the abdomen closed with two layers of silk sutures. The rat was placed in a restraining cage and allowed to recover. It was given 0.45% NaCl solution to drink and rat nuts to eat ad libitum.

Collection and preparation of chylomicrons. On the day after the operation, rats with thoracic duct fistulae were given a feed of 0.5-1.0 ml. of fat through the gastrostomy tube. The resulting fatty chyle was collected during the period of fat absorption into vessels containing penicillin. No anticoagulants were used. Radioactive chylomicrons were collected by

dissolving the required ^{14}C-labelled fatty acid in the fat before feeding it to the rats. The chyle was centrifuged on two occasions to remove the clots that formed.

The chylomicrons were separated from the smaller lipoprotein complexes and unesterified fatty acids bound to albumin by centrifuging the chyle at 20,000 x g for 30 min in a Beckman-Spinco Model L Ultracentrifuge. The chylomicrons separated as a cream-like layer on the top of the tubes. The lower clear fraction was removed with a syringe and long needle and the packed chylomicrons were redispersed in physiological saline or Kreb's bicarbonate Ringer solution. The concentration of the total esterified fatty acids in these preparations was measured, and the preparations diluted to provide the right concentration for the infusions. In any series of experiments, the same amount of radioactivity was infused at all rates of infusion of total lipid. The rate of infusion of total lipid was increased by adding unlabelled chylomicrons to the labelled ones.

Preparation of mixed glycerides suitable for feeding by gastrostomy tube. In order to obtain chylomicrons consisting predominantly of palmitic, oleic and linoleic acids, a sample of fat was prepared by transesterification in the presence of an acid catalyst. A simple mixture of triglycerides was unsatisfactory because the physical

characteristics of the tripalmitin precluded it being fed by gastrostomy tube and because such a mixture was poorly absorbed (cf. Deuel & Hallman, 1940). Tripalmitin, triolein and trilinolein, in amounts calculated to yield fat of the desired composition, were heated together with concentrated H_2SO_4 (1 ml./g fat) at 80°C for 8 hr. The resulting dark reaction mixture was dissolved in hexane and washed several times with 5% $NaHCO_3$ solution. The hexane solution was dried over anhydrous Na_2SO_4 and then applied to an untreated column of Florisil (Floridin Co., Fla.) at the rate of 1 g fat per 4 g of Florisil. The triglycerides were eluted with 15% diethyl ether in hexane (v:v) until the eluent contained no further lipid. The ether-hexane was removed under reduced pressure and a yellow fat was obtained which melted below body temperature. A yield in excess of 80% of theoretical was obtained. The colored decomposition products were adsorbed firmly onto the Florisil and could not be eluted until more polar solvents were used.

Isolated heart-lung preparation. This preparation was devised to study the oxidation of chylomicron fatty acids by the isolated working heart, autoperfused through its own coronary vessels with its own unaltered blood in the absence of heparin or any anticoagulant. The preparation was based on the observation that if all the

systemic blood vessels were tied off at the heart, leaving the coronary and pulmonary circulations intact, the heart would continue beating, provided the blood volume of the preparation was appropriate. The blood was pumped from the left heart into the aortic stump at arterial pressure. It then passed from the aortic stump through the coronary vessels to the right heart and from there it was pumped through the pulmonary circulation back to the left heart. As the blood did not come in contact with any foreign surfaces, it did not clot and no anticoagulants were required. A simple procedure was developed for establishing the preparation.

The rat was anaesthetized with ether and a long midline incision was made from the jaw to the pelvis. The trachea was dissected and left accessible. The abdomen was opened and the abdominal aorta was cut. A stopwatch was started to check the times taken for the following procedures. The ventral half of the thoracic cage was removed rapidly and a clamp placed on the aorta about $\frac{1}{4}$ in. from the base of the heart which was quite flaccid but still beating. The heart soon pumped sufficient blood into the aortic stump to restore the coronary circulation. When this occurred, the heart's action again became vigorous, but the heart and blood were very dark in color. The trachea was then cannulated and the lungs inflated several times with 5% CO_2 in O_2. The color

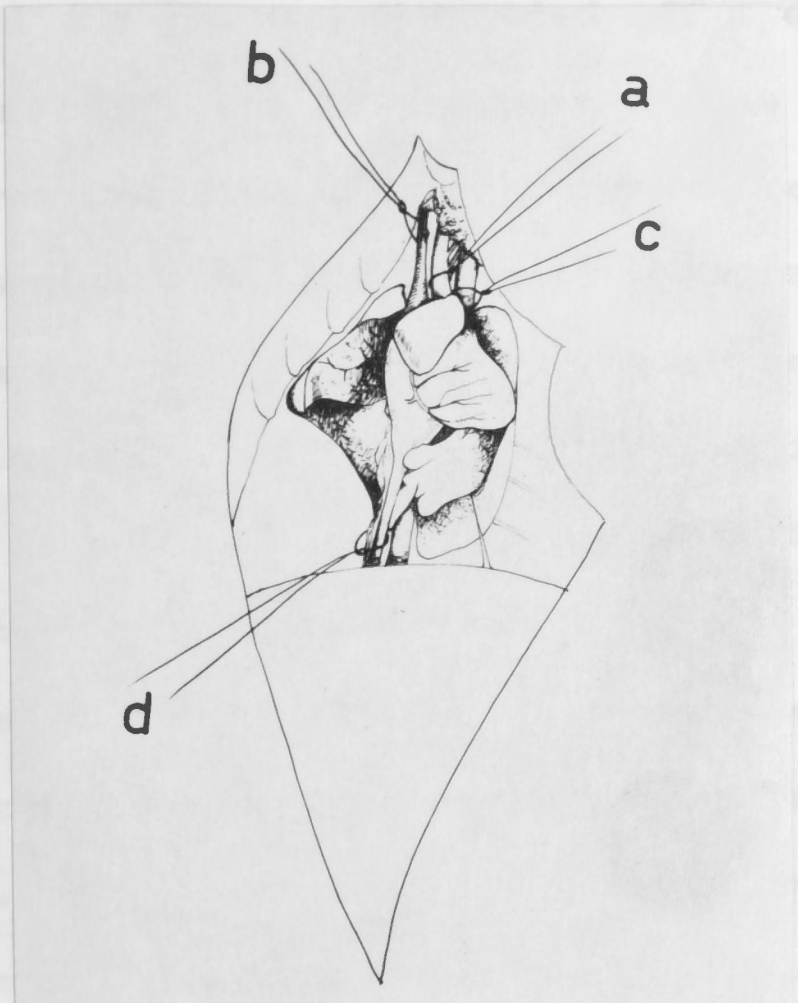


FIGURE 2.

Dissection of a rat's thorax to demonstrate the vessels which must be tied to isolate the heart and lungs. Ties are shown placed around the aorta (a), right and left superior venae cavae (b, c) and inferior vena cava (d).

of the heart and lungs changed dramatically as the blood became fully oxygenated. The lungs were ventilated intermittently throughout the rest of the operative procedure. The aorta was then tied off and the clamp removed. The right and left superior vena cavae were dissected free and tied off; care was taken to tie the vena azygous with the left superior vena cava. A tie was placed around the inferior vena cava and a polyethylene cannula (PE10 Intramedic) was inserted into it and tied in place. The infusion of metabolites into the preparation was started immediately.

The heart and lungs were then removed from the rat and placed in a special organ chamber made from an epoxy resin. The cavity of the chamber was cast in the shape of the rat's thoracic cage. The trachea was tied onto the tracheal cannula and the infusion cannula was led out through a seal in the chamber. The chamber was closed and filled with bicarbonate Ringer solution which had been heated to 38°C. The lungs floated towards the top of the chamber and the heart hung freely between them. The chamber was washed through with several volumes of warm electrolyte solution and the lungs were then inflated by closing the fluid outlet of the chamber and withdrawing 5 cc. of fluid from the chamber. The lungs were then passively ventilated by pumping fluid into and out of the chamber with a reciprocating syringe. A stroke

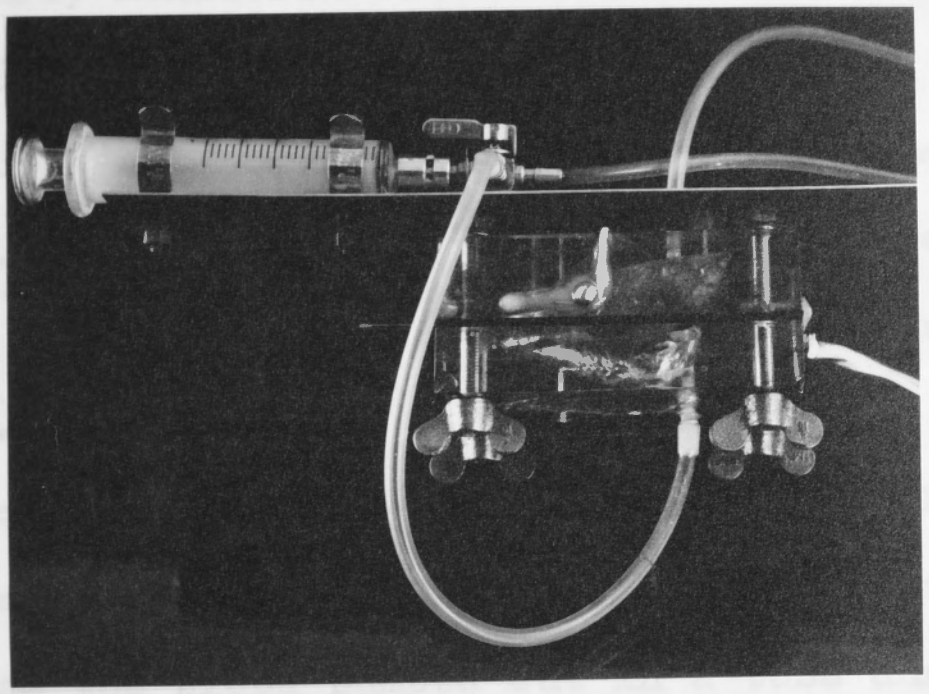


FIGURE 3.

The organ chamber used for experiments with the isolated rat heart-lung preparation. The chamber was made of an epoxy resin and cast in two halves which were held together by screws. The syringe shown was used to wash the organs with pre-warmed electrolyte solution, and to inflate the lungs initially.

volume of 1 cc. and a rate of 60/min were used. The respiratory gas was 5% CO_2 in O_2. The organ chamber which is shown in Figure 3, was placed in a water bath maintained at 38°C.

Platinum electrodes were cast into the walls of the organ chamber and the electrocardiogram of the heart continuously monitored with a cathode ray oscilloscope (New Electronic Products, U.K.) and recorded at intervals with a photographic recording camera (New Electronic Products, U.K.). The disposition of the organs and the relative position of the E.C.G. electrodes are shown in Figure 4.

Blood samples were collected from the heart at the end of an experiment by cutting the aortic stump and collecting the blood pumped out in a tube dusted with heparin powder.

Analytical methods

Extraction of lipids. The lipids in small volumes of chyle or plasma were extracted with 10 ml. boiling ethanol-ether (3:1, v:v). The precipitated protein was filtered off and the solvent removed under reduced pressure. Larger volumes of chyle were extracted with chloroform-methanol (2:1, v:v) by the method of Korn (1959). One ml. of chyle was vigorously mixed with 25 ml. of the chloroform-methanol and allowed to stand for 30 min.

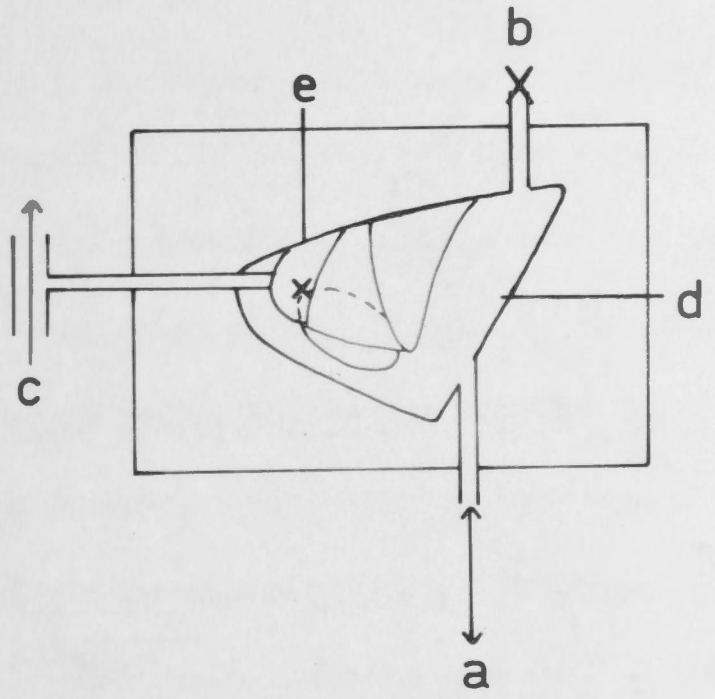


FIGURE 4.

The disposition of the heart and lungs in the organ chamber. The lungs were ventilated by pumping fluid into and out of the chamber at (a). The other fluid outlet (b) was closed during an experiment. 5% CO_2 in O_2 was pumped past the tracheal cannula at (c). The active E.C.G. lead was taken from the electrode (d) and the neutral lead was connected through 5000 ohm resistors to two electrodes one on either side of the chamber at the position marked (x). The preparation was earthed at (e).

Four ml. of water were then added and the two phases allowed to separate overnight. The methanol-water phase was sucked off and the lower chloroform phase, which contained all the lipid, was taken to dryness under reduced pressure. The lipid residue was extracted with petroleum ether and the solvent removed under reduced pressure.

Finely ground tissue was extracted twice with boiling chloroform-methanol (2:1, v:v). The combined extracts were filtered and an equal volume of 2% KH_2PO_4 solution (Olivecrona, 1962a) was added and the two phases were allowed to separate. The lower chloroform phase was removed and dried over anhydrous Na_2SO_4 and then the solvent was removed under reduced pressure. Some samples of tissue were extracted with boiling ethanol-ether (3:1, v:v). The extract was filtered and the solvent removed under reduced pressure. The residue was extracted with petroleum ether. The lipid extracted from samples of tissue was dissolved in a measured volume of chloroform or petroleum ether. Samples of these solutions were pipetted into tared glass shells and the solvent evaporated under an infra-red lamp. The lipid was then weighed.

Protein-free filtrates of blood. Whole blood or plasma was deproteinized with $Ba(OH)_2$ and $ZnSO_4$ by the method of Nelson (1944). The mixture was centrifuged and

the clear supernatant filtered.

Isolation of plasma glucose as potassium gluconate.

Blood glucose was isolated for radioassay as potassium gluconate by the method of Blair & Segal (1960). A weighed quantity of carrier glucose was added to a sample of a protein-free filtrate of the blood. The glucose concentration in another sample of the filtrate was measured. The filtrate was taken to dryness and extracted with hot 90% methanol. The methanol extract was reduced to a thick syrup and re-extracted with a small volume of 90% methanol. This was transferred to a small flask and for each 100 mg of glucose to be oxidized, 280 mg iodine dissolved in 4 ml of absolute methanol was added. The flask was placed in a water bath at 40°C and 5.5 ml. of 4% KOH in absolute methanol per 100 mg of glucose was added dropwise with constant stirring. The flask was then set aside at room temperature for 40 min to allow the complete precipitation of the potassium gluconate. The precipitate was filtered on a small filter stick, washed with absolute methanol and dried on the filter stick with acetone. The potassium gluconate was recrystallized once by dissolving it in a small volume of water and adding 30 ml. of hot absolute methanol. Diethyl-ether was added dropwise until a faint cloudiness appeared. The flask was then put in a refrigerator for 30 min for the precipitation to be

completed. The potassium gluconate was filtered, washed and dried as before. The fine white needle-like crystals melted at 177°C; this was within the temperature range given by Blair and Segal (1960). Elemental analysis for hydrogen and carbon gave figures which agreed with the theoretical composition. The specific activity of the blood glucose was calculated from that of the potassium gluconate by the following equation

$$\text{Specific activity blood glucose} = \frac{\text{molecular weight potassium gluconate}}{\text{molecular weight of glucose}} \times \frac{\text{weight of carrier glucose + blood glucose}}{\text{weight of blood glucose}} \times \text{specific activity of potassium gluconate.}$$

Isolation of glucose by paper chromatography.

One sample of radioactive glucose did not appear to be radiopure. The glucose in this preparation was isolated on paper using an N-isopropanol:ethyl acetate:water (7:1:2, v:v:v) descending solvent system as described by Depocas (1959) for the chromatographic separation of plasma glucose directly from plasma. Whatman No. 1 chromatography paper, cut into 8 in. strips was used. Four spots, 2 in. apart were applied at the origin. Each spot consisted of 0.02 ml of carrier glucose solution (20 g/ml.) and 0.02 ml. of radioactive glucose

solution (5 μc/ml.). The spots were dried by gently heating the paper under an infra-red lamp and the paper was then placed in the chromatography jar. The atmosphere was equilibrated for 1 hr with the solvent system and the chromatograms were then developed for 12 hr. The papers were dried in a fume cupboard. The glucose in the two 2 in. side strips was located with an aniline phthalate reagent (Partridge, 1949). The carrier glucose moved as a single spot which was completely contained in a 2 in. square. Corresponding 2 in. squares were cut for the two centre strips and the glucose was eluted with 5 ml. of water. Blank 2 in. squares of paper, and 2 in. squares which had been spotted as the origin were eluted with 5 ml. of water. Samples of the eluates were used for the determination of glucose and for radioassay. The blank determination was subtracted from the glucose measurements. The glucose was found to have moved 7 in. in 12 hr and 94% of the glucose applied to the origin was recovered from the glucose spot. The specific activity of the glucose recovered from the glucose spot was 80% of that applied to the origin.

Separation of lipid extracts into neutral lipid-unesterified fatty acid and phospholipid fractions by silicic acid column chromatography. The procedure described by Borgström (1952a) was used to separate the lipid extracts. Silicic acid (Baker, 100 mesh) was

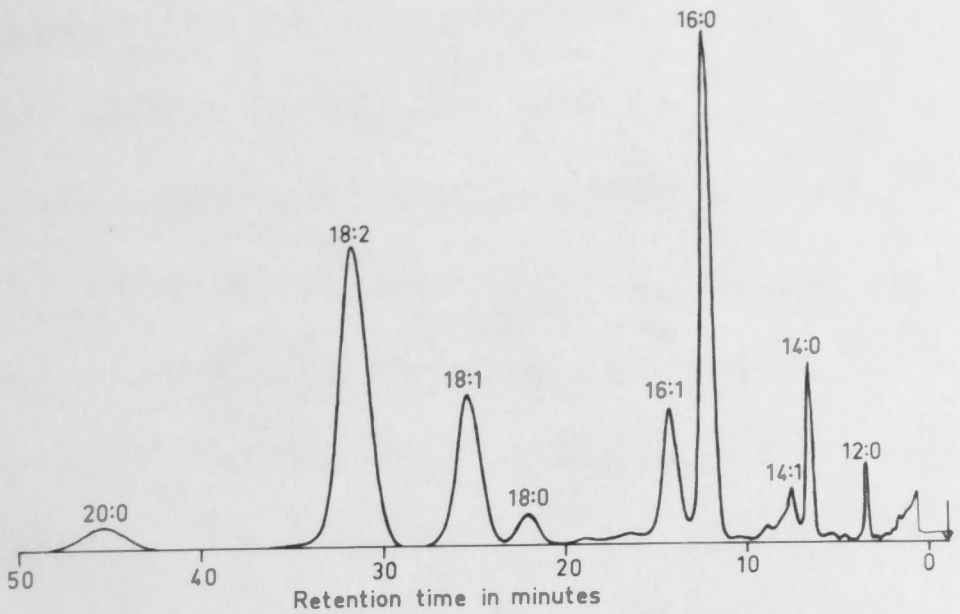
activated in an oven at 110°C overnight. One gram of the activated silicic acid was slurried with chloroform into a 1 cm diam. chromatography tube fitted with a sintered glass disc at the bottom on which rested a circle of filter paper. The sample of lipid dissolved in about 5 ml. of chloroform was applied to the top of the column. The neutral lipid-free fatty acid fraction was eluted with 30 ml. of chloroform and the phospholipid fraction with 30 ml. of methanol. Recovery of radioactive lipid from these columns was in excess of 95% of that applied and no radioactivity could be detected in the final portion of either fraction. When the free fatty acids were separated from the neutral lipids, the chloroform from the first fraction was removed under reduced pressure and the lipids redissolved in petroleum ether. The free fatty acids were separated by partitioning between alkaline ethanol and petroleum ether (Borgström, 1952b).

Silicic acid thin layer chromatography. Lipid extracts were screened by separating them on thin layers of silicic acid on microscope slides. A slurry of silicic acid was spread on the microscope slide in essentially the same way a blood smear is prepared, and the thin layer dried in an oven. Two samples of lipid could be chromatographed on one slide. These thin layers were found particularly useful for detecting the presence of

triglycerides and unesterified fatty acids, and plasma samples could be applied directly to the silicic acid without prior extraction of the lipids. The solvent system used was petroleum ether:diethyl ether:glacial acetic acid (90:10:1, v:v:v) and the chromatograms were developed by ascending chromatography in Coplin jars. The lipid fractions were located with iodine vapor. With this solvent system the phospholipids remain at the origin and the other lipids move at rates increasing in the order partial glycerides, free cholesterol, unesterified fatty acids, triglycerides and cholesterol esters.

Gas liquid chromatography. The fatty acid composition of the chylomicron preparations and the various fats used was determined by gas liquid chromatography.

Preparation of methyl esters. Methyl esters of the fatty acids were prepared in the following way. Chyle containing approximately 50 mg of total esterified fatty acids was extracted with chloroform-methanol. The extracted fat was hydrolyzed by refluxing with 10 ml. of a 2% solution of KOH in ethanol for 1-2 hr. The ethanol was removed under reduced pressure and the products of hydrolysis dissolved in 10 ml. of water. Any unsaponifiable lipid soluble material was removed by washing twice with hexane. The reaction mixture was acidified to methyl orange and the fatty acids extracted



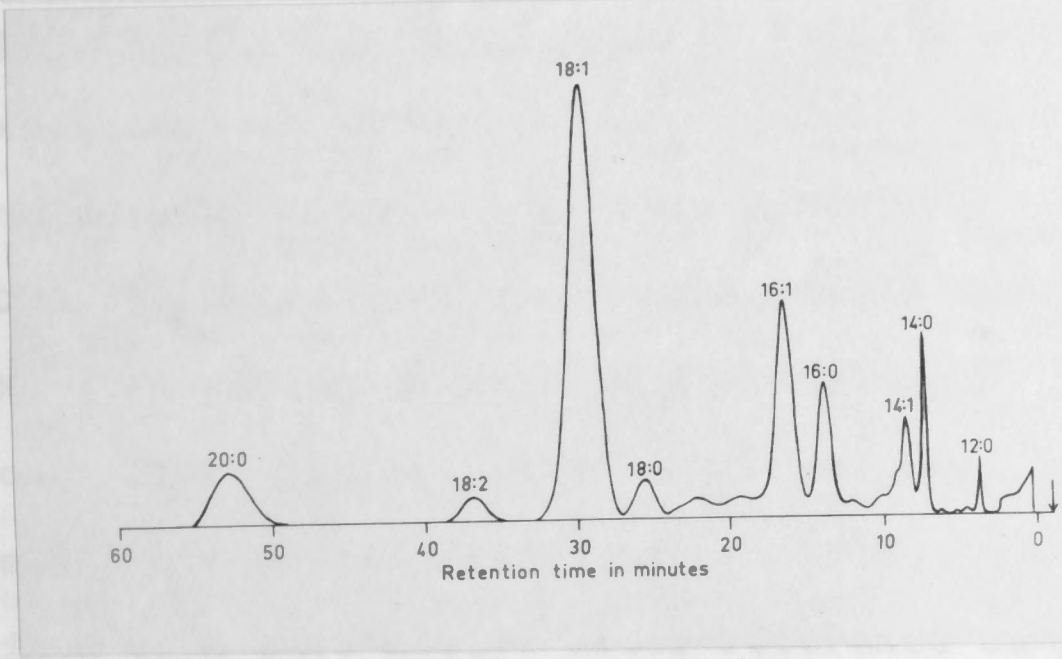


FIGURE 5.

Gas chromatograms of the methyl esters of fatty acids obtained from two chylomicron preparations. The differences between the retention times of the corresponding methyl esters on the two chromatograms were due to a change in the characteristics of the column between the two analyses. In both chromatograms the logarithm of the retention time of the members of an homologous series of fatty acid esters was proportional to the fatty acid chain length. The stationary phase in the column used for these chromatograms was a succinate polyester of diethylene glycol and nitrogen was used as the carrier gas.

into hexane. The hexane solution of fatty acids was dried over anhydrous Na_2SO_4 for 24 hr. and the solvent then removed under reduced pressure. The fatty acids were methylated by refluxing with 10 ml. of BF_3 in methanol for 10 min. An equal volume of water was added and the methyl esters were extracted into hexane. The hexane solution was dried over Na_2SO_4 and then concentrated under a stream of nitrogen.

Chromatography of methyl esters. The mixtures of methyl esters dissolved in hexane were analyzed on a gas chromatography apparatus (Perkin Elmer Vapor Fractometer Model 154D) using a column packed with a succinate polyester of diethylene glycol as the stationary phase. A hydrogen flame ionization detector was used to record the peaks which were identified by comparison with those obtained with pure methyl ester reference compounds (Applied Science Labs., Pasadena). Nitrogen was used as the carrier gas and the column was operated at a temperature of about 190°C. Two representative chromatograms are shown in Figure 5. The methyl esters emerged in order of increasing chain length, but as the stationary phase was polar, unsaturated fatty acid esters emerged after the esters of saturated fatty acids of the same chain length. As the retention times of the methyl ester of a 20 carbon saturated fatty acid and methyl linolenate were similar on this column, the $C_{20:0}$ ester

was positively identified by using an Apiezon L grease Golay capillary column. This was a non-polar column and the methyl esters of unsaturated fatty acids of a given chain length emerged before the saturated fatty acids of the same chain length. The areas of the peaks were measured with an automatic printing integrator and the relative concentrations of the esters determined.

Biochemical analyses

Determination of tissue glycogen. Tissue glycogen was isolated by the method of Good, Kramer & Somogyi (1933). The tissue was digested by heating with 30% KOH solution for 30 min on a boiling water bath. The solution was cooled and the glycogen precipitated by adding 95% ethanol to give a final concentration of 50% ethanol. The mixture was heated to boiling and then allowed to cool. The mixture was centrifuged and the glycogen-free supernatant fluid was decanted. The glycogen precipitate was hydrolyzed by heating with 0.6N-HCl for $2\frac{1}{2}$ hr on a boiling water bath. The hydrolysate was cooled, neutralized to methyl orange with NaOH solution and then made up to a volume such that the glucose concentration was suitable for determination; 1 ml. samples of this dilution were taken for glucose determination.

Determination of glucose. The glucose in protein-free filtrates of whole blood or plasma, eluates from chromatograms or hydrolysates of glycogen was determined

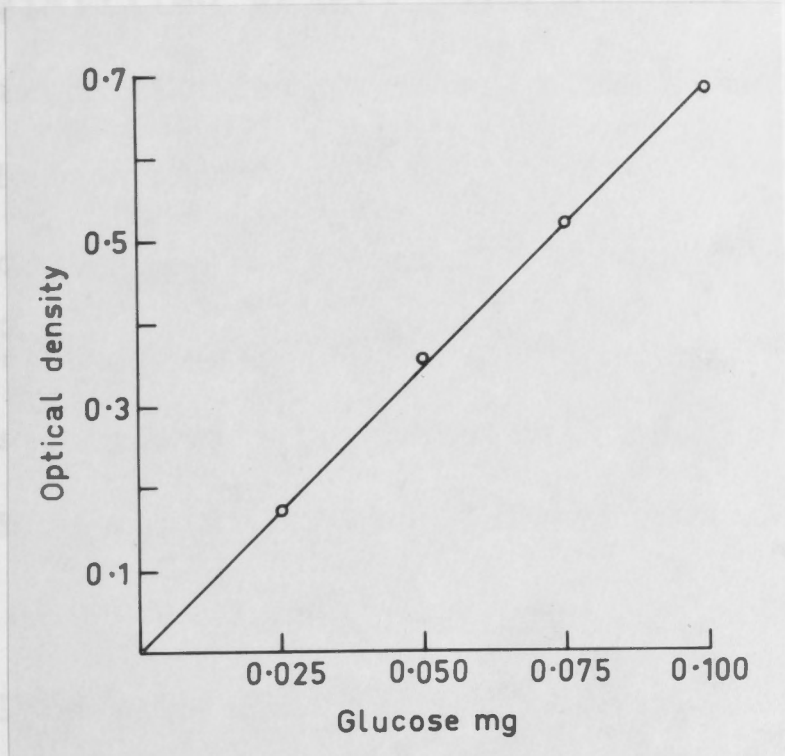


FIGURE 6.

Calibration line for the estimation of glucose by the method of Nelson (1944). Wavelength 660mμ.

by the colorimetric method of Nelson (1944). One ml. of the solution was pipetted into a Folin Wu tube; 1 ml. of alkaline copper reagent was added and the mixture heated on a boiling water bath for 20 min. Arsenomolybdate reagent was added and the reaction mixture made up to volume with distilled water. The optical density of the reaction mixture was measured at a wave length of 660 mμ. In each batch of determinations a reagent blank and a glucose standard were included. Glucose standards containing 0.025, 0.050, 0.075 and 0.100 mg/ml. were prepared in saturated benzoic acid. A standard curve obtained with 1 ml. of each of these standards is shown in Figure 6.

Determination of total esterified fatty acids. The concentration of esterified fatty acids in chylomicron preparations and in plasma was measured by the colorimetric method of Stern and Shapiro (1953). Samples of plasma (0.2 ml.) and chylomicron preparations (0.02 ml.) were extracted with 10 ml. of boiling ethanol-ether (3:1, v:v). The precipitated proteins were removed by filtration and 3 ml. of the lipid extracts were reacted with hydroxylamine hydrochloride and NaOH for 20 minutes. The mixture was then acidified with HCl and the color developed by adding $FeCl_3$. The optical density of the reaction mixture was measured at a wavelength of 520 mμ. A standard curve for the color reaction with triacetin

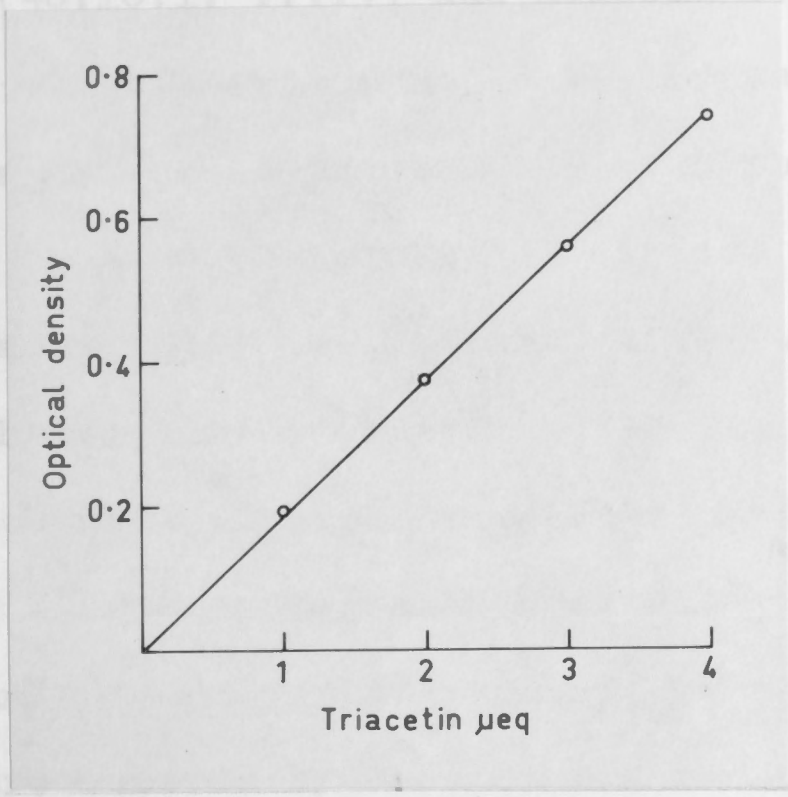


FIGURE 7.

Calibration line for the estimation of total esterified fatty acids by the method of Stern & Shapiro (1953). Wavelength 520 mμ.

is shown in Figure 7.

Determination of plasma unesterified fatty acids. Plasma unesterified fatty acids were determined by the titrimetric method of Dole (1956). One ml. of plasma was mixed with 5 ml. of a mixture of isopropanol, heptane and N-H_2SO_4 (40:10:1, v:v:v) and allowed to stand for 10 min. Two ml. of heptane and 3 ml. of water were then added and the mixture separated into two phases. One ml. of the upper heptane phase which contained all the unesterified fatty acids was titrated with 0.01N-NaOH using 1 ml. of a thymol blue indicator. The two phases were mixed with a stream of nitrogen bubbled into a titration vessel. One titration vessel was used for all the titrations in a series; the contents of the previous titration were removed by suction and the indicator added. This was titrated to the end point with the alkali before adding the heptane extract and the indicator again titrated to the end point. Some heptane was found to give a high blank titration and this was always checked. The procedure was tested by titrating a standard solution of palmitic acid both directly and after it was put through the extraction procedure. Both methods gave identical titrations.

Determination of plasma ketone bodies. The ketone bodies in plasma were determined as their acetone equivalent in protein-free sugar-free filtrates of the

plasma by the method of Werk, McPherson, Hamrick, Myers and Engel (1955). One ml. of plasma was mixed with 5 ml. of H_2O, 2 ml. of 2.5% $ZnSO_4$ solution, 1 ml. of 0.3N-$Ba(OH)_2$ solution, 2 ml. of 20% $CuSO_4$ solution and 2 ml. of a 10% $Ca(OH)_2$ suspension. The mixture was centrifuged and 2 ml. of the clear supernatant taken for analysis. All of the following procedures were carried out in tightly stoppered tubes to prevent the loss of the volatile ketones. The acetoacetate was decarboxylated by heating with H_2SO_4 in an oil bath at 110-120°C. Potassium dichromate was then added and the mixture heated at the same temperature to convert the β-hydroxybutyrate to acetone. The excess dichromate was destroyed by adding sodium sulphite and the amount of acetone in the reaction mixture was determined by adding 2,4 dinitrophenylhydrazine. The color developed was extracted into CCl_4. The aqueous phase was removed and the CCl_4 washed twice with distilled water and then shaken with NaOH solution. The optical density of the CCl_4 extract was then determined at a wavelength of 420 mμ. A standard curve was constructed by measuring the color developed with known amounts of acetone and is shown in Figure 8. Good duplicates could not be obtained with this method, however it was sufficiently accurate to demonstrate differences in plasma ketone levels of the order of magnitude encountered in some of the experimental treatments.

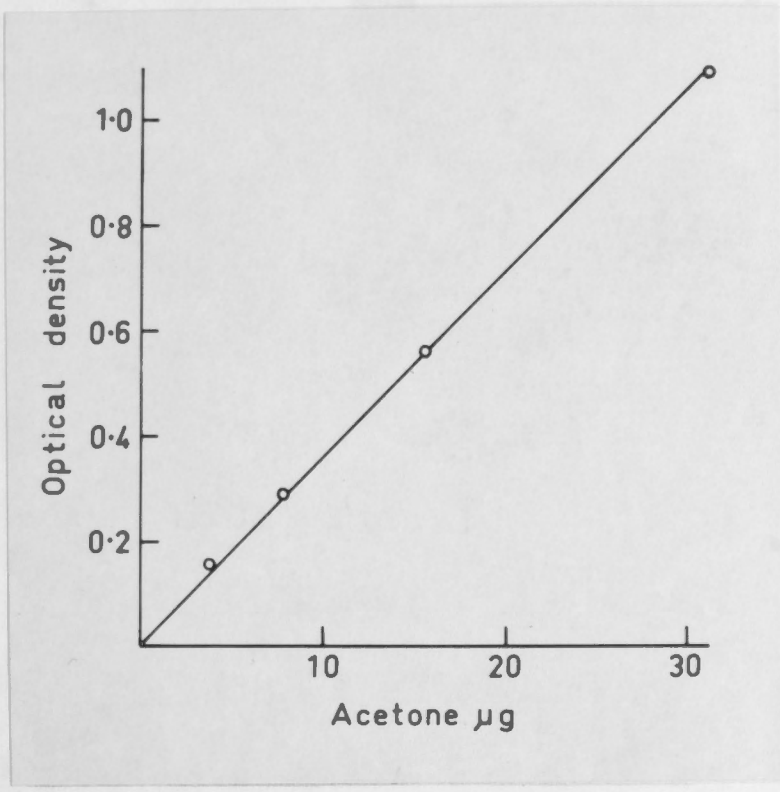


FIGURE 8.

Calibration line for the estimation of acetone by the method of Werk, McPherson, Hamrick, Myers & Engel (1955). Wavelength 420 mµ.

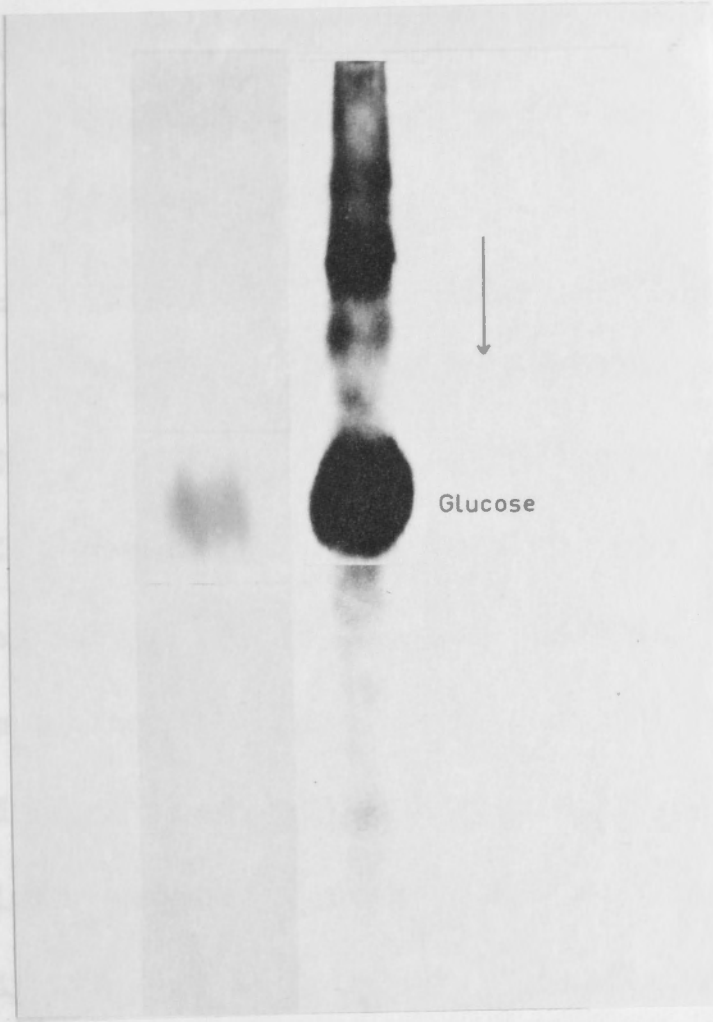


FIGURE 9.

A paper chromatogram of a sample of radioactive glucose and carrier glucose (left) and an auto-radiograph prepared from it (right). The carrier glucose has been stained with aniline phthalate.

Radioisotope methods

Autoradiographs. The presence of radioactivity on paper chromatograms was detected by exposing a non-screen X-ray film in close contact with the chromatograms. A paper chromatogram and an autoradiograph prepared from it is shown in Figure 9.

Solid counting of ^{14}C radioactivity

Assay of ^{14}C in $BaCO_3$. Radioactive CO_2 was counted as $BaCO_3$. The CO_2, as Na_2CO_3, was precipitated with an excess of $BaCl_2$ in a special filtration apparatus. A perforated polyethylene planchet fitted with a circular disc of Whatman No. 54 filter paper was placed in the apparatus and the precipitated $BaCO_3$ was allowed to settle on to the filter paper disc. Suction was then applied and the precipitate was washed successively with distilled water, acetone:water (1:1, v:v) and acetone. The planchets were dried under an infra-red lamp and the weight of the $BaCO_3$ determined. The radioactivity of the $BaCO_3$ was measured with an ultra-thin end-window G-M tube and scaler. Either a Philips manually operated counter or a Tracerlab Multimatic counter with automatic sample changer and scaler was used. To determine the effects of the sample mass on the counting rate, increasing amounts of $BaCO_3$ of constant specific activity were plated and a self-absorption curve was constructed for each counter used. These self-absorption curves are

shown in Figure 10. The regression lines calculated from these data were used to correct observed counting rates to counting rates at zero mass. The planchets used for the $BaCO_3$ mounts were 2 cm^2 in area.

Assay of ^{14}C in 'infinitely thin' solid mounts. Most of the samples of lipid could be counted as virtually infinitely thin preparations and self-absorption was disregarded. Sample weights of less than 1 mg cm^{-2} were regarded as being infinitely thin. Lipid extracts dissolved in petroleum ether or chloroform were pipetted directly on to lens tissues on polyethylene planchets 2 cm^2 in area or else directly on to stainless steel planchets 3.142 cm^2 in area. The solvents in which the lipids were dissolved, were evaporated carefully under an infra-red lamp. It was important not to put too large a volume of solvent on the planchets as with heating, the solvent tended to 'creep' up the sides of the planchets and this introduced errors into the counting geometry, and loss of radioactivity from the planchet. Small volumes of the chylomicron preparations were also plated directly on to the planchets as described. Water was added to the chylomicrons to spread them over the surface of the planchets before drying under an infra-red lamp.

Assay of ^{14}C in 'infinitely thick' layers. Some of the samples of lipid extracted from adipose tissue had very

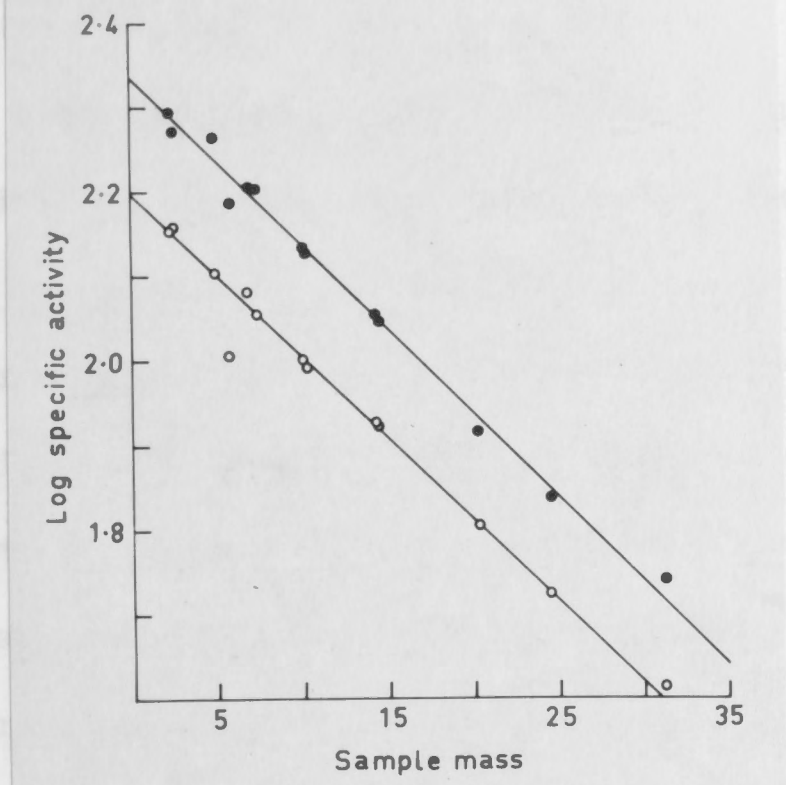


FIGURE 10.

$BaCO_3$ self-absorption curves for Philips (O) and Tracerlab (●) G-M counters. The regression lines calculated from these data to correct observed specific activities for sample self-absorption were

$$\text{Log}\ \frac{\text{S.A at zero mass}}{\text{S.A observed}} = 0.0189X \qquad \text{(Philips)}$$

$$\text{Log}\ \frac{\text{S.A at zero mass}}{\text{S.A observed}} = 0.0197X \qquad \text{(Tracerlab)}$$

where X mg is the sample mass.

low specific activities and as relatively large amounts of this lipid were available for radioassay, samples were counted as infinitely thick layers on stainless steel planchets 3.142 cm^2 in area. Because of the surface tension properties of the lipid, most of it tended to aggregate at the sides of the planchet and to ensure reproducible counting geometry with true infinite thickness over the entire planchet, comparatively large amounts of lipid had to be plated. It was found that total sample weights in excess of 80 mg gave satisfactory preparations. The melted lipid, 0.15 ml. (ca. 120 mg) was plated directly on to a planchet and heated with an infra-red lamp to spread the lipid over its surface. In order to compare the radioactivity of the adipose tissue with that of the infused chylomicron lipid, the lipid in a sample of chylomicrons was extracted and dissolved in a known weight of olive oil. This oil was then plated as described. These low activity samples were counted in a windowless gas flow proportional counter with an automatic sample changer (Tracerlab) and scaler. The proportional gas used was 10% methane in argon.

Counting procedures. All samples were counted for at least 1000 counts. The counting rate was calculated and the background counting rate of the counter used was subtracted to give the true counting rate of the sample. Duplicate counts agreed within ±5%.

Ionization chamber assay of $^{14}CO_2$. A combination of an ionization chamber, vibrating reed electrometer and recorder was used to record continuously the excretion of $^{14}CO_2$ by intact animals and isolated heart-lung preparations during the infusion of ^{14}C-labelled substrates. The radioactivity in the substrates used for infusion was also measured in this way. Although this method of assay has been well documented (cf. Tolbert, 1956) some of its aspects will be discussed in connection with the various metabolism systems which were used for the experiments in this thesis.

Principle of the ionization chamber. The β-particles emitted during the radio decay of ^{14}C atoms ionize some of the molecules of any gas through which they pass. The number of ions and electrons produced in any given gas is a function of the number and energy of β-particles emitted. The ionization chamber is an apparatus devised to collect the electrons produced by this ionization. It consists of a hollow metal chamber with a polished metal probe, which is highly insulated from any other conductors. A negative potential is applied to the walls of the chamber and a potential difference is created between them and the centre probe. Any electrons produced by ionization in the chamber migrate towards the centre probe, under the influence of this electric field, where they accumulate because of the

high resistance of the probe insulator. The rate of accumulation of electrons, which is proportional to the amount of radioactivity in the chamber, constitutes an electric current. By measuring this current the amount of radioactivity in the chamber can be determined. Each ionization chamber has a characteristic background current due to radioactive contaminants in the materials from which it is constructed and from cosmic radiation which penetrates through its walls. This cosmic radiation and the β-particles arising from the chamber walls produce a very steady background current which is quite reproducible and can be subtracted from measured currents to give the true current originating from the $^{14}CO_2$ within the chamber. Another component of the background current is due to individual α-particles emitted from the chamber walls and from radon gas within the chamber, and these are not predictable statistically. Each α-particle produces a comparatively large number of electrons causing a large current to flow momentarily. By continuously recording the current with a strip-chart recorder, these individual events can be identified and a correction made for them.

Two ionization chambers (Applied Physics Corporation, Calif.) were used for the experiments to be described. They were equipped with sapphire insulators and were 250 cc and 4000 cc in volume. (Figure 11).

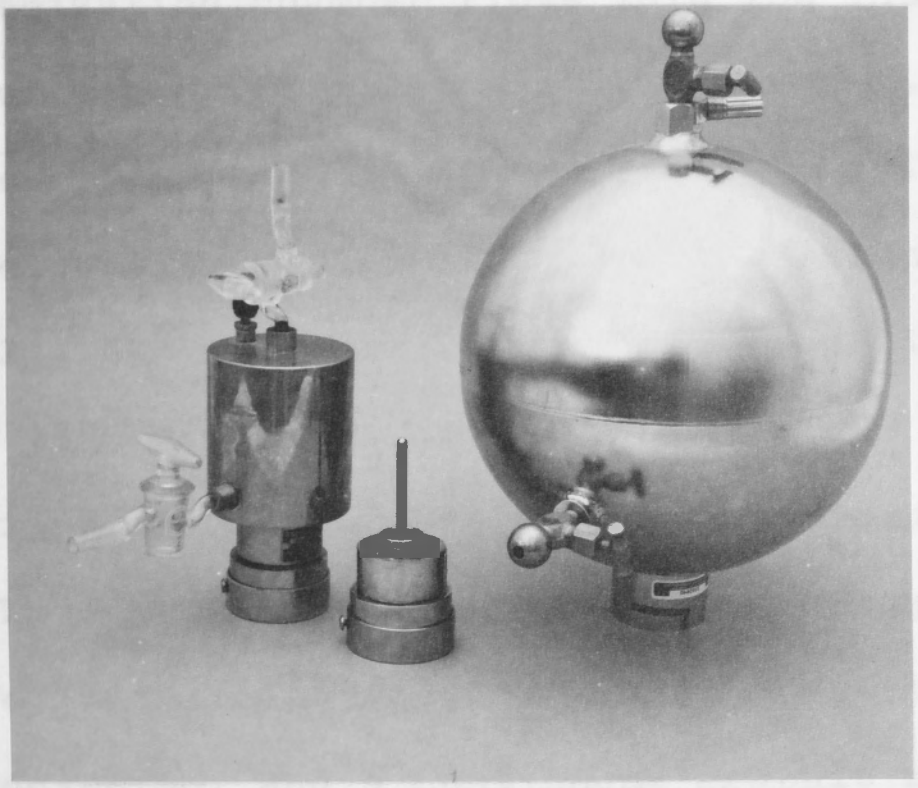


FIGURE 11.

The two ionization chambers which were used in the experiments described in this thesis. The charge collection probe for another 250 cc. chamber is also shown.

The vibrating reed electrometer. The current originating in the ionization chamber was measured with a vibrating reed electrometer (Cary Model 31, Applied Physics Corporation, Calif.). The principle of operation of the vibrating reed electrometer is as follows. The current from the ionization chamber flows into the input capacitance of the electrometer which is highly insulated. The capacitance of this input is small (ca. 10 $\mu\mu$f) and a small number of electrons accumulating on it will charge it to a relatively high potential. The input capacitance is cyclically varied at a frequency of about 450 c.p.s. and the a.c. signal generated by this changing capacity is amplified by a very stable amplifier employing 100% negative feedback. The potential of the electrometer input is indicated, and a continuous graphic record of this can be obtained with an ink writing strip-chart recorder.

The electrometer can be operated in two ways. The charge flowing into the electrometer input can be allowed to accumulate when the potential of the input will continue to increase. This is known as the rate of charge method of operation. The current, i amp, flowing into the electrometer when operated in this way is given by

$$i = C\frac{dE}{dt} \qquad \ldots \qquad (1)$$

where C farads is the effective capacitance of the electrometer input and E volts is the potential to which it is charged. Alternatively, the electrometer input can be connected to earth by a high resistance leak when the current flowing into the electrometer is given by

$$i = \frac{E}{R} + C\frac{dE}{dt} \qquad \ldots\ldots \qquad (2)$$

where R ohms is the high resistance leak. It can be seen that, if the current is constant, the electrometer input will attain a constant potential which is directly proportional to the current and the resistance of the leak according to the following relationship

$$E = iR(1 - e^{-t/RC}) \qquad \ldots\ldots \qquad (3)$$

where t is measured in seconds. This is known as the <u>high resistance leak</u> method of operation. The term $(1 - e^{-t/RC})$, which tends to unity, is a time function for any high resistance leak-electrometer input combination which determines the rate at which the electrometer input attains a new equilibrium potential when the current changes from one value to another. This relationship is of importance when the current is continually changing as it does in the various experimental procedures to be described and is considered more fully in Appendix I. The electrometer used was fitted with a turret switch assembly in its input circuit so that it could

be operated in the rate of charge method or with high resistance leaks of 10^{10}, 10^{11}, 10^{12} or 10^{13} ohms. This switch was also used for grounding the input circuit.

Vibrating reed electrometers of the quality used are extremely precise instruments. The Cary Model 31 has a background rate of drift stable to less than 10^{-17} amp, which is equivalent to about 60 electrons a second. Care was taken to ensure that it was never allowed to exceed the maximum voltage in any voltage range.

Measurement of $^{14}CO_2$ with an ionization chamber, vibrating reed electrometer, recorder combination. This combination can be used in two ways to measure $^{14}CO_2$ activity. The $^{14}CO_2$ can be introduced directly into the chamber and the current produced by it measured, or the $^{14}CO_2$ can be swept through the chamber and the amount of $^{14}CO_2$ present in the chamber at any time continuously recorded. If the electrometer is operated by the rate of charge method, the amount of radioactivity in the chamber at any time is proportional to the measured rate of charge minus the background rate of charge. If a high resistance leak is used, then, within the response time characteristics of the electrometer input-high resistance leak combination, the amount of radioactivity in the chamber is indicated by the electrometer input potential.

Tolbert (1956) stated that when used for assaying $^{14}CO_2$ in a flow system, the vibrating reed electrometer

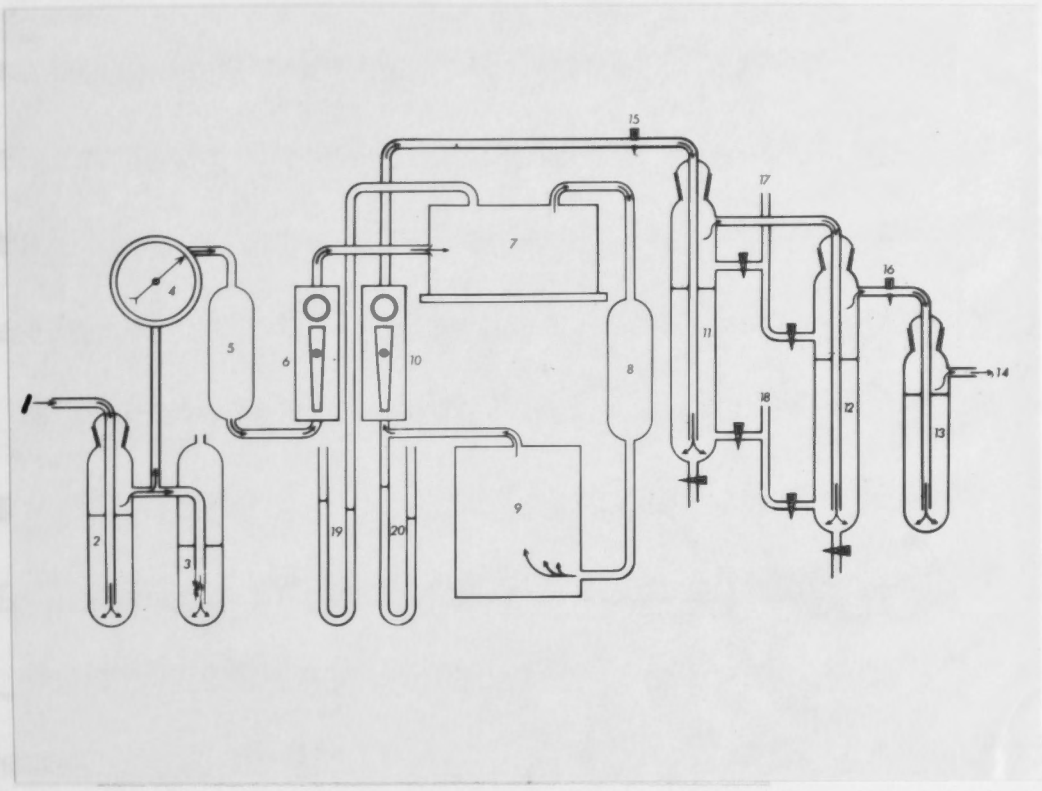


FIGURE 12.

The metabolism system which was used for continuously recording the excretion of $^{14}CO_2$ in the breath of intact rats. See text for description.

could only be used with a high resistance leak. It was found however that if the air flowed into the bottom of the ionization chamber and out the top it could be used by the rate of charge method; if the air flow was reversed, the air stream impinging on the collection probe in the ionization chamber seemed to interfere with the insulation characteristics of the sapphire.

Ionization chambers can be calibrated in absolute units but for most biological experiments, only relative measurements of the radioactivity are required and these can be conveniently expressed in terms of the electrometer input potential.

Continuous recording of the excretion of $^{14}CO_2$ by intact unanaesthetized rats. This system was similar to that described by Tolbert, Hughes, Kirk & Calvin (1956) but was modified so that the labelled substances could be infused continuously throughout the experiments and the total CO_2 produced by the animals could be collected and measured.

The metabolism system is shown diagrammatically in Figure 12. Air which had been kept in a cylinder for at least a month to reduce its radon content, was admitted to the system at (1) and was scrubbed free of CO_2 in a tower (2) which contained concentrated NaOH solution. The pressure of the air as it left the scrubbing tower was controlled by a regulator (3); the flow of air was

adjusted to provide the desired flow through the system plus a steady stream of bubbles which escaped to the atmosphere through the regulator (3). The air which passed through the rest of the system flowed through a wet test gas meter (4) and then through a drying tube (5) which contained silica gel to remove excess water vapor. The air then flowed through a rotameter (6) into the metabolism cage (7) in which the experimental animal was confined. The air which left the metabolism cage, together with the rat's expired CO_2, passed through a drying tube (8) which contained anhydrous $Mg(ClO_4)_2$. It was essential for the air to be dried completely as water vapor affects the efficiency of the ionization chamber. The air entered the ionization chamber (9) which was mounted on the head of the vibrating reed electrometer. A 250 cc ionization chamber was used in this system and the electrometer input was earthed by a 10^{12} ohm high resistance leak. The rate of flow of air as it left the ionization chamber was checked with a second rotameter (10) and the CO_2 was then removed in two scrubbing towers (11, 12) connected in series, each containing approximately 50 cc of initially CO_2-free N-NaOH solution. The efficiency of these scrubbing towers was checked by finally passing the air through a tower (13) which contained saturated $Ca(OH)_2$ solution. Suction was applied at (14) with a venturi vacuum pump. Two sets of towers

containing NaOH solution were connected in parallel so that serial collections of the expired CO_2 could be made; the air was diverted through the appropriate set of towers by means of three-way stopcocks (15, 16). When the contents of the scrubbing towers were collected during an experiment, air was admitted into the scrubbing towers through a soda-lime guard tube (17). The scrubbing towers could be washed with CO_2 free water and refilled with NaOH solution through tube (18). The pressures of the air in the metabolism cage and in the ionization chamber were measured with water manometers (19, 20). The flow rate of air was kept constant during the experiments by controlling these pressures accurately with the needle valves on the rotameters. The pressures were maintained at a level of a few cm of water below atmospheric. The amount of air which flowed through the system was measured accurately throughout the course of an experiment by means of the wet-test gas meter.

The metabolism cage used in this system was made of perspex and was essentially an enclosed Bollman type restraining cage (Bollman, Cain & Grindlay, 1948). Removable sides were coated with a thin layer of stopcock grease to ensure an air-tight seal. The air inlet was close to the animal's head and the outlet was at the opposite end of the cage. The dead space in the cage was approximately 1000 cc.

The amount of $^{14}CO_2$ which passed through the ionization chamber in consecutive intervals during the course of the experiments was determined by measuring the area under the recorded trace with a planimeter for each time interval and multiplying this area by the rate of flow of air during the interval. The areas were measured in mV. min and the rate of flow of air in cc./min. The amount of radioactivity was thus expressed as mV.cc.

The rate of flow of air used in this system was approximately 250 cc./min and the volumes of the metabolism cage and ionization chamber were 1000 cc. and 250 cc. respectively. The fractional rates of turnover of air in these two compartments was thus 0.25 and 1.0 min^{-1}. Because of the relatively slow fractional rates of turnover of the air in these compartments, the recorded $^{14}CO_2$ excretion curves were distorted from their true shape. The degree of distortion introduced into the recorded curves is shown in Appendix II and methods of calculating the true rate of excretion of $^{14}CO_2$ by the animals during the course of the infusions are given.

The CO_2 produced by the experimental animals was measured as follows. At the end of a period of collecting, the contents of the scrubbing towers were run into a 200 ml. volumetric flask. The towers were washed with CO_2-free water and these washings were added to the flask. After the contents were made up to volume,

samples of the NaOH solution (10 ml.) were mixed with an excess of N-$BaCl_2$ solution and the Na_2CO_3 precipitated as $BaCO_3$. The $BaCO_3$ was centrifuged and washed three times with distilled water and then reacted with an excess of 0.1 N-HCl and the excess acid back-titrated with 0.1 N-NaOH solution. The amount of $BaCO_3$ was calculated from the amount of acid remaining. Duplicates determined in this way seldom differed by more than 3%.

This metabolism system was used for all experiments reported in Chapters 3 and 4 of this thesis. The respiratory quotients of these animals were not expected to vary much and, under these conditions, the total CO_2 production was used as a measure of the animals' metabolic rates. In individual experiments fluctuations occurred in the recorded $^{14}CO_2$ excretion curves during periods of spontaneous activity. These fluctuations were smoothed by pooling the results of a group of similar experiments. However, for kinetic analysis of the excretion curve, it was apparent that it would be an advantage to have a simultaneous measure of the animals' oxygen consumption.

Continuous recording of the $^{14}CO_2$ excretion and O_2 consumption of intact unanaesthetized rats. This metabolism system was designed to be used in experiments in which the respiratory quotients of the experimental animals were expected to differ with the various experimental treatments. Under these conditions, the

oxygen consumption of the animals gives a better index of metabolic rate than the CO_2 production. With this system, simultaneous continuous records of $^{14}CO_2$ excretion and O_2 consumption of the experimental animals could be made during each experiment. In addition, the CO_2 produced by the animals during intervals could be measured. Faults which became obvious in the first system described above were in large measure eliminated in this system. The electrometer was operated by the rate of charge method and the resulting increase in sensitivity enabled higher rates of flow of air to be used without the need for the use of increased amounts of activity. It was found that the higher rate of flow could be adjusted with sufficient precision by means of the rotameters and could be kept constant to within $\pm 1\%$ during an experiment and between different experiments. There was thus no longer any need to incorporate a wet test gas meter in the system. As the flow rate was constant in all experiments, it was not necessary to use this in the calculation of amounts of radioactivity passing through the ionization chamber. The dead space in the metabolism cage was virtually eliminated and the fractional rate of turnover of air in the ionization chamber increased by a factor of 3 compared with that in the first system. Under these conditions the distortion introduced into the recorded $^{14}CO_2$ excretion curves was minimal and the rate at which radioactivity

passed through the ionization chamber could be used as a measure of the rate at which the animals excreted it. By using the electrometer in the rate of charge method, a continuous integrated record of the amount of $^{14}CO_2$ which passed through the ionization chamber was obtained. The amount of $^{14}CO_2$ which passed through the ionization chamber in any interval was simply measured as the increase in scale deflection during the interval, minus the increase due to background and the contribution of α- particles. This obviated the tedious procedure of measuring the area under the recorded trace with a planimeter.

The metabolism system is shown in Figure 13. Air was circulated through the apparatus by a diaphragm aquarium pump (A) at a rate of 750 ml./min. The air flowed through a two litre flask (B) and then through the glass metabolism cage (C) which was made to fit the experimental animals comfortably. The air inflow and outflow were near to the animal's head and the dead space in the cage was kept to a minimum (ca. 20 cc). The air which left the metabolism cage together with the expired CO_2, was dried in tubes which contained self-indicating silica gel (D) and anhydrous $Mg(ClO_4)_2$ (E). The dried air passed through the rotameter (F) calibrated to 950 ml./min before flowing through the 250 ml ionization chamber. It then passed through a second flow

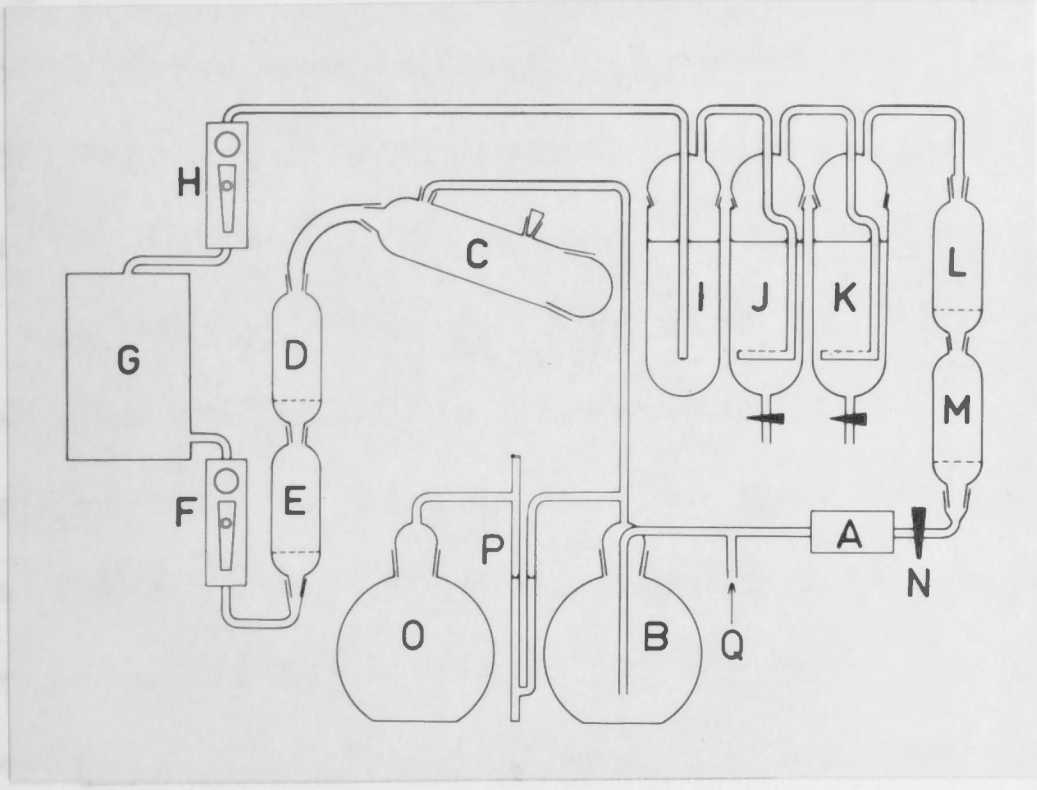


FIGURE 13.

The metabolism system which was used for simultaneously measuring the $^{14}CO_2$ excretion and O_2 consumption of intact rats. See text for description.

meter (H) and then through a set of three scrubbing towers (I, J, K), the first of which contained dilute HCl and the next two a solution of N-NaOH. The air was saturated with water vapor in the first tower to prevent evaporative losses in the towers containing NaOH. The scrubbing towers were fitted with fine sintered glass discs which faced upwards and dispersed the air into very fine bubbles. After being scrubbed free of CO_2, the air was dried in tubes (L, M) before it was recirculated through the system. The rate of flow of air through the system was controlled by a needle valve (N) which was as close to the pump as possible. The pressure of the air as it left the flask (B) was referred to a constant pressure in a two litre flask (O) through the electro-manometer (P). The flask (B) was included in the flow system to prevent the animal's movements affecting the pressure at the reference point. Any oxygen consumed by the animal reduced the pressure of the air in the system as the CO_2 produced by the animal was completely removed in the NaOH solution and the system had a constant volume. When the pressure of the air at the reference point fell below the set value, the electromanometer activated the oxygen delivery pump and oxygen was pumped into the system through the connection (Q).

Details of the electromanometer are shown in Figure 14. It was made of 2 mm internal bore glass

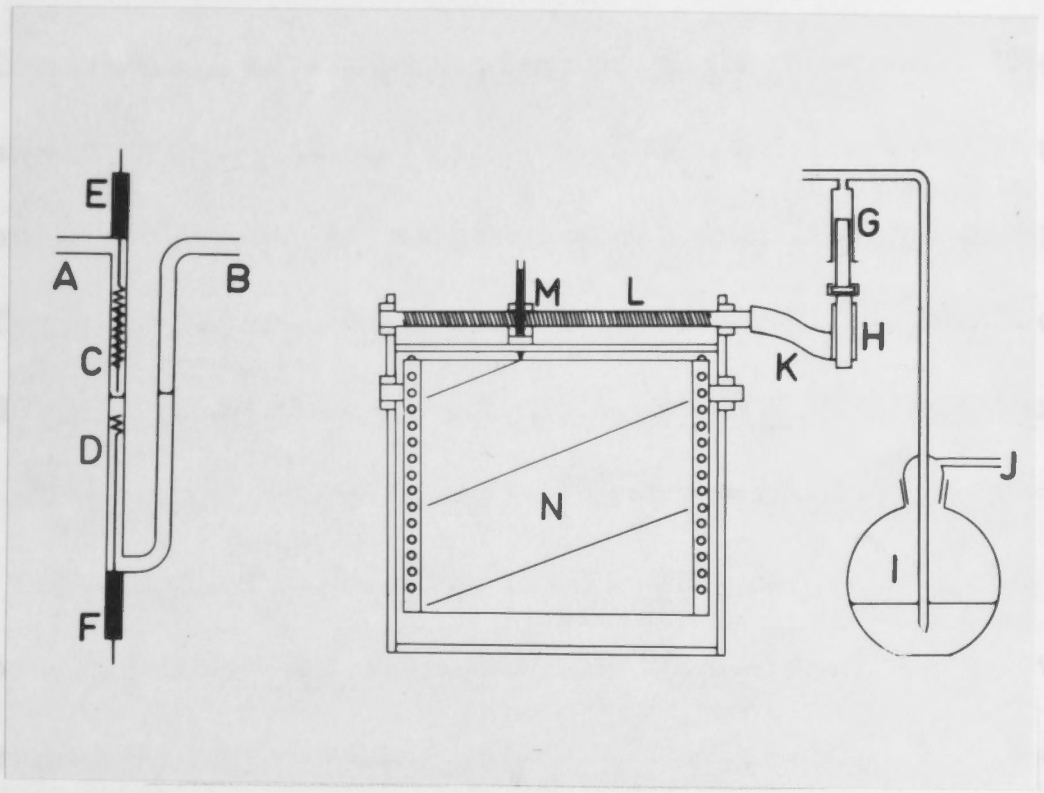


FIGURE 14.

The electromanometer and apparatus which were used for recording the O_2 consumption of intact rats. See text for description.

tubing and was connected to the constant pressure flask at (A) and to the reference point in the metabolism system at (B). The manometer fluid was 15% $CuSO_4$ solution. Two copper electrodes (C, D) were fixed into one arm of the manometer so that their tips were as close together as possible and when the pressure in the metabolism system fell, electrical contact between them through the manometer fluid was broken. Care was needed in the construction of the upper electrode to ensure that the fluid surface broke evenly from it when the column of fluid descended. The electrode tip was sharpened to a point and soldered into a helix of copper wire which fitted neatly into the tube so that the tip of the electrode lay as close to the centre of the tube as possible. The electrodes were connected to a thyratron relay through the two seals (E, F). A small amount of Teepol (Shell) was added to the manometer fluid to reduce its surface tension and promote free movement of the fluid in the tube.

The apparatus for delivering oxygen and the recorder are shown in Figure 14. When electrical contact was broken in the manometer by a fall of pressure in the metabolism system, water was pumped into an oxygen reservoir by means of a Brewer Automatic Pipetting Machine and oxygen was displaced from the reservoir into the metabolism system. The syringe (G) and the driving eccentric (H) of the pipetting machine are shown in the

figure. The water displaced oxygen from the reservoir (I) through a tube (J) which was connected to the metabolism system by a length of fine-bore tubing to prevent the back diffusion of air from the metabolism system into the oxygen reservoir. The eccentric of the pipetting machine was connected by a length of thick walled rubber tubing (K) to a long screw (L) across the top of the oxygen consumption recorder. The thread of the screw engaged the pen carriage (M) and every turn of the pipetting machine delivered a constant volume of water into the oxygen reservoir, which displaced a similar volume of oxygen into the metabolism system. At the same time, the pen was moved a constant distance across the recorder chart (N). The chart was driven at a constant rate by a small synchronous motor. A linearly integrated record of oxygen consumption was thus obtained. The paper used for recording oxygen consumption was graduated from 0-12 and the thread of the screw was such that when the pen had reached between 10 and 12 it could be returned manually to an exactly comparable position between 0 and 2. The stroke volume of the pipetting machine and its rate of delivery were adjusted so that the pump was being continually turned on and off and the pen travel matched to the rate of oxygen consumption. This gave a continuous precise record of the oxygen consumption. A pen travel of

approximately 10 inches for 100 cc was used. Although the dead space of the system was of the order of 5 litres, the oxygen delivery pump was actuated by a pressure change induced by withdrawing as little as 0.2 cc of air from the system.

The oxygen consumed during any interval was measured from the increase in the oxygen consumption record during the interval. The recorder was calibrated in one of two ways. A known volume of air was withdrawn from the metabolism cage whilst the air was flowing and the pen travel noted, or alternatively, the pen travel was determined when a given amount of water was pumped by the pipetting machine into a graduated flask. The second method was more convenient for routine checking, but as the oxygen which leaves the reservoir is saturated with water vapor, the S.V.P. of the water had to be subtracted from the pressure of the oxygen when its volume was converted to S.T.P. Both methods of calibration gave essentially the same calibration constant.

The amount of CO_2 absorbed in the scrubbing towers was determined in samples taken from the towers at intervals during an experiment. The towers were filled initially with 200 cc of CO_2-free N-NaOH solution. When a sample was taken from the towers, 20 cc of CO_2-free NaOH was injected into the tower through the tap at the bottom by means of a syringe. This was rapidly mixed with the contents of the tower by the air bubbles, but to

ensure a representative sample of the tower contents, the syringe plunger was drawn up and down several times and then rapidly withdrawn to obtain a sample of exactly 20 cc. The whole procedure took about 1 min. The amount of CO_2 absorbed in the NaOH was determined as described above or by the following procedure. Five cc of the NaOH was reacted with 10 cc of 0.5N-$BaCl_2$ and the $BaCO_3$ was centrifuged down taking care to protect the mixture from atmospheric CO_2. Three cc. of the supernatant were reacted with an excess of 0.1 N-HCl and the excess acid was back-titrated to pH 5 with 0.01 N-NaOH solution using an automatic titrator (Radiometer). Blank titrations were performed by using water instead of the $BaCl_2$. The amount of CO_2 precipitated as $BaCO_3$ was found by difference between the two titrations. This method gave results identical to the previous method but duplicate determinations seldom differed by more than 1% and the method was more convenient for routine use. The volume of NaOH in the towers at the time of sampling was 220 cc and 20 cc were removed in each sample. The amount of CO_2 produced during an interval was calculated by subtracting 10/11 of the amount of CO_2 present in the tower at the beginning of the interval from the amount present in the tower at the end of the interval. The volume in the towers remained constant during the course of a 4 hr experiment and a negligible amount of CO_2 was present in

the second tower after a similar interval. The blank titration of the NaOH did not change during an experiment. The sampling procedure was checked by using a dye in the tower contents and measuring the change in optical density of the contents when successive samples were taken and replaced with dye-free NaOH. The ratio of the optical density of the consecutive samples indicated that 1/11 of the tower contents were removed with each sample. Any error in the sampling technique would not introduce a consistent error into the CO_2 measurement. One falsely high measurement would tend to be followed by a low measurement.

The exact time at which a sample of NaOH was removed from the tower was recorded automatically on the oxygen consumption record. When the volume of NaOH was injected into the tower, the pressure in the system was increased and the oxygen delivery pump automatically stopped. When the sample was removed, oxygen delivery was immediately recommenced to replace the volume of oxygen consumed during the sampling procedure.

This apparatus was used for experiments described in Chapter 5. The animals were placed in the metabolism cage one hour before the start of the experiment and the system was purged for 30 min with air of low radon content. During this period, the oxygen reservoir was filled with oxygen and the oxygen delivery pump calibrated. This

calibration was very constant from day to day. The recorder was turned on and the background rate of charge of the electrometer measured. After 30 minutes the system was closed and the oxygen consumption apparatus turned on. After the oxygen consumption apparatus had been functioning for 30 min, the infusion of the labelled substrates was begun. The system seemed to require about 30 min to stabilize with respect to the temperatures and pressures within the various compartments. As the pressure of the air at the reference point in the system was referred to a constant volume of gas at the same temperature as the air at the reference point, the measurement of oxygen consumption was not altered by changes in atmospheric temperature or pressure.

During an experiment, it was possible for one operator to write down the oxygen consumption and $^{14}CO_2$ excretion from the recordings for 5 minute intervals and to determine the CO_2 produced during 30 minute periods. The electrometer input was grounded momentarily when the scale deflection reached 80-90% of the full scale.

The oxygen consumption and $^{14}CO_2$ excretion records were very well synchronized. Two such sets of records are shown in Figure 15. Although these two animals showed extreme fluctuations in the rate at which they consumed oxygen, it can be seen that for every fluctuation in the consumption of oxygen, there was a corresponding

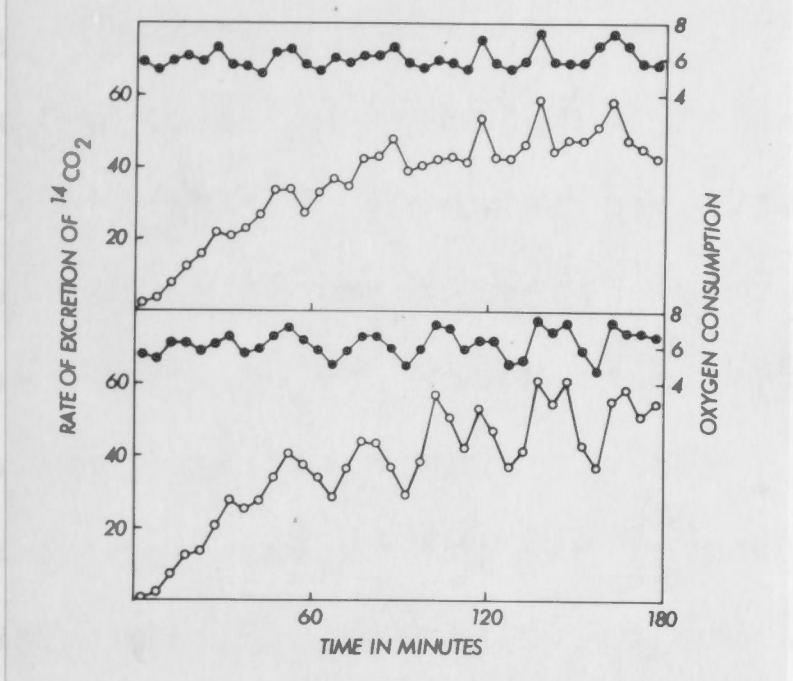


FIGURE 15.

Simultaneous records of the $^{14}CO_2$ excreted and the O_2 consumed in successive 5 minute intervals during the continuous intravenous infusion of ^{14}C-labelled chylomicrons by two intact rats. The excretion of $^{14}CO_2$ is plotted as a percentage of the rate of infusion of ^{14}C and the oxygen consumption is measured in cc./min.

fluctuation in the excretion of $^{14}CO_2$. By equating the excretion of $^{14}CO_2$ to the simultaneous consumption of oxygen, the excretion curves in individual experiments were smoothed in large measure and were much more suitable for kinetic analysis.

Apparatus used to measure the excretion of $^{14}CO_2$ by the isolated heart-lung preparation. This was designed for use with the isolated heart-lung preparation. In these experiments the CO_2 produced was allowed to accumulate in the system during an experiment. To prevent the partial pressure of the CO_2 rising too much, the volume of gas in the system was large and by using a large spherical ionization chamber, most of the gas in the system at any time was present in the ionization chamber. The system is shown in Figure 16. It was initially filled with 5% CO_2 in oxygen. This gas was circulated by the pump (A) from which it passed through the oxidation apparatus (B) which is described below. It then passed through a T-piece (C), through two drying tubes (D, E) through a rotameter (F) and then through the ionization chamber (G) back to the pump. The tracheal cannula of the organ preparation was connected to the T-piece at (H) and the pressure of the gas was measured with a water manometer (I). With this system a constant proportion of the $^{14}CO_2$ present in the total system was contained in the ionization chamber. By operating the vibrating reed electrometer

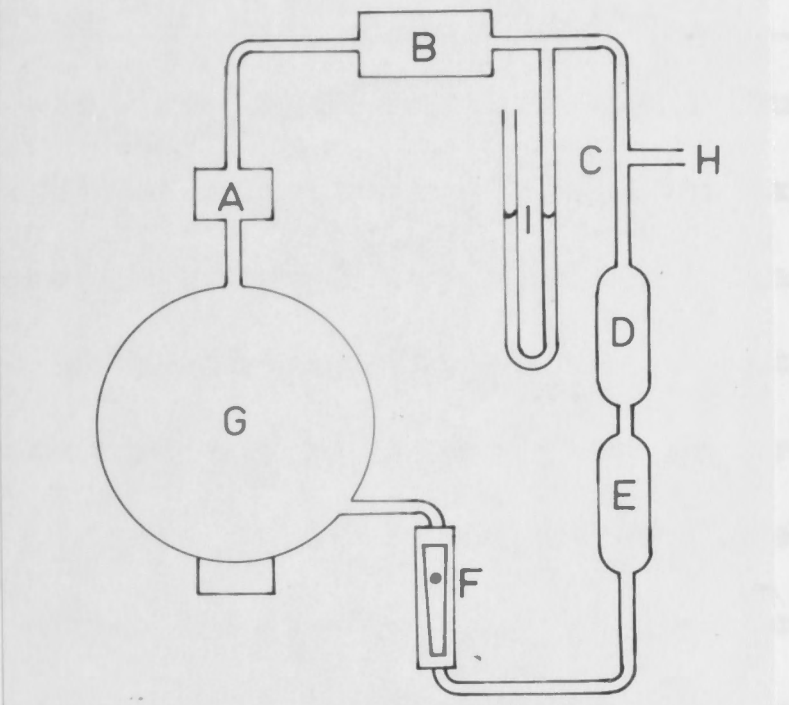


FIGURE 16.

The apparatus which was used for continuously measuring the excretion of $^{14}CO_2$ by the isolated rat heart-lung preparation. See text for details.

with a high resistance leak, a cumulative record of $^{14}CO_2$ excretion by the heart-lung preparation was obtained. The flow rate of gas was not critical in this system but was maintained at about 1 litre per minute to promote prompt mixing within the various compartments.

Apparatus for oxidizing radioactive samples to CO_2.

An apparatus was developed so that the infusion mixtures used in the various experiments could be oxidized to CO_2 and the CO_2 passed through the various metabolism systems. In this way, the radioactivity of the substrates used in the experiments and the radioactivity expired by the experimental animals could be compared directly. The apparatus is shown in Figure 17. It replaced the metabolism cages in the systems used for intact animals and was permanently incorporated into the system used for isolated heart-lung preparations. The route taken by the air through the system during the assay of a sample is shown in the figure. Air entered the apparatus through a three way stopcock at (1) and then flowed through the generation flask (2), two spiral cold traps (3, 4), the stopcock (5) and the compensating volume (6). From there it flowed into the metabolism system. During the oxidation of a sample the flow of air through the apparatus was diverted by turning stopcock (1) and closing stopcock (5). The air flow through the rest of the system was not interrupted. In the two closed systems the

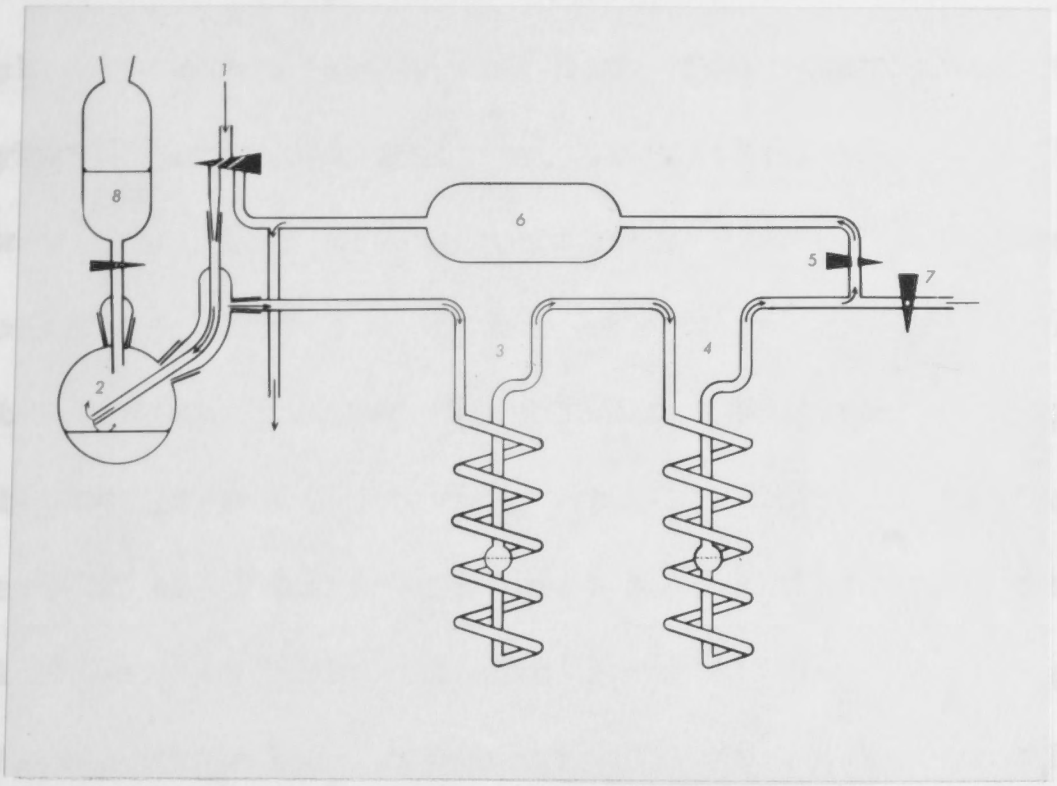


FIGURE 17.

The apparatus which was used for the oxidation of ^{14}C-labelled compounds to $^{14}CO_2$ for radioassay with the ionization chamber, vibrating reed electrometer and recorder combination. See text for details.

metabolism systems were purged with air or carbogen during the oxidation procedure. The sample which was to be oxidized was placed in the generation flask together with a chip of silicon carbide and the flask was fitted to the apparatus as shown in Figure 17. The spiral cold traps were immersed in liquid air, stopcock (7) was opened and the apparatus evacuated. Van Slyke-Folch oxidizing mixture (Van Slyke & Folch, 1940) was run into the generation flask from the reservoir (8), and the flask was heated for 5-10 minutes until the oxidation was complete. The cold traps prevent the loss of any CO_2 during the oxidation. They were fitted with sintered glass discs in their central tubes to prevent the loss of any CO_2 fog which might form. The CO_2 froze in the first coils of the first trap; the second trap acted only as a guard. When the oxidation was complete, stopcock (7) was closed and the liquid air was removed from the cold traps and the CO_2 was allowed to evaporate. When the CO_2 had evaporated, stopcock (5) was cautiously opened and air or carbogen allowed into the apparatus. The manometers incorporated into the various systems provided checks on the pressure in the systems. When the pressure had returned to its previous level, systems in which the gas was to recirculate were closed. Stopcock (1) was then reversed and the air allowed to flow through the

apparatus. The recorder was left on during the oxidation. In the first system, as the radioactive CO_2 passed through the ionization chamber a peak was recorded. The amount of air which flowed through the system as the peak was recorded was noted from the wet-test meter. The amount of activity was measured by multiplying the area of the peak which was measured with a planimeter, by the rate of flow of air whilst the peak was recorded. In the second system, as the radioactive CO_2 passed through the ionization chamber, the rate of charge of the electrometer increased from background and then returned to background. The amount of activity was measured as the increase in scale deflection above that due to background before the background rate of charge was resumed. In the third system, as the radioactive CO_2 equilibrated throughout the system, the electrometer potential increased to a new plateau value. The amount of radioactivity was measured simply by the increase in scale deflection. The records made during radio assays in the various systems are shown in Figures 18, 19 and 20. In the first metabolism system it was important to determine whether the amount of radioactivity passing through the ionization chamber was recorded accurately by the electrometer when the rate of transfer of the radioactivity was of the same temporal order as the response time of the electrometer. It is shown

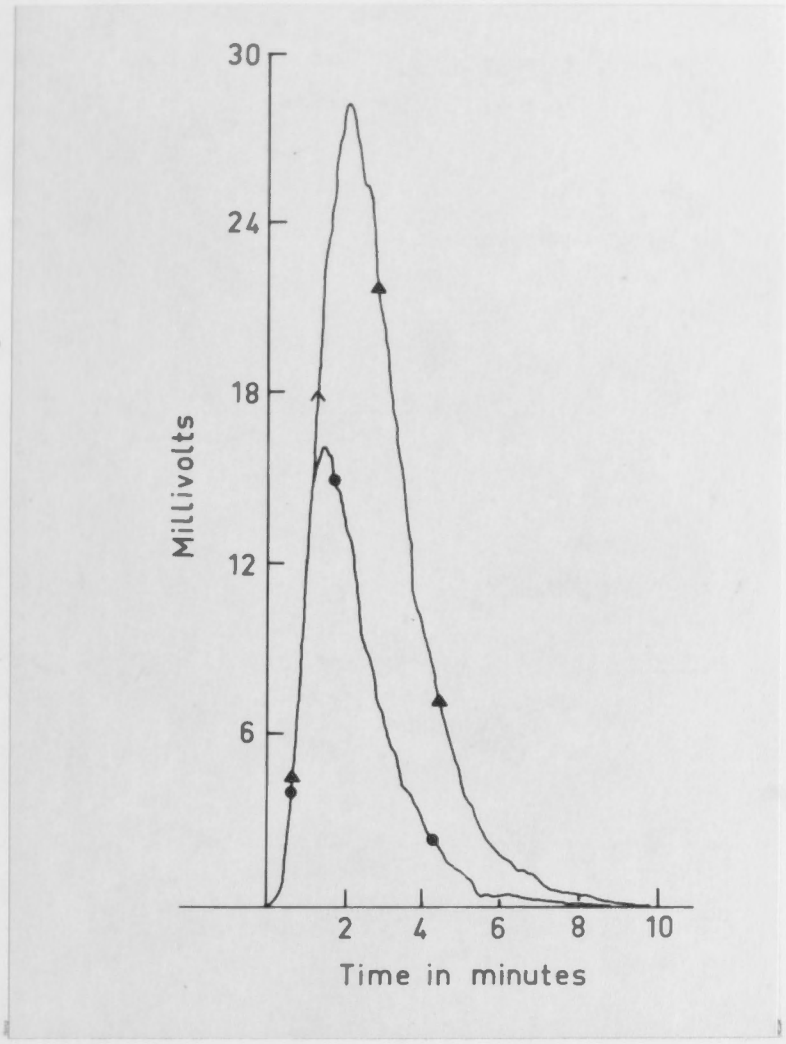


FIGURE 18.

The traces recorded during the assay of the $^{14}CO_2$ liberated by the oxidation of 0.05 ml. and 0.10 ml. samples of a radioactive chylomicron preparation. The electrometer input was earthed by a 10^{12} ohm high resistance leak and the electrometer was operated in the 300 mV full scale deflection range.

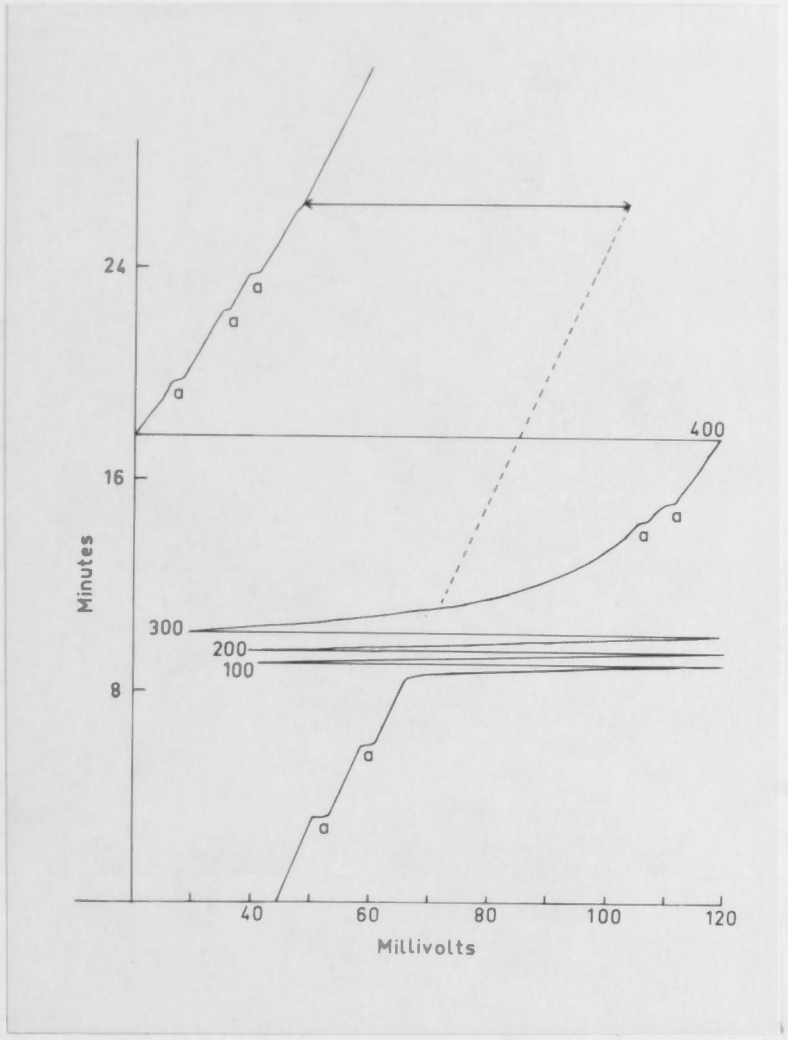


FIGURE 19.

The trace recorded during the assay of the $^{14}CO_2$ liberated by the oxidation of 0.10 ml. of a radioactive chylomicron preparation, using the electrometer in the rate of charge method of operation. The discontinuities in the recorded trace due to individual α-particles are indicated (α). The electrometer was operated in the 1 V full scale deflection range.

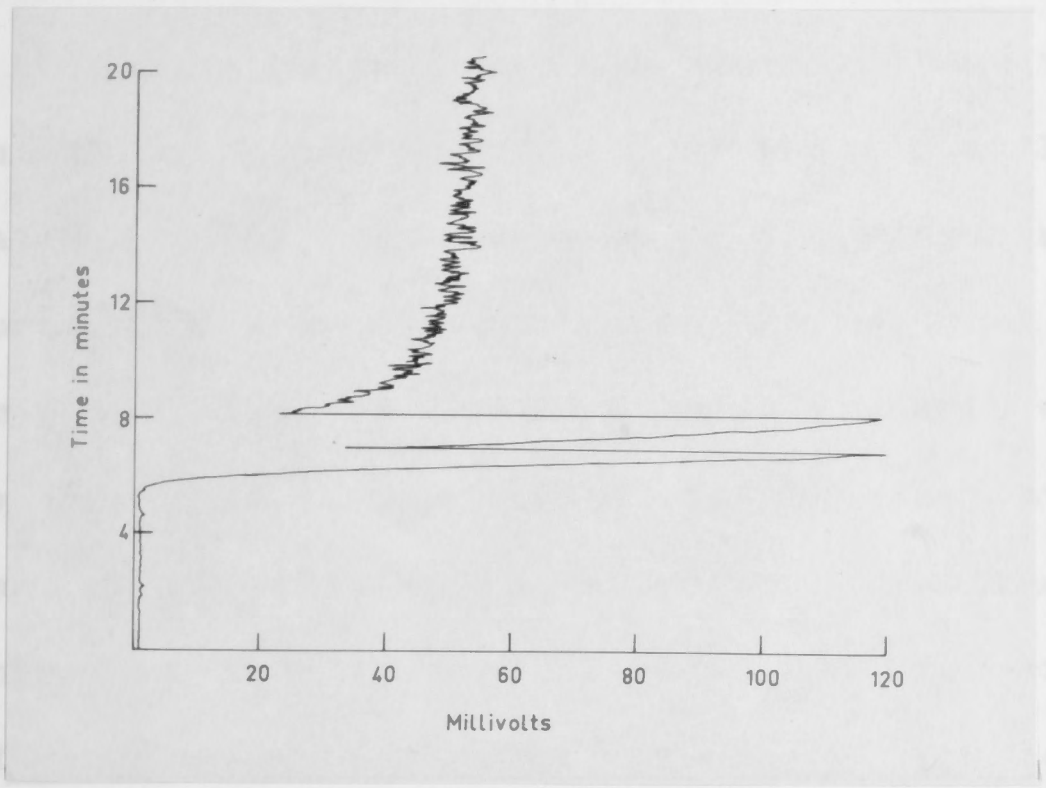


FIGURE 20.

The trace recorded during the assay of the $^{14}CO_2$ liberated by the oxidation of 0.01 ml. of a radioactive chylomicron preparation. The apparatus for studying on the rat heart lung preparation was used and the $^{14}CO_2$ allowed to accumulate in the system. The electrometer was operated in the 1 V full scale deflection range and a 10^{12} ohm resistance leak was used in the electrometer input.

theoretically in Appendix I that the area under the recorded trace will always be proportional to the area under the true activity-time curve in any interval, provided that the electrometer scale deflection is the same at the beginning and the end of the interval. In any assay, the electrometer recording always returned to the base line potential. The amount of activity in the ionization chamber changed more rapidly with higher flow rates and the response time of the electrometer introduced a greater distortion in the recorded trace. However, the product of the area under the recorded trace and the flow rate of air will always be constant for a given amount of activity, provided the efficiency of the ionization chamber remains constant. The effects of different rates of flow of air on the true activity-time curves and the recorded curves are shown in Figure 21.

Assay of potassium gluconate. Samples of potassium gluconate were oxidized to CO_2 and the CO_2 frozen out in the cold traps as described. The CO_2 was allowed to evaporate into an evacuated ionization chamber which was then filled with non-radioactive CO_2 to atmospheric pressure. The ionization chamber was then placed on the vibrating reed electrometer and the current produced by the $^{14}CO_2$ measured. The radioactive glucose infused

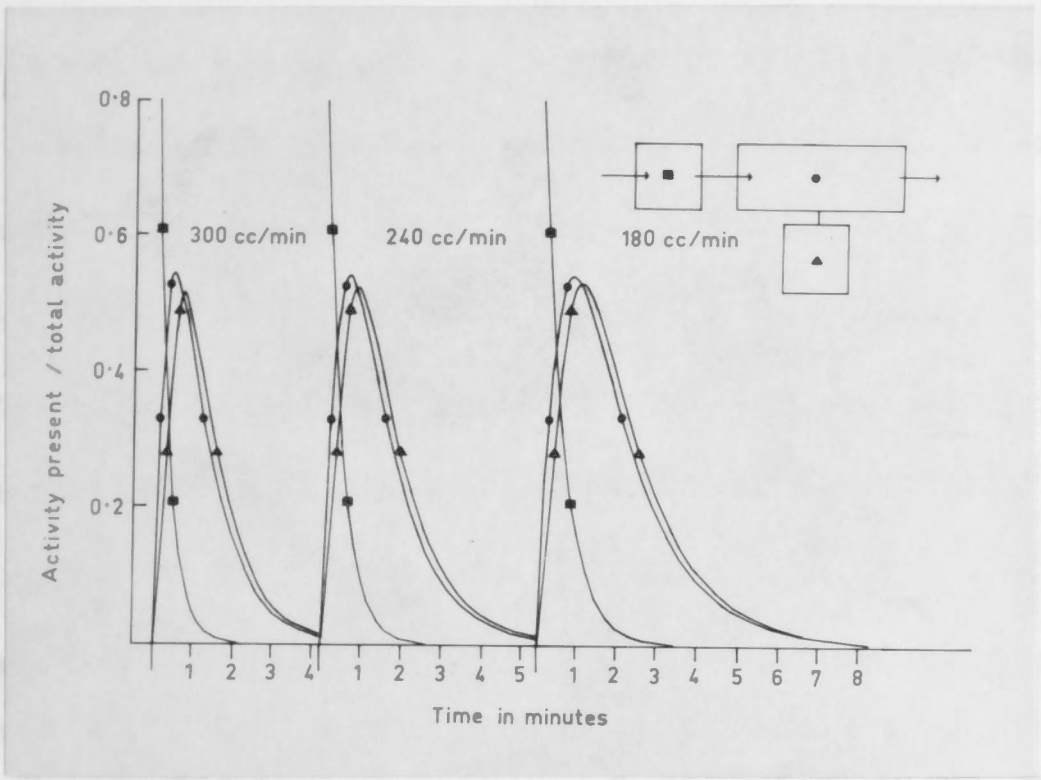


FIGURE 21.

The effect of the rate of flow of air on the rate of transfer of the $^{14}CO_2$ liberated by the oxidation of a sample of a ^{14}C-labelled substance through the ionization chamber, and the distortion introduced into the recorded curve when the electrometer input is earthed with a 10^{12} ohm resistance. The amount of $^{14}CO_2$ present in the generation flask (■), ionization chamber (●) and the recorded amount of activity in the ionization chamber (▲) are shown. These curves were derived theoretically assuming complete and instantaneous mixing of the air in the generation flask and ionization chamber. The volumes of the generation flask and ionization chamber were 100 cc and 250 cc respectively.

into the animals, from which the samples of potassium gluconate were prepared, was also assayed in this way.

Testing of the ionization chamber system for the assay of $^{14}CO_2$.

Use of a high resistance leak in the electrometer input. It is shown in Appendix 1, that, provided the efficiency of the ionization chamber remains constant, when identical amounts of $^{14}CO_2$ are passed through the ionization chamber, the area under the recorded peak is inversely related to the rate of flow of air. This was tested experimentally and the results are shown in Table 1.

Table 1

The amount of radioactivity measured when identical amounts of $^{14}CO_2$ were passed through a 250 cc. ionization chamber at different rates of flow of air. The amount of activity was measured by multiplying the area under the recorded peak, measured in mV. min, by the rate of flow of air measured as cc./min.

Rate of flow of air (cc/min)	Measured activity (mV x cc x 10^{-3})	
200	21.32	
206	21.62	mean = 21.63
209	21.96	
268	21.10	
263	22.31	mean = 22.04
267	22.72	
298	22.07	
298	22.40	mean = 22.04
298	21.64	
Overall mean 21.91 $\pm$ 0.176		

These results indicated that the efficiency of the ionization chamber remained constant at rates of flow of air from 200 to 300 cc./min.

The precision of this form of assay can be assessed by comparing the measurement of the $^{14}CO_2$ liberated from duplicate oxidations of labelled substances. A series of duplicate determinations is tabulated in Table 2.

Table 2

Determination of the $^{14}CO_2$ produced by duplicate oxidations of different samples of chylomicrons labelled with different fatty acids. The ^{14}C-labelled fatty acid in the chylomicrons and the total amount of fatty acids in the sample taken are also shown.

Sample	Labelled fatty acid	Total acids (mg)	Activity measured (mV.cc./cc x 10^{-3})	Difference between duplicates (%)
1	Palmitic	3.6	14.39	
			14.64	1.72
2	Palmitic	5.4	14.00	
			14.70	4.88
3	Palmitic	7.2	14.85	
			14.65	0.48
4	Oleic	3.6	23.29	
			23.37	0.34
5	Oleic	5.4	23.41	
			23.60	0.81
6	Oleic	7.2	22.31	
			22.72	1.82
7	Linoleic	3.6	20.35	
			20.30	0.25
8	Linoleic	5.4	19.90	
			19.83	0.37
9	Linoleic	7.2	19.15	
			19.75	3.08
10	Palmitic	1.5	14.72	
			15.21	3.27
11	Oleic	0.6	9.51	
			9.55	0.42
12	Linoleic	1.5	19.07	
			18.66	2.17

The mean difference between these 12 sets of duplicate oxidations was 1.63%. On rare occasions when the difference between two duplicates was more than 5% a third oxidation was performed, and the two closest values used. The samples containing the same labelled fatty acid listed in Table 2 (samples 1-9), were prepared to contain the same amount of radioactivity, the total amount of fatty acids being varied by adding unlabelled chylomicrons to the labelled ones. These results indicated that the oxidation of the carboxyl carbon atom of these fatty acids to CO_2 was complete over the range of total fatty acids examined.

The activity of samples as determined with the ionization chamber were compared with the activity of the samples when determined by conventional counting methods. These comparisons are shown in Tables 3 and 4.

The ratios in Table 3 were calculated from data which were obtained over some period of time. There was no systematic variation in this ratio demonstrating the stability of the instrument with time.

The ratio in Table 4 is different to that in Table 3 because the geometry of counting was different. There is more inherent variation in counting CO_2 as $BaCO_3$ mainly due to errors in weighing the $BaCO_3$.

Table 3

The activity of chylomicron preparations as determined with the ionization chamber-vibrating reed electrometer combination compared with the activity determined by plating the chyle directly on lens tissues on polyethylene planchets (2 cm^2 in area) and counting with a G-M tube.

Sample	Activity with ionization chamber / Activity with Geiger counter (mV.cc./counts per minute)
1	7.41
2	7.54
3	7.89
4	7.38
5	7.62
6	7.29
7	7.44
8	7.37
9	7.39
Mean	7.48 ± 0.061

Table 4

The amount of $^{14}CO_2$ which passed through the ionization chamber as determined from the recorded electrometer input potential, compared with the activity of $BaCO_3$ plates prepared from the CO_2 which was collected in NaOH scrubbing towers. The activity of the $BaCO_3$ was measured with a G-M tube and observed counting rates were corrected to counting rates at zero mass.

Sample	Source of $^{14}CO_2$	Activity with ionization chamber Activity as $BaCO_3$ (mV.cc./counts per minute)	
1	Expired CO_2	5.89	
2	rat 57	5.42	mean 5.69
3		5.77	
4	Expired CO_2	5.87	
5	rat 58	5.94	mean 5.72
6		5.35	
7	Oxidation	5.18	
8		5.85	mean 5.52
		Overall mean 5.55	

These figures indicated that there was no difference in the performance of the ionization chamber-vibrating reed electrometer combination when used for experiments with animals or for oxidation procedures.

Use of the electrometer by the rate of charge method.

The precision of radio assay using the rate of charge method was good and two sets of triplicate assays are given in Table 5.

Table 5

Determination of $^{14}CO_2$ produced by triplicate oxidations of two chylomicron preparations.

Sample	Activity measured (mV)
1	332
	332
	331
2	337
	338
	341

When the electrometer was used in this way, it was incorporated into metabolism systems in which the air was recirculated and in each cycle, the air was completely saturated with water vapor in the NaOH scrubbing towers. The drying tubes incorporated into the flow system had to be very efficient to remove all of the water vapor. If there was any water vapor in the air in the ionization chamber, the current produced by a given amount of activity was increased. This was checked in every experiment. The background rate of charge was closely watched when the system was closed initially. Inefficient drying was indicated by an increase in this background rate of charge. Similarly the air flow was maintained for a period after the animal was removed at the end of an experiment to ensure the driers had not deteriorated during the experiment. If the background rate of charge did not return to the value determined before the experiment, the experimental results were discarded.

CHAPTER THREE

THE USE OF $^{14}CO_2$ EXCRETION PATTERNS IN THE STUDY OF THE OXIDATION OF ^{14}C-LABELLED COMPOUNDS IN INTACT ANIMALS

The oxidation of various ^{14}C-labelled compounds by intact animals can be studied by following the excretion of $^{14}CO_2$ in the animals' expired breath. The excretion of $^{14}CO_2$ cannot be related to the oxidation of the labelled substance with any precision however, unless information is available about the excretion of metabolically produced CO_2. An important preliminary to studies on the oxidation of ^{14}C-labelled fatty acids and ^{14}C-labelled glucose was to study the pattern of elimination of CO_2 from the body. As the experiments to be reported in this thesis have involved the use of continuous infusions of ^{14}C-labelled substances, the pattern of $^{14}CO_2$ excretion has been studied in rats given continuous intravenous infusions of $NaH^{14}CO_3$ under the same conditions as in the metabolism experiments.

The excretion of $^{14}CO_2$ during the continuous intravenous infusion of $NaH^{14}CO_3$ in fasted unanaesthetized rats. Twelve rats were starved for 24 hr and then infused continuously for 3 hr with $NaH^{14}CO_3$ (ca. 1.4 μc/hr) dissolved in physiological saline. The animals were trained to the experimental conditions by placing them in restraining cages, similar to the one used during the

experiment, for the 24 hr during which they were starved. Each rat was infused through an indwelling cannula and the rate of infusion of fluid was 1.4 ml./hr. These experiments were carried out with the metabolism system first described in Chapter 2. The amount of $^{14}CO_2$ which passed through the ionization chamber in successive 4 minute intervals during the course of each infusion was calculated and equated to the amount of radioactivity infused during this interval.

For most of the experiment, the rats rested or spent their time grooming themselves and cleaning their faces. They were permitted a degree of movement and were restrained only sufficiently to prevent them biting through their cannulae. During periods of spontaneous activity, the excretion of $^{14}CO_2$ increased significantly. In many cases, activities such as face cleaning were enough to almost double the rate of excretion of $^{14}CO_2$. Irregularities in the excretion of $^{14}CO_2$ by individual animals were smoothed out by plotting the pooled data from all the rats as these irregularities appeared to be distributed randomly with time. The mean excretion curve is shown in Fig. 22, where the rate of excretion of ^{14}C as $^{14}CO_2$, expressed as a percentage of the rate of infusion of ^{14}C as $[H^{14}CO_3]^-$, is plotted against time. The pattern of excretion showed an initial rapid phase and by the end of the first 20 minutes, the rate

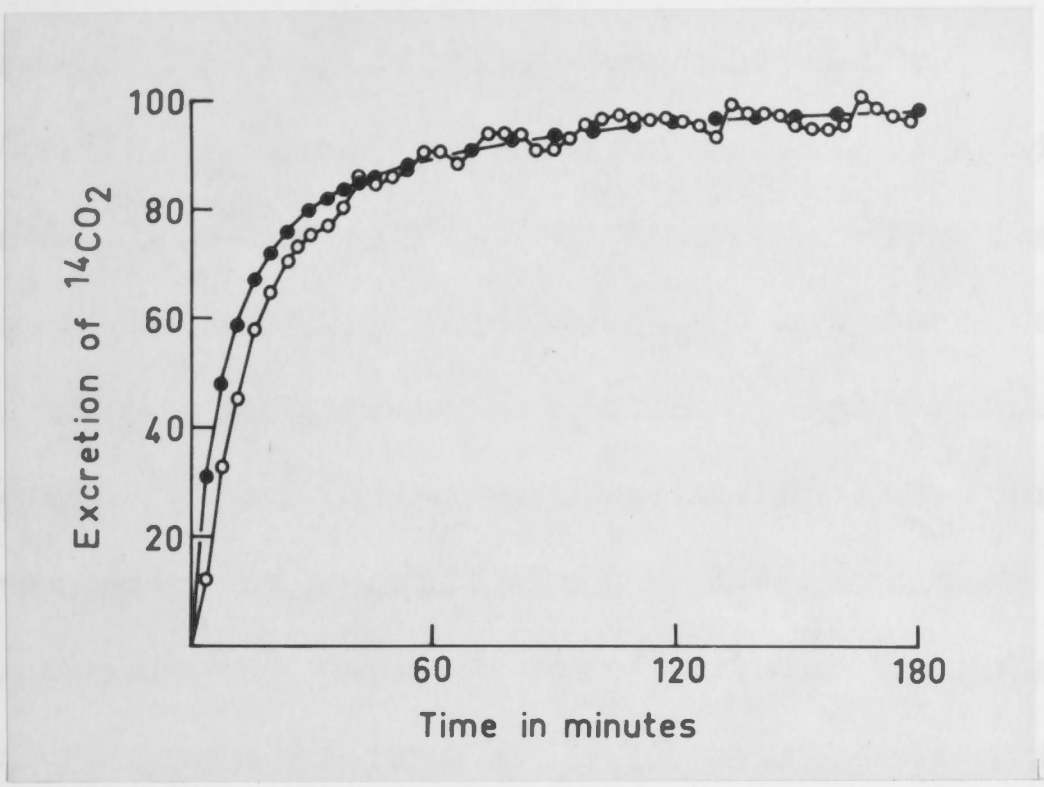


FIGURE 22.

The excretion of $^{14}CO_2$ by rats during the continuous intravenous infusion of $NaH^{14}CO_3$. The fractional rate of excretion of radioactivity as $^{14}CO_2$, expressed as a percentage of the rate of infusion of radioactivity is plotted against time. The mean curve constructed from the recorded excretion curves of 12 rats is shown (O), together with the derived true mean excretion curve (●) corrected for the distortion introduced by the compartments of the metabolism system.

of excretion of label was more than 60% of the rate of infusion. By the end of 60 minutes the rate of excretion of $^{14}CO_2$ had levelled off and reached 90% of the rate of infusion. Subsequently, the rate of excretion continued to rise slowly.

The recorded curve was resolved into exponential components. The pooled data were plotted as log(1 - rate of excretion/rate of infusion) against time. This curve is shown in Fig. 23 together with the two exponential components into which it was resolved. The exponential components were fitted by the method of least squares. Small irregularities in the excretion curve were greatly magnified in the logarithmic transformation when the rate of excretion was approaching its theoretical maximum. The rate at which radioactivity left the ionization chamber at any time, t minutes, during the infusion is given by

$$\frac{\text{Rate of excretion}}{\text{Rate of infusion}} = 1 - f_t - 0.88e^{-0.090t} - 0.29e^{-0.016t} \qquad (4)$$

The term, f_t, has been included in the above expression as the rate of excretion equals 0 when t equals 0. This term would include components contributed by the metabolism system as well as any more rapid components which may be associated with the excretion of the $^{14}CO_2$ by the rats.

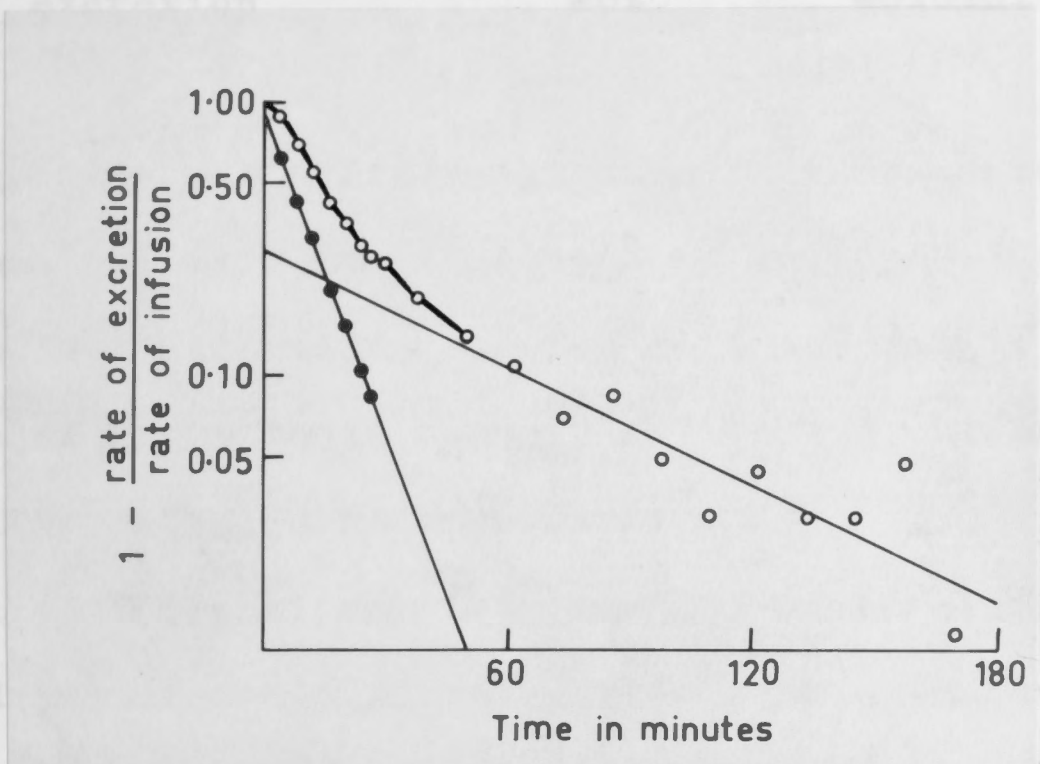


FIGURE 23.

The excretion of $^{14}CO_2$ by rats during the continuous intravenous infusion of $NaH^{14}CO_3$. The mean curve constructed from the recorded excretion curves of 12 rats was plotted as log(1 - rate of excretion of radioactivity as $^{14}CO_2$/rate of infusion of radioactivity) against time. A straight line was fitted to the terminal part of this curve by the method of least squares. This line was subtracted from the parent curve (O) to yield a second series of points (●). A second line was fitted to these points by the method of least squares. The curve was thus resolved into two exponential components.

The recorded excretion curve was corrected for the distortion introduced by the metabolism system (Appendix II). When corrected in this way, the true rate of excretion of radioactivity by the animals at any time, t minutes, is given by

$$\frac{\text{Rate of excretion}}{\text{Rate of infusion}} = 1 - 0.22e^{-bt} - 0.51e^{-0.090t} - 0.27e^{-0.016t} \qquad (5)$$

This equation was differentiated with respect to time so that some estimate might be made of the constant 'b' and so that this data might be used in interpreting the results of experiments using ^{14}C-labelled metabolites. When differentiated it becomes :

(Fraction of the bicarbonate in the extracellular pool expired per minute)x(Fraction of a single intravenous injection of $NaH^{14}CO_3$ in this pool at time, t minutes)

$$= 0.22be^{-bt} + 0.046e^{-0.090t} + 0.004e^{-0.016t} \qquad (6)$$

The derivation of this equation is given in Appendix III (equation 23). The amount of extracellular bicarbonate in the rats used was about 1.7 m-mole (cf. Shipley, Baker, Incefy & Clark, 1959), and the rate of CO_2 production was about 0.17 m-mole per minute. The fraction of the extracellular CO_2 expired per minute was thus 1/10th. For this rate of excretion the value of b becomes 0.227, and the fraction of an intravenous injection of $NaH^{14}CO_3$,

(F_t), remaining in the extracellular fluid pool at time t minutes following the injection is given by the following equation,

$$F_t = 0.50e^{-0.227t} + 0.46e^{-0.090t} + 0.04e^{-0.016t} \qquad (7)$$

<u>The effect of these characteristics of $^{14}CO_2$ excretion on the pattern of excretion of $^{14}CO_2$ produced during the oxidation of a ^{14}C-labelled metabolite</u>. When $^{14}CO_2$ enters the blood in a continuous fashion, the amount which enters during each small consecutive interval of time can be regarded as a single intravenous injection of $[H^{14}CO_3]^-$. By using the data presented above, it is possible to calculate how much of each 'injection' will remain in the extracellular fluid at any time after the injection. An approximation of the total amount of $^{14}CO_2$ present in the extracellular fluid at any time can be made by summing all the fractions of the individual 'injections' remaining at this time. As the number of single injections becomes very large, and the intervals of time become very small, this approximation will come closer to the true value. This is essentially the method used in Appendix III where the time course of excretion of $^{14}CO_2$ has been calculated for the special general case when the rate of entry of $^{14}CO_2$ into the extracellular fluid is itself a multi-exponential function of time. Thus it has been shown that, except for a few unique cases,

the excretion curve will be a pure multi-exponential function of time made up of the exponential components associated with both the formation of the CO_2 and its excretion from the bicarbonate pools.

The simplest type of oxidation pattern will be considered first, that is one that can be described by a single exponential function of time, such that during the continuous infusion of a ^{14}C-labelled compound, the rate of oxidation of the compound to $^{14}CO_2$ is given by the following equation,

$$\frac{\text{Rate of oxidation}}{\text{Rate of infusion}} = 1 - e^{-bt} \qquad (8)$$

By using the equations derived in Appendix III and equation (7) above, the rate of excretion of $^{14}CO_2$ during the oxidation will be given by the following equation,

$$\frac{\text{Rate of excretion}}{\text{Rate of infusion}} = 1 - a_1 e^{-0.227t} - a_2 e^{-0.090t} - a_3 e^{-0.016t} - a_4 e^{-bt} \qquad (9)$$

where $a_1 + a_2 + a_3 + a_4 = 1$, and the values of a_1, a_2, a_3, and a_4 are independent of time but are dependent on the value of b. The values of a_1, a_2, a_3 and a_4 are shown in Fig. 24 for all possible values of b.

a_1, a_2 and a_3, which are associated with the excretion of the $^{14}CO_2$ once it is produced, are related to b in a qualitatively similar fashion. When b is

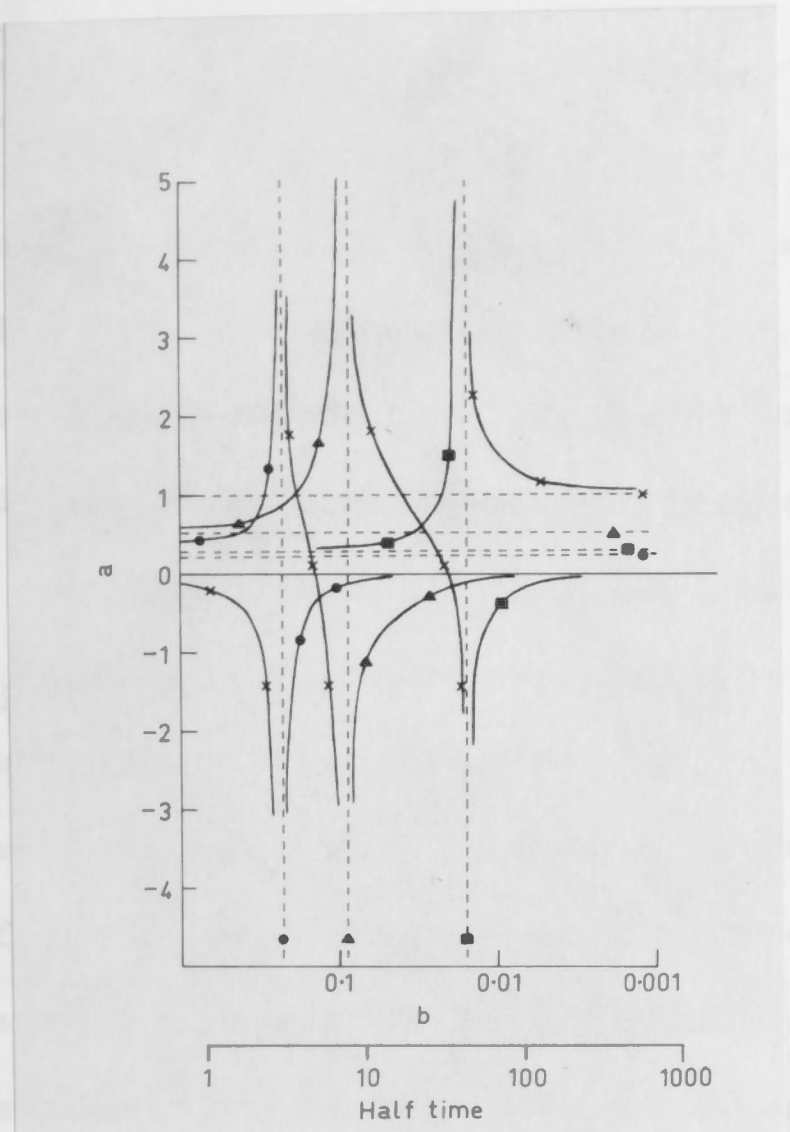


FIGURE 24.

The effects of the bicarbonate pools on the excretion of metabolically produced $^{14}CO_2$. The values of a_1 (●), a_2(▲), a_3(■) and a_4(×) in the equation

$$\frac{\text{Rate of excretion}}{\text{Rate of infusion}} = 1 - a_1e^{-0.227t} - a_2e^{-0.090t} - a_3e^{-0.016t} - a_4e^{-bt}$$

are shown for various values of b. This equation describes the excretion of $^{14}CO_2$ during the continuous infusion of a ^{14}C-labelled substrate which is oxidized in the body according to the following equation.

$$\frac{\text{Rate of oxidation}}{\text{Rate of infusion}} = 1 - e^{-bt}$$

Time is measured in minutes and b in reciprocal minutes.

large, the values of a_1, a_2 and a_3 tend towards those found when $[H^{14}CO_3]^-$ was infused continuously. As b decreases towards 0.227, 0.090 or 0.016 then a_1, a_2 or a_3 is positive and becomes very large and in the limiting case when b equals 0.227, 0.090 or 0.016, a_1, a_2 or a_3 is indeterminate and is no longer independent of time. When b is less than 0.227, 0.090 or 0.016, then a_1, a_2 or a_3 respectively becomes negative and is initially very large but decreases rapidly towards zero.

The value of a_4, which is associated with the formation of the $^{14}CO_2$, changes in a more complex fashion with changes in b. For very large values of b, a_4 tends to zero. As the value of b decreases towards 0.227, a_4 is negative and becomes very large. When b decreases from 0.227 to 0.090 and from 0.090 to 0.016, a_4 is initially positive and very large, becomes progressively smaller passing through zero when it becomes negative and very large again. When b equals, 0.227, 0.090 or 0.016 then a_4 is indeterminate and is no longer independent of time. When b is less than 0.016, a_4 is initially positive and very large but decreases as b becomes smaller and approaches 1 for very small values of b. There are two single values of b for which a_4 is equal to 0 and in fact the exponential involving b will not appear in such an excretion curve.

From Fig. 24 it can be seen that the rate of

formation of $^{14}CO_2$ can be calculated from observed $^{14}CO_2$ excretion curves for only some of the possible rates at which the formation of the $^{14}CO_2$ reaches its theoretical maximum. For an oxidative process taking less than 10 minutes for the rate of formation of $^{14}CO_2$ to reach 50% of the maximum it is impossible to separate the oxidative process from the processes of excretion. For processes taking between 10 and 20 minutes to reach 50% of the maximum $^{14}CO_2$ production, the component in the excretion curve describing the oxidation will not be affected very much by the kinetics of the bicarbonate pools, whilst for oxidation patterns that take longer than 70 minutes for the rate of oxidation to reach 50% of the theoretical maximum, the effects of the bicarbonate pools will be minimal.

The interactions between the rate at which the formation of $^{14}CO_2$ approaches the theoretical maximum and/the effects of the bicarbonate pools on the excretion of the $^{14}CO_2$ are shown graphically in Fig. 25 where simultaneous rates of formation and excretion of the $^{14}CO_2$ are plotted for different oxidation patterns. It can be seen that in the most rapid oxidation patterns, the $^{14}CO_2$ excretion curves are distorted very much, but as the rate of formation of the $^{14}CO_2$ takes longer to reach the theoretical maximum, distortion becomes less until its only effect is to make the excretion of the

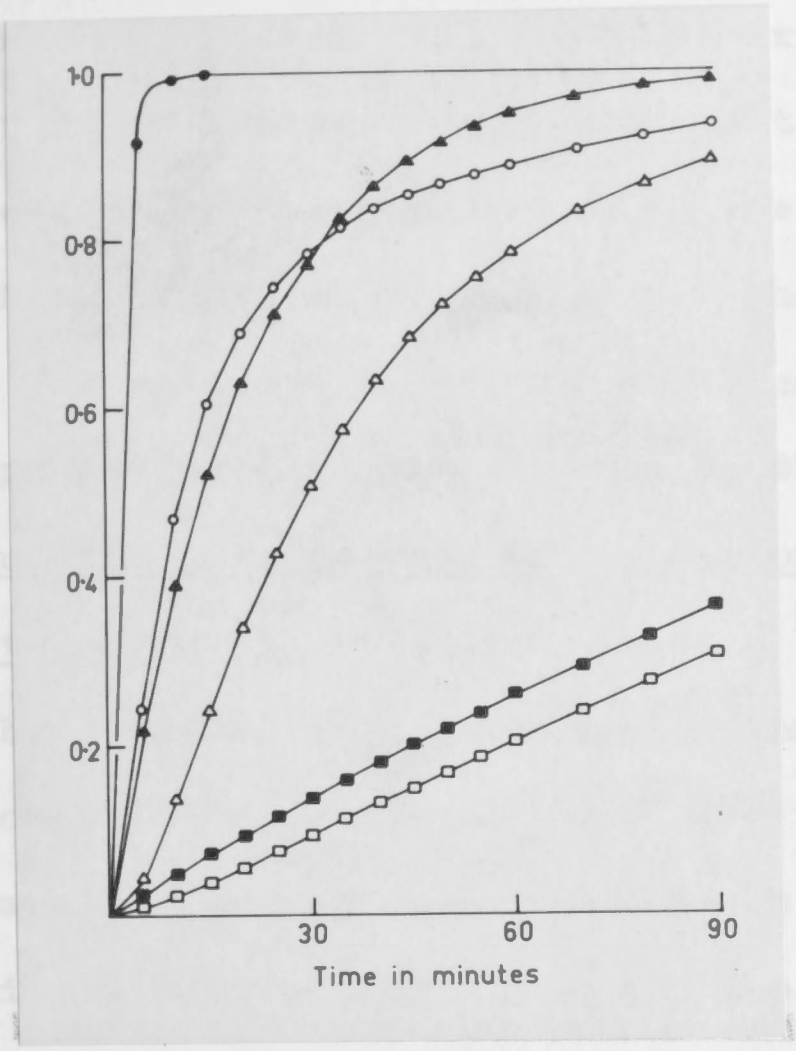


FIGURE 25.

The effects of the bicarbonate pools on the excretion of metabolically produced $^{14}CO_2$. Simultaneous rates of formation and excretion of $^{14}CO_2$ during the continuous infusion of ^{14}C-labelled substrates are shown for oxidations which can be described by

$$\frac{\text{Rate of oxidation}}{\text{Rate of infusion}} = 1 - e^{-bt}$$

Rates of formation (closed symbols) and rates of excretion (open symbols) of $^{14}CO_2$, given by the ordinate as a fraction of the theoretical maximum, are plotted against time for b = 0.5 (O) b = 0.05 (Δ) and b = 0.005 (□) in the above equation.

$^{14}CO_2$ lag behind its formation.

Most oxidation patterns will not be of the simple type discussed above, but will be multi-exponential functions of time. However each component will appear in the excretion curves, together with the components due to the bicarbonate pools except for the unique cases mentioned. The relative effect of the bicarbonate pools on each component will be the same as in the simple case.

Calculation of patterns of oxidation from observed $^{14}CO_2$ excretion curves. If the recorded $^{14}CO_2$ excretion curves can be resolved into exponential components, it should be possible to decide which of the various components are due to the formation of the $^{14}CO_2$, which are due to its excretion, or which are due to a mixture of both of these processes. These excretion curves must be analyzed graphically and it is not usually possible to resolve curves into more than 3 components (cf. Robertson, 1957). It is impossible also to separate components with similar half-lives. However, by knowing that certain components should appear in the excretion curves, it is possible to estimate their magnitude and by a series of successive approximations separate their effects from the other components. To illustrate this point, a hypothetical oxidation pattern will be considered such that during the continuous infusion of a ^{14}C-labelled metabolite, the rate of formation of $^{14}CO_2$ will be given

by

$$\frac{\text{Rate of oxidation}}{\text{Rate of infusion}} = 1 - 0.5e^{-0.05t} - 0.5e^{-0.005t} \qquad (10)$$

For such an oxidation pattern the rate of excretion of $^{14}CO_2$ would be given by

$$\frac{\text{Rate of excretion}}{\text{Rate of infusion}} = 1 + 0.03e^{-0.227t} + 0.34e^{-0.090t} - 0.13e^{-0.016t} - 0.66e^{-0.05t} - 0.57e^{-0.005t} \qquad (11)$$

When this function was plotted and analyzed graphically two components were separated and the rate of excretion of $^{14}CO_2$ as described by these components was

$$\frac{\text{Rate of excretion}}{\text{Rate of infusion}} = 1 - f_t - 0.58e^{-0.045t} - 0.63e^{-0.0055t} \qquad (12)$$

Both of these components are obviously associated with the oxidation of the substance, but from what has been said above, there should be components involving $e^{-0.227t}$, $e^{-0.090t}$ and $e^{-0.016t}$ and these components would be difficult to separate graphically from the ones determined in the first approximation of the excretion curve. From the components determined it is possible to calculate the magnitude of the components involving $e^{-0.090t}$ and $e^{-0.016t}$. When these were calculated they were found to be $+ 0.31e^{-0.090t}$ and $- 0.118e^{-0.016t}$. These components were subtracted from the observed excretion curve yielding a second curve

which was analyzed into exponential components. In this analysis the two components associated with the oxidation of the labelled substance were found to be $-0.63e^{-0.094t}$ and $-0.57e^{-0.0051t}$. The components associated with the bicarbonate pools were recalculated from this second approximation and found to be $+ 0.32e^{-0.090t}$ and $- 0.131e^{-0.016t}$. These second approximations are very close to the true values and it is obvious that in a third approximation they would be closer still. As the two components which were found to be associated with the oxidation of the labelled compound were sufficient to describe the oxidative process completely, it was not necessary to consider more rapid components. In practice it is doubtful if there would be any virtue in more than a second approximation. The oxidation pattern calculated from such an approximation would enable an estimate to be made of any more rapid components associated with the oxidation which could not be separated from the effects of the bicarbonate pools.

CHAPTER FOUR

THE OXIDATION OF CONTINUOUSLY INFUSED CHYLOMICRON FATTY ACIDS BY UNANAESTHETIZED RATS.

In order to study the oxidation of chylomicron fatty acids under conditions which simulate their normal mode of entry into the circulation from the gut, a series of experiments was carried out in rats in which ^{14}C-labelled chylomicrons of defined fatty acid composition were infused continuously into the blood stream. The rate of oxidation of ^{14}C-palmitic, ^{14}C-oleic and ^{14}C-linoleic acids incorporated into chylomicron triglycerides was studied.

In an initial series of experiments, the oxidation of ^{14}C-palmitic acid incorporated into chylomicrons was studied in rats infused at rates commensurate with normal rates of entry of fat into the blood from the gut. The effects of higher rates of infusion of chylomicron fat on the esterified fatty acid concentration of the circulating plasma were then studied and the results of these experiments were used in the design of experiments in which the oxidation of labelled palmitic, oleic and linoleic acids were compared when they were incorporated into chylomicrons of similar or varying fatty acid composition.

Experimental procedures. All the rats used in the experiments described in this chapter were carefully matched for age and weight and weighed 220 g and were 10 weeks of age when taken from their food. The rats were starved for 24 hr and were cannulated for infusion or for simultaneous infusion and blood sampling. The rate of infusion of fluid was 1.4 ml./hr in all experiments.

In experiments in which the levels of lipid in the plasma were followed, three groups of four rats were infused with chylomicrons at rates of 50, 75 and 100 mg total esterified fatty acid per hour. In addition several rats were infused at rates of up to 200 mg of total esterified fatty acid per hour. Blood samples (0.8 ml.) were collected at the beginning, and at the end of the 1st, 2nd and 3rd hr of each infusion. After each blood sample was collected, an equal volume of defibrinated rat's blood was injected into the animal. The plasma was separated from the blood by centrifugation at 4°C. The haematocrit value and the total esterified fatty acid concentration of the plasma were measured. No anticoagulants were injected into any of the animals.

In experiments in which the oxidation of chylomicron fatty acids was studied, five series of infusions were carried out. The infusions were made in random order in each series and were all completed within 10 days of preparing the infusion mixtures; four rats were infused

at each level. In the first series, the chylomicrons were used without further treatment after the clot had been removed by centrifugation. In all other experiments, the chylomicrons were concentrated by centrifugation at 20,000 x g for 30 min and resuspended in physiological saline. In the first four series of experiments, the chylomicrons were obtained from rats with thoracic duct fistulae which had been fed 'triolein'. In the first series, chylomicrons labelled with ^{14}C-palmitic acid were infused at rates of 5, 10, 20 and 40 mg total esterified fatty acid per hour. In the second, third and fourth series, chylomicrons labelled with ^{14}C-palmitic, ^{14}C-oleic and ^{14}C-linoleic acid respectively, were infused at rates of 50, 75 and 100 mg of total esterified fatty acid per hour. All of the rats in these series were infused for 3 hr. In the fifth series of infusions, chylomicrons were obtained from rats fed a special fat which had been prepared to contain approximately equal proportions of palmitic, oleic and linoleic acids. These chylomicrons, labelled with either ^{14}C-palmitic, ^{14}C-oleic or ^{14}C-linoleic acids, were infused at the rate of 40 mg total esterified fatty acid per hour for 4 hr.

The metabolism system described first in chapter 2 was used for these experiments. The amount of $^{14}CO_2$ which passed through the ionization chamber in consecutive

8 minute intervals during the infusions was calculated from the recorded traces. The total amount of CO_2 produced in each hour of the infusions was measured. At the end of the experiments, the animals were anaesthetized with pentobarbitone intravenously and the livers rapidly excised, washed and frozen in liquid air. The livers were either freeze-dried or stored at -59°C until the lipids were extracted and their radioactivity measured.

Results

The fatty acid composition of the total chylomicron lipids. The fatty acid composition of the chylomicron preparations used for the various experiments closely resembled that of the fed fats. The percentage fatty acid composition of the chylomicron preparations is given in Table 6.

Although the 'triolein' used in these experiments was laboratory reagent grade, only 36% of its total fatty acids was oleic acid. Traces of fatty acids other than those shown in Table 6 were present in the chylomicrons and these were also present in the fats which were fed.

The distribution of the labelled fatty acids in the chylomicron lipids. In the chylomicron preparations labelled with ^{14}C-palmitic or ^{14}C-oleic acids, no more than 5% of the label was present in phospholipid fatty

TABLE 6.

The mean fatty acid composition of the total chylomicron lipids infused in series 1-5. The fatty acids are classified by their chain length and degree of unsaturation.

Fat fed	Fatty acids (percentage)								
	12:0	14:0	14:1	16:0	16:1	18:0	18:1	18:2	20:0
'Triolein'	2	9	8	10	26	2	36	1	6
Special fat	2	5	3	25	8	2	15	36	4

acids. In chyle labelled with ^{14}C-linoleic acid, 18% of the label was present in phospholipid fatty acids when the concentration of linoleic acid in the chylomicrons was low (1%). When the concentration of linoleic acid was higher (36%) only 5% of the label was in this fraction. To verify this result, two further rats whose thoracic ducts had been cannulated were fed ^{14}C-linoleic acid dissolved in 'triolein'; 12% and 16% of the labelled fatty acid in the chylomicrons collected was in the phospholipids. This would seem to indicate that linoleic acid is preferentially esterified into phospholipids (cf. Whyte, Karmen & Goodman, 1962), but the total amount of the acid incorporated into phospholipids is limited by the absolute amount of phospholipid synthesized during fat absorption and the amount of linoleic acid present during absorption. When large amounts of linoleic acid are being absorbed, only a

small proportion of the total is incorporated into phospholipids. In the chyle labelled with ^{14}C-palmitic acid used in the first series of experiments, 1-2% of the label was present as unesterified fatty acid. In the other chylomicron preparations which were prepared by centrifugation, the radioactivity in the unesterified fatty acid fraction was not measured but this fraction would be expected to contain a smaller proportion of the label (cf. Olivecrona, 1962).

The oxidation of continuously infused chylomicrons labelled with ^{14}C-palmitic acid. The results of the first series of experiments are shown in Fig. 26. The mean rate of excretion of $^{14}CO_2$, expressed as a percentage of the rate of infusion of ^{14}C, is plotted against time for each infusion rate. Over a range of rates of infusion from 5-40 mg of total esterified fatty acid per hour, there was no significant decrease in the percentage of the infused material oxidized. The mean amounts of infused label excreted as $^{14}CO_2$ in each hour of the infusions are shown in Table 7.

As there appeared to be no change in the $^{14}CO_2$ excretion pattern when rats were infused with chylomicrons at rates of 5-40 mg of total esterified fatty acid per hr, it was decided to use higher rates of infusion of fatty acid in subsequent experiments. Before these rates of infusion were chosen, the effect of higher

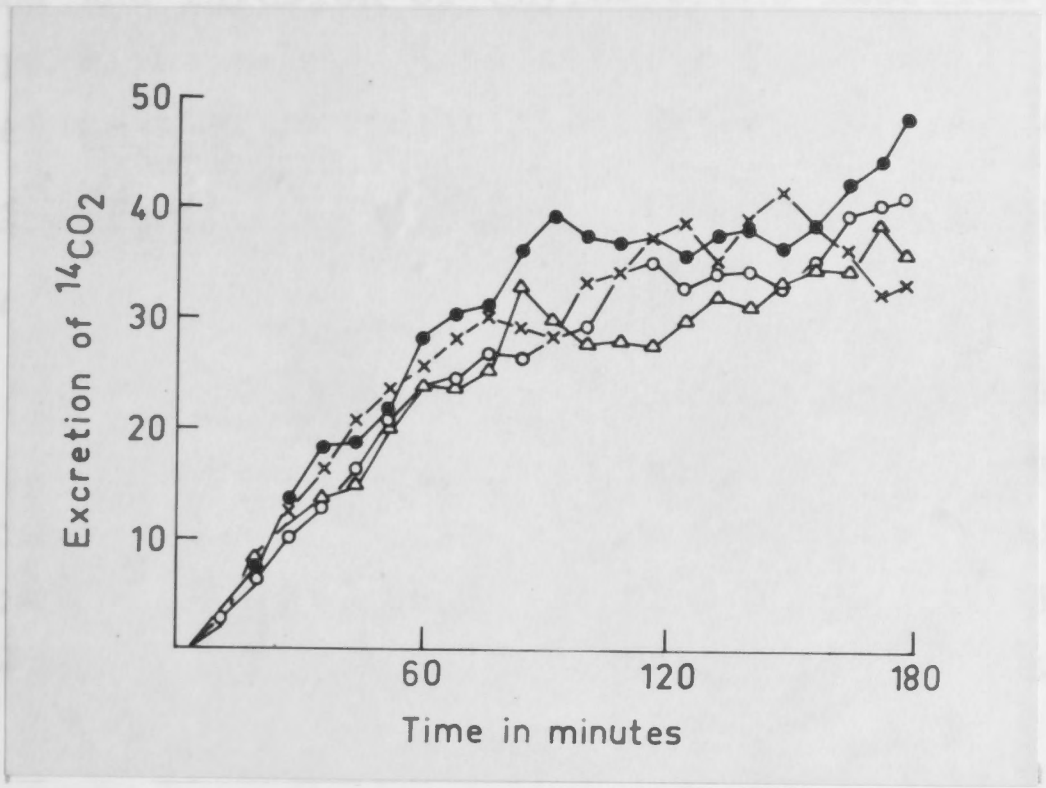


FIGURE 26.

The excretion of $^{14}CO_2$ by rats during the continuous intravenous infusion of chylomicrons labelled with ^{14}C-palmitic acid (Series 1). The fractional rate of excretion of radioactivity as $^{14}CO_2$, expressed as a percentage of the rate of infusion of radioactivity, is plotted against time. Chylomicrons were infused at rates of 5 (●), 10 (○), 20 (△) and 40 (×) mg of total esterified fatty acid per hour. Each curve is the mean result of 4 animals.

rates of infusion on the concentration of esterified fatty acids in the plasma was studied.

TABLE 7

The percentage of the amount of ^{14}C which was infused hourly excreted as $^{14}CO_2$ in each hour during the infusion of chylomicrons labelled with ^{14}C-palmitic acid. Mean results for four animals are given together with their standard errors.

Rate of infusion (mg/hr)	Percentage of infused ^{14}C excreted as $^{14}CO_2$		
	0-60 min.	60-120 min.	120-180 min.
5	13.1±1.69	35.0±4.85	39.7±3.68
10	10.8±1.02	29.0±0.51	36.0±2.65
20	11.1±0.81	27.6±0.60	33.5±2.23
40	13.1±0.68	31.2±1.50	37.1±2.62

The esterified fatty acid concentration in the plasma during the intravenous infusion of chylomicrons.

The results of these experiments are given in Table 8. The mean concentration of total esterified fatty acid in the plasma at the end of each hour of the 3 hr infusion period is shown for each rate of infusion. The fractional rate of turnover of the chylomicron fatty acids and their circulating half-lives were calculated assuming that the increased concentration of esterified fatty acids in the plasma was due to the presence of chylomicron lipid. The mean concentration

TABLE 8

The concentration of total esterified fatty acids in the plasma of rats during the continuous intravenous infusion of chylomicron fatty acids. The number of animals in each group is given in brackets. Mean results are given for each group together with their standard errors.

Rate of infusion of total esterified fatty acids (mg/hr)	Plasma concentration of total esterified fatty acids (mg/100 ml)			Amount in plasma (mg)	Fractional turnover rate (min^{-1})	Half-life (min)
	60min.	120min.	180min.			
50 (4)	170± 6	181±14	178± 7	4.9	0.17	4
75 (3)	253±10	259±20	255±23	11.0	0.11	6
100 (4)	483±80	534±80	616±124	40.0	0.042	16
150 (2)	717 910	1147 1298	1461 1616	114	0.022	31

of the total esterified fatty acids in the plasma of the rats at the start of the infusions was 117 mg/100 ml. of plasma. The plasma volume of the rats was taken to be 8 ml. (Metcoff & Favour, 1944). These calculations are shown in Table 8.

It can be seen that as the rate of infusion of total fatty acid increased from 50 to 100 mg/hr, the circulating half-life of the fatty acids increased about fourfold. The circulating half-life calculated for the infusion rate of 50 mg/hr was about 4 min. This rate of infusion is about equal to the maximum rate at which fat is absorbed by a rat given a meal of 1 ml. of olive oil. It could be assumed that with rates of infusion approaching more usual rates of absorption of fat from the gut (10-20 mg/hr), the circulating half-life of the chylomicron fat would be of the order of 1-2 minutes. This is close to the circulating half-life of free fatty acids (Laurell, 1959). It can be seen from the results that there was no linear relationship between the rate of infusion of fatty acids and their concentration in the plasma. At rates of infusion above 75 mg/hr, the concentration of fatty acids in the plasma rose very steeply and the plasma became milky. There was much more variation in the plasma levels at the higher infusion rates. The plasma concentration reached a reasonable plateau value by the end of the first hour

with the lower infusion rates. At infusion rates above 75 mg/hr, the plasma concentration rose during the second and third hours of the infusions. None of the rats appeared to be disturbed by the infusion of these large amounts of fat. In view of these results, 100 mg/hr was chosen as the highest rate of infusion for subsequent experiments.

The oxidation of ^{14}C-palmitic, ^{14}C-oleic and ^{14}C-linoleic acids incorporated into chylomicrons. The mean rates of excretion of $^{14}CO_2$, expressed as a percentage of the rate of infusion of ^{14}C, for each rate of infusion in Series 2, 3 and 4 are plotted against time in Figures 27, 28 and 29. For each series of infusions there was no significant difference in the percentage oxidized for the different rates of infusion of total esterified fatty acid. When the results for each labelled fatty acid and for each infusion rate were pooled, there were no significant differences between the rates of oxidation of the different labelled chylomicron fatty acids. However the initial rise in the rate of excretion of $^{14}CO_2$ tended to occur more slowly with the higher rates of infusion of fat. The mean amounts of label excreted in each hour of the infusions are given in Table 9. The mean excretion curves constructed from the pooled results in each series of infusions (1-4) are shown in Figure 30.

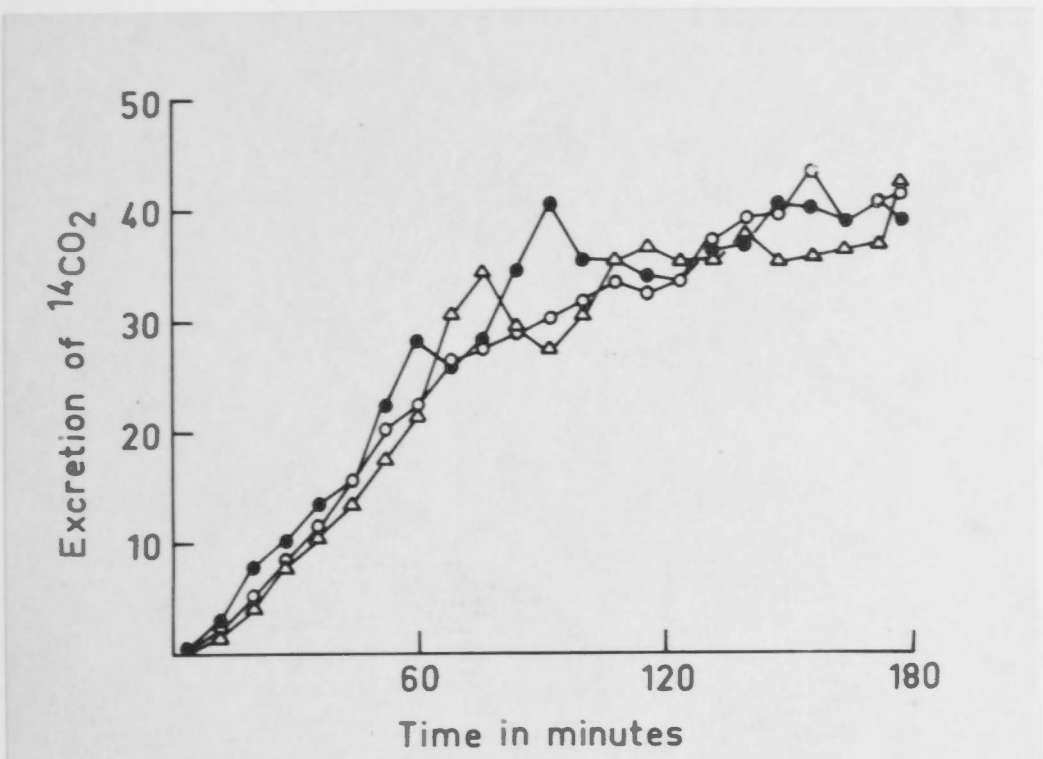


FIGURE 27.

The excretion of $^{14}CO_2$ by rats during the continuous infusion of chylomicrons labelled with ^{14}C-palmitic acid (Series 2). The fractional rate of excretion of radioactivity as $^{14}CO_2$, expressed as a percentage of the rate of infusion of radioactivity, is plotted against time. Chylomicrons were infused at rates of 50 (●), 75 (○) and 100 (△) mg total esterified fatty acids per hour. Each curve is the mean result of 4 animals.

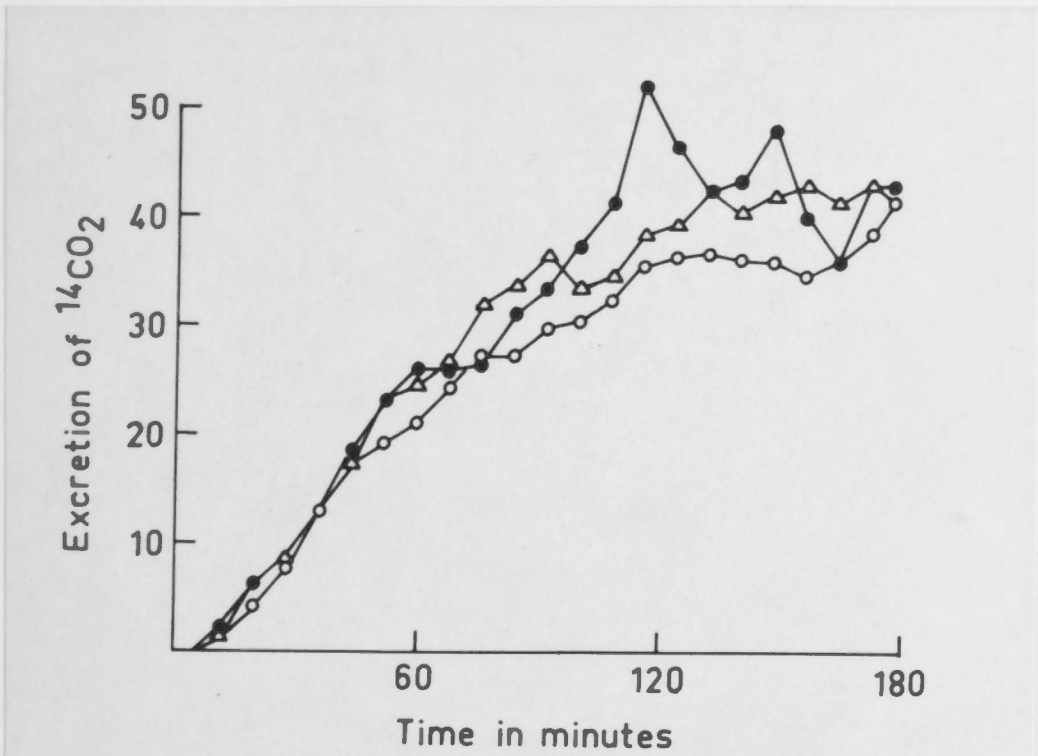


FIGURE 28.

The excretion of $^{14}CO_2$ by rats during the continuous infusion of chylomicrons labelled with ^{14}C-oleic acid (Series 3). The fractional rate of excretion of radioactivity as $^{14}CO_2$, expressed as a percentage of the rate of infusion of radioactivity, is plotted against time. Chylomicrons were infused at rates of 50 (●), 75 (○) and 100 (△) mg total esterified fatty acids per hour. Each curve is the mean result of 4 animals.

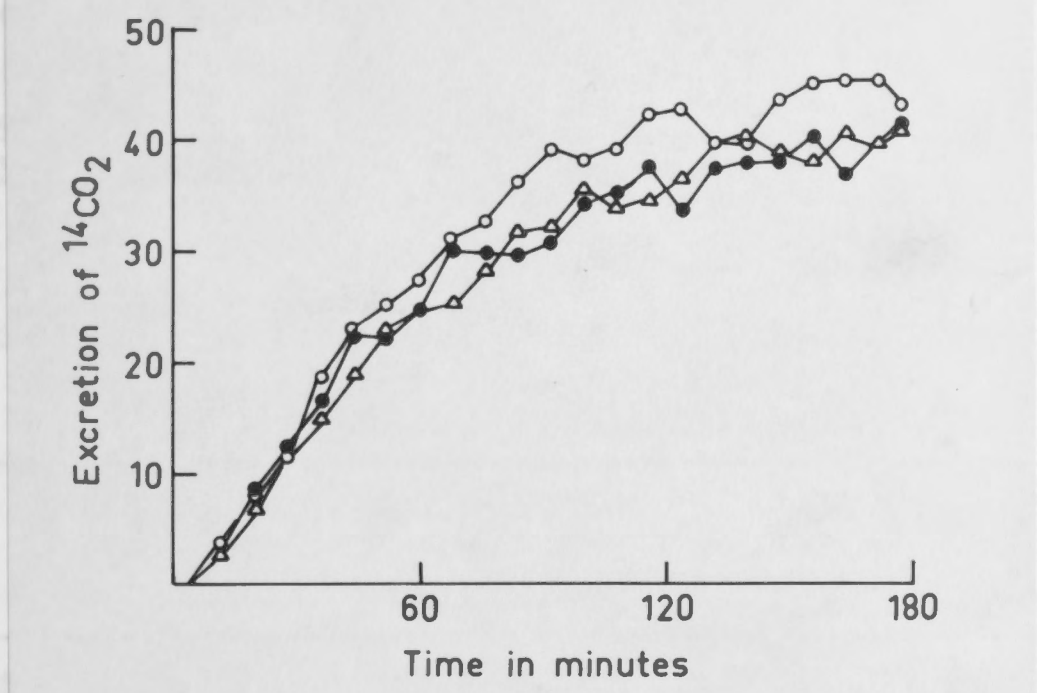


FIGURE 29.

The excretion of $^{14}CO_2$ by rats during the continuous infusion of chylomicrons labelled with ^{14}C-linoleic acid (Series 4). The fractional rate of excretion of radioactivity as $^{14}CO_2$, expressed as a percentage of the rate of infusion of radioactivity, is plotted against time. Chylomicrons were infused at rates of 50 (●), 75 (○) and 100 (▲) mg total esterified fatty acids per hour. Each curve is the mean result of 4 animals.

TABLE 9

The amount of $^{14}CO_2$, expressed as a percentage of the amount of ^{14}C infused hourly, excreted during each hour when rats were infused continuously with chylomicrons labelled with ^{14}C-palmitic, ^{14}C-oleic and ^{14}C-linoleic acids (Series 2, 3 and 4 respectively). Mean results for 4 animals are given together with their standard errors. The pooled results are the mean of 12 animals.

Labelled fatty acid	Rate of infusion	Amount of $^{14}CO_2$ excreted as p.c. of ^{14}C infused hourly		
		0-60 min.	60-120 min.	120-180 min.
Palmitic	50	11.7±0.99	33.1±1.45	38.2±2.11
	75	9.9±1.96	29.6±3.34	39.1±3.21
	100	8.9±2.64	31.3±2.62	36.7±1.84
Oleic	50	10.9±1.90	34.9±2.52	43.0±2.36
	75	9.8±0.82	29.2±2.37	37.0±1.61
	100	11.2±2.03	33.3±2.30	41.5±2.39
Linoleic	50	13.1±0.91	32.0±2.05	38.0±2.28
	75	14.1±2.38	36.4±5.62	43.1±3.94
	100	12.1±1.79	31.1±2.46	39.1±3.27
Pooled palmitic		10.2±1.09	31.3±1.42	38.0±1.32
Pooled oleic		11.1±0.93	32.4±1.45	40.6±2.42
Pooled linoleic		13.1±0.97	33.2±2.46	40.1±1.81
	Pooled 50	12.4±0.72	33.3±1.13	39.7±1.36
	Pooled 75	11.3±1.14	31.7±2.32	39.7±1.78
	Pooled 100	10.8±1.21	31.9±1.58	39.2±1.49

These curves show the essential similarity between the oxidation of the three labelled fatty acids. In all experiments the excretion of $^{14}CO_2$ was detectable in the first few minutes of the infusion. The rate of excretion rose rapidly for the first hour of the infusion and a relative plateau was attained in the second hour.

<u>The relationship between the rate of oxidation of the labelled fatty acids and the total CO_2 production</u>. The relationship between the rate of excretion of $^{14}CO_2$ and the rate of production of CO_2 was obscure. There was no significant correlation between the rate of excretion of $^{14}CO_2$ and the rate of excretion of CO_2 between different rats. However for individual rats, periods of spontaneous activity were always associated with temporary increases in CO_2 production accompanied by the excretion of increased amounts of $^{14}CO_2$. This was probably due to an increased rate of excretion of previously formed $^{14}CO_2$ present in the animals' bicarbonate pools. However, the individual variations in the rate at which the infused material was oxidized by different rats obscured any systematic relationship between the rate of oxidation of the infused fat and the metabolic rate as reflected by CO_2 production. The data for CO_2 production for all the animals in Series 2, 3 and 4 are summarized in Table 10.

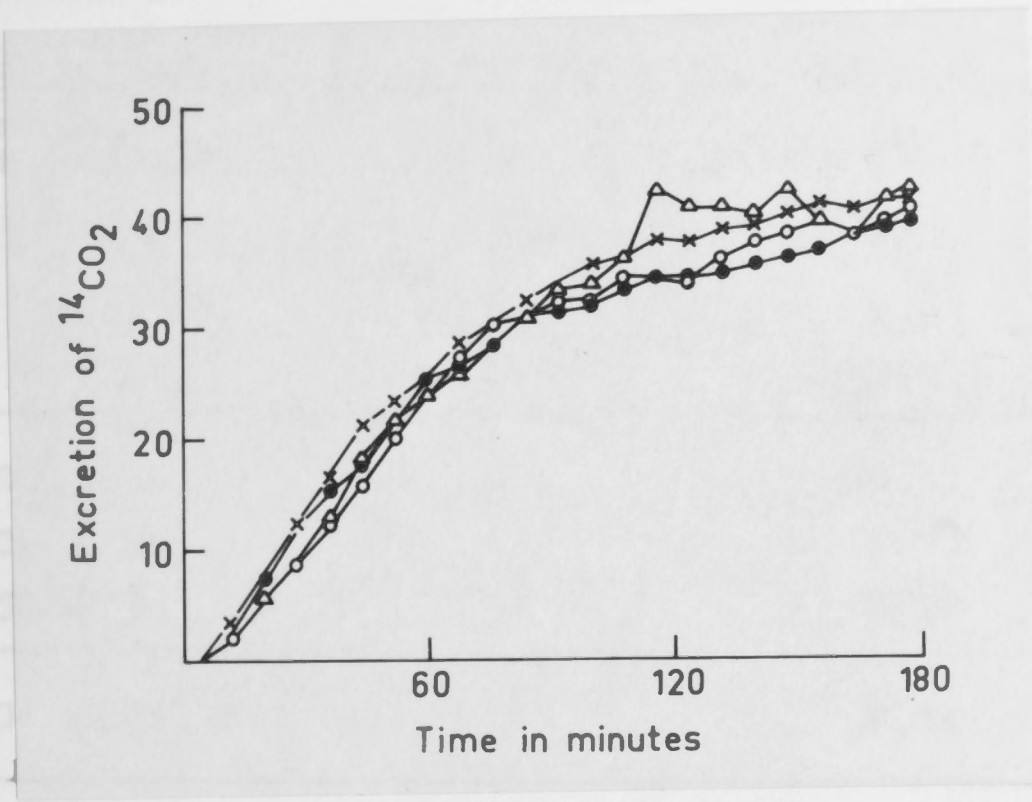


FIGURE 30.

The mean fractional rate of excretion of radioactivity as $^{14}CO_2$, expressed as a fraction of the rate of infusion of radioactivity, of all the rats in Series 1 (●), Series 2 (○), Series 3 (△) and Series 4 (×) plotted against time.

TABLE 10

The amount of CO_2 produced by the rats in Series 2, 3 and 4. The mean hourly CO_2 production for the different rates of infusion of lipid and for successive hours of the infusions are given together with their standard errors. The number of measurements is indicated in brackets.

Rate of infusion of lipid (mg/hr)		Hour of infusion	Mean CO_2 production (m-mole/hr)
50	(36)	1-3	9.43 ± 0.17
75	(36)	1-3	9.84 ± 0.23
100	(36)	1-3	9.95 ± 0.16
50-100	(36)	1	9.81 ± 0.19
50-100	(36)	2	9.82 ± 0.21
50-100	(36)	3	9.59 ± 0.18
50-100	(108)	1-3	9.74 ± 0.11

There was no significant difference between the mean hourly CO_2 production for any hour of the infusion and there was no systematic relationship between the rate of infusion of lipid and the total CO_2 production. However the rats infused with 100 mg of chylomicron fatty acids per hr produced about 5% more CO_2 than those infused with 50 mg/hr ($P < 0.05$). The individual fluctuations in CO_2 production were randomly distributed with time and this was seen in the smoother nature of the excretion curves when they were pooled (Figure 30).

By equating the excretion of $^{14}CO_2$ to the rate of production of total CO_2, a measure of the percentage of the animals' total CO_2 production which came from the oxidation of the infused material was made. These figures are given in Table 11. Some significant differences are seen in the results of the first series of infusions when expressed in this way. The percentage oxidized in the 5 mg/hr infusion experiments was significantly higher than in the 10 and 20 mg/hr infusion experiments but the biological significance of these results cannot be explained in relation to the other results obtained. The chyle used in the first series of experiments was not concentrated by centrifugation and some of the radioactivity in this preparation would be present in small lipoprotein complexes. The results of the first series of experiments therefore cannot be compared validly with those of the other series. It can be seen however that slightly less of the infused lipid was oxidized in the first series of experiments.

The effect of the chylomicron infusions on the liver lipids. The amount of lipid recovered from the livers of rats infused with chylomicrons in Series 2, 3 and 4 is shown in Table 12. These livers were freeze dried and the total lipid in samples of the dried livers was extracted and the weight of lipid and its radioactivity determined.

TABLE 11

The percentage of the chylomicrons infused hourly which was oxidized to CO_2 in the third hour of the infusion per 10 m-mole of CO_2 expired. The percentage of the CO_2 derived from oxidation of the chylomicrons was calculated on the assumption that the mean chain length of the fatty acids in the chylomicrons was 16, and their mean molecular weight was 256. Mean results are given for 4 rats in each group together with their standard errors.

Labelled fatty acid	Rate of infusion	Percentage of ^{14}C excreted per 10 m-mole CO_2	Percentage of CO_2 from chylomicrons
Palmitic	5	41.9 ± 1.53	1.3 ± 0.05
Series 1	10	34.9 ± 1.90	2.1 ± 0.11
	20	34.3 ± 1.41	4.3 ± 0.18
	40	36.7 ± 1.49	9.2 ± 0.37
Palmitic	50	41.8 ± 1.50	13.1 ± 0.47
Series 2	75	37.1 ± 2.20	17.4 ± 1.03
	100	38.2 ± 2.45	23.9 ± 1.53
Oleic			
Series 3	50	43.8 ± 2.02	13.7 ± 0.63
	75	41.5 ± 1.72	19.4 ± 0.80
	100	40.5 ± 1.37	25.3 ± 0.86
Linoleic			
Series 4	50	42.9 ± 1.31	13.4 ± 0.41
	75	45.5 ± 4.96	21.3 ± 2.32
	100	41.7 ± 2.82	26.1 ± 1.77

TABLE 12

The lipid content of livers taken from rats infused with chylomicrons labelled with ^{14}C-palmitic, ^{14}C-oleic and ^{14}C-linoleic acids at various rates for 3 hr. The mean results are given together with their standard errors. The number of livers examined is shown in brackets.

Rate of infusion (mg/hr)	% lipid in liver (g/100 g dry weight)
50 (8)	18.5 ± 1.3
75 (10)	19.1 ± 0.9
100 (12)	18.2 ± 0.7

Labelled chylomicrons	Amount of infused ^{14}C in liver (% of hourly infusion/mg liver lipid)
Palmitic (7)	0.058 ± 0.005
Oleic (12)	0.048 ± 0.004
Linoleic (11)	0.112 ± 0.005

There was no apparent relationship between the total amount of lipid recovered in the liver and the rate of infusion of lipid. The percentage of the amount of radioactive lipid infused each hour which was present in the liver at the end of the experiment was the same for each rate of infusion. These results show that although the total amount of labelled lipid taken up by the liver increased with increasing rates of infusion of lipid, the total amount of lipid in the liver did not change. This suggested that some adjustment occurred in the relative

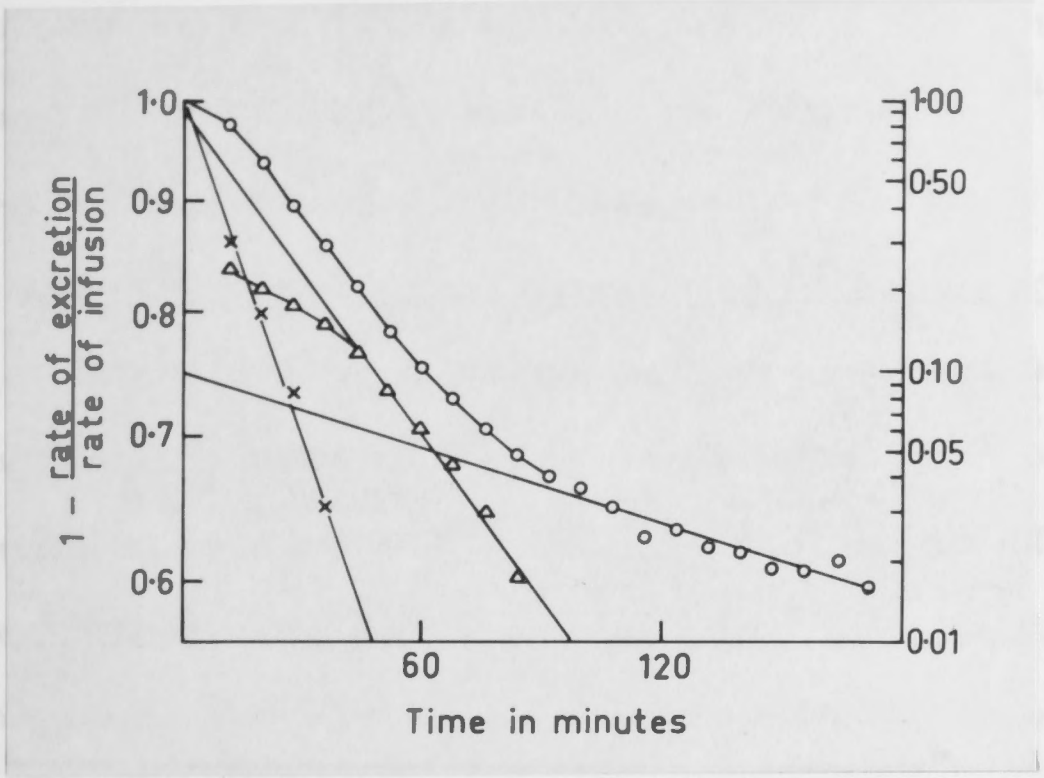


FIGURE 31.

The characteristics of the excretion curve for $^{14}CO_2$ in rats infused continuously with ^{14}C-labelled chylomicrons. The parent curve (O) is the mean result of experiments in Series 2, 3 and 4, plotted as log(1 - rate of excretion of radioactivity as $^{14}CO_2$/rate of infusion of activity) against time. A straight line has been fitted to the terminal part of this curve by the method of least squares. This line was subtracted from the parent curve to yield a second curve (Δ). By repeating this process a third curve was obtained (×). The parent curve was thus resolved into three exponential components. The ordinate for the parent curve and the slowest component is on the left of the graph, whilst that for the two derived curves and more rapid components is on the right of the graph.

amounts of endogenous and exogenous lipid which entered the liver at the different rates of infusion. A significantly higher proportion of the infused radioactivity was present in the liver at the end of infusions of ^{14}C-linoleic labelled chylomicrons than with ^{14}C-palmitic or ^{14}C-oleic labelled chylomicrons ($P < 0.001$).

Kinetic considerations of the $^{14}CO_2$ excretion curves. The relative plateau which was attained in the $^{14}CO_2$ excretion curves during the second hour of the infusions, suggested a possible partitioning of the infused chylomicron fatty acids. In order to investigate this further, the excretion curves were analyzed kinetically. The pooled results of experiments in Series 2, 3 and 4 were plotted as log(1 - rate of excretion of ^{14}C as $^{14}CO_2$/rate of infusion of ^{14}C) against time. The mean curve obtained in this way was resolved into a series of exponential components. From this analysis, which is shown in Figure 31, it can be seen that as a first approximation, at any time t minutes after the start of the infusion of chylomicrons, the rate at which the animals excreted the infused label, as detected in the ionization chamber, is given by

$$\frac{\text{Rate of excretion}}{\text{Rate of infusion}} = 1 + 1.02e^{-0.094t} - 0.92e^{-0.046t} - 0.75e^{-0.0013t} + f_1(t) \qquad (13)$$

When this is corrected for the distortion introduced by the metabolism system, the infused animals' true mean rate of excretion to give such a recorded curve is given by

$$\frac{\text{Rate of excretion}}{\text{Rate of infusion}} = 1 + 0.58e^{-0.094t} - 0.72e^{-0.046t} - 0.75e^{-0.0013t} - f_2(t) \qquad (14)$$

The terms $f_1(t)$ and $f_2(t)$ have been included in the above equations because the rate of excretion equals 0 when t equals 0. It can be inferred that the term $0.58e^{-0.094t}$ which delays the excretion of the $^{14}CO_2$ is associated with the excretion of $^{14}CO_2$ from the bicarbonate pools. A component similar to this appeared in the $^{14}CO_2$ excretion curves which were obtained when rats were continuously infused with $NaH^{14}CO_3$ (cf. Chapter 3). However the mean circulating half-life of the chylomicrons is also of the same order as this component and it is likely that this too will contribute to the component. Once this term has become negligible, that is when the circulating level of chylomicrons has attained a plateau value and the rate of excretion of $^{14}CO_2$ is approximately equal to its rate of production, any subsequent rise in the rate of excretion of $^{14}CO_2$ is due to the two slower components. From a consideration of these two components and the dynamics of the bicarbonate pools, it is possible to calculate the rate at which the chylomicron fatty acids were being oxidized at any time

during the infusions so as to give such a $^{14}CO_2$ excretion curve. This is given in the following equation,

$$\frac{\text{Rate of oxidation}}{\text{Rate of infusion}} = 1 + 0.36e^{-bt} - 0.61e^{-0.046t} - 0.75e^{-0.0013t} \quad (15)$$

This oxidation pattern was then used to recalculate the components due to the bicarbonate pools which should have appeared in the $^{14}CO_2$ excretion curves so that they could be subtracted from the recorded curve and a second approximation made of the oxidation pattern. As the half-life of the term involving e^{-bt} in this pattern is expected to be short, it would be impossible to predict its effect on the excretion curves and so it was disregarded and only the two longer half-life components used in the second approximation. The calculated rate of excretion from this oxidation pattern was

$$\frac{\text{Rate of excretion}}{\text{Rate of infusion}} = 1 + 0.52e^{-0.090t} - 0.72e^{-0.046t} - 0.15e^{-0.016t} - 0.75e^{-0.0013t} - f_3(t) \quad (16)$$

and this would be recorded by the electrometer as

$$\frac{\text{Rate of excretion}}{\text{Rate of infusion}} = 1 + 0.87e^{-0.090t} - 0.92e^{-0.046t} - 0.16e^{-0.016t} - 0.75e^{-0.0013t} - f_4(t) \quad (17)$$

The two terms due to the bicarbonate pools, $+ 0.87e^{-0.090t}$

and $-0.16e^{-0.016t}$, were subtracted from the pooled curve and this corrected curve was analyzed as before. This analysis is shown in Figure 32.

The true rate of excretion of $^{14}CO_2$ by the animals calculated from this analysis was

$$\frac{\text{Rate of excretion}}{\text{Rate of infusion}} = 1 + 0.52e^{-0.090t} - 0.62e^{-0.052t} - 0.15e^{-0.016t} - 0.70e^{-0.0011t} - f_5(t) \qquad (18)$$

The second approximation of the rate of oxidation of the chylomicrons calculated from this was

$$\frac{\text{Rate of oxidation}}{\text{Rate of infusion}} = 1 + 0.26e^{-bt} - 0.56e^{-0.052t} - 0.70e^{-0.0011t} \qquad (19)$$

The value of the constant b which largely describes the removal of the chylomicrons from the circulation, will depend on the interconnections in the three pool system required to account for the oxidation pattern. It is profitless at this stage to speculate as to the nature of the pool system, but it is of interest that the oxidation of the chylomicron fatty acids can be partitioned into a rapid and a slow component and this information might be of interest in experiments in which the oxidation of the chylomicron fatty acids is altered by experimental procedures.

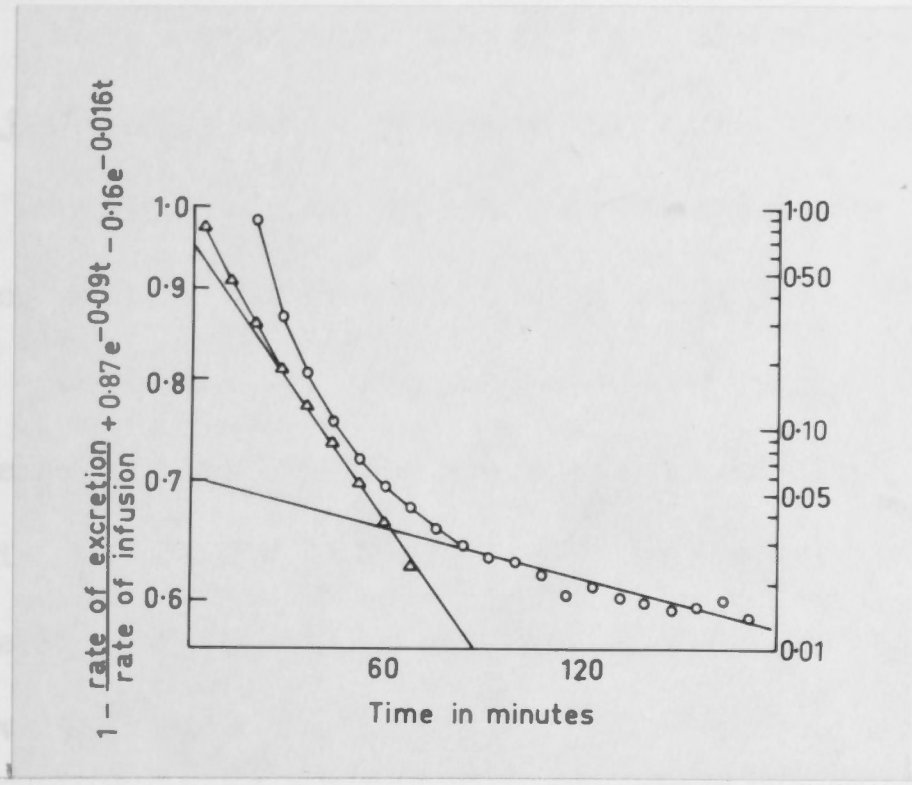


FIGURE 32.

The characteristics of the excretion curve for $^{14}CO_2$ in rats infused continuously with ^{14}C-labelled chylomicrons, second approximation. See text for explanation.

The effect of the fatty acid composition of chylomicrons on the oxidation of palmitic, oleic and linoleic acids incorporated into them. The oxidation of the labelled fatty acids can only be compared validly when they are introduced into the animal in chylomicrons containing approximately equal amounts of the three fatty acids. In the experiments described above, oleic acid was the major fatty acid present in the chylomicrons used; the concentration of palmitic acid was less than half that of oleic whilst linoleic acid was present in only trace amounts. It was decided to perform a series of experiments in which the concentrations of palmitic and linoleic acids in the chylomicrons were increased, and to extend the duration of the infusions to see whether the relative plateau in the $^{14}CO_2$ excretion curve persisted throughout the fourth hour of such infusions. The results of these experiments are shown in Figure 33 where the excretion of $^{14}CO_2$, expressed as a percentage of the rate of infusion of ^{14}C, is plotted against time for each labelled fatty acid. It can be seen that the excretion curves are of the same general form as the ones described above and the relative plateau persisted through the fourth hour of the infusions. The oxidation of the ^{14}C-oleic and ^{14}C-linoleic acids was quite similar but the oxidation of the palmitic acid was reduced. The amount of the infused label excreted in each hour of the

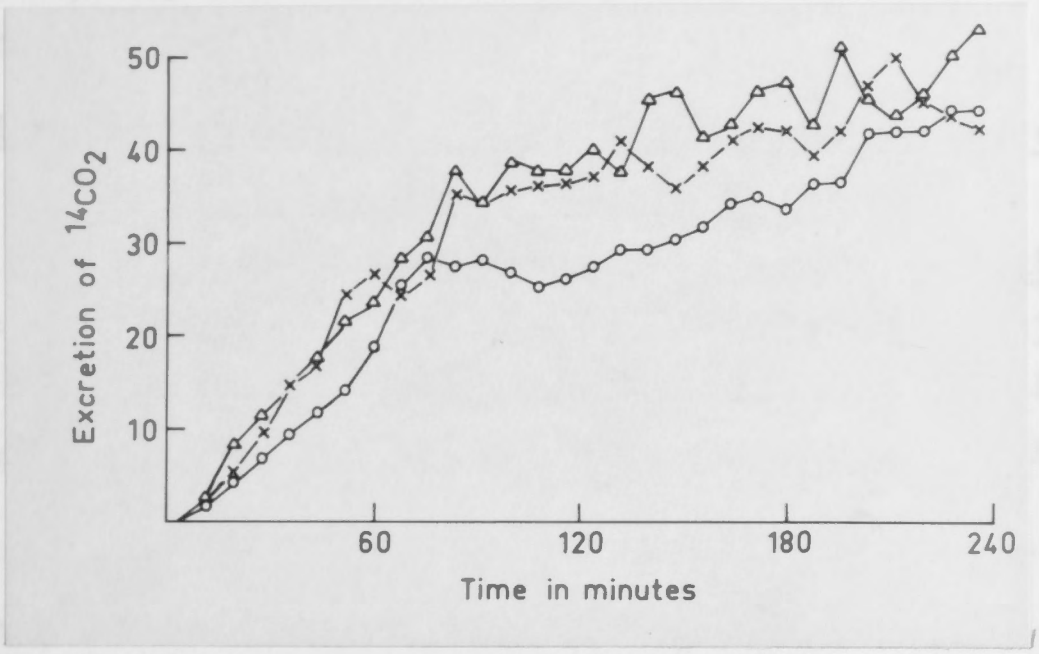


FIGURE 33.

The excretion of $^{14}CO_2$ by rats during the continuous infusion of chylomicrons labelled with ^{14}C-palmitic (O), ^{14}C-oleic (Δ) and ^{14}C-linoleic (×) acids (Series 5). The fractional rate of excretion of radioactivity, expressed as a percentage of the rate of infusion of radioactivity, is plotted against time. Chylomicrons were infused at a rate of 40 mg of total esterified fatty acids per hour. Each curve is the mean result of 4 animals.

infusions for each labelled acid per 10 m-mole CO_2 expired is given in Table 13.

TABLE 13

The percentage of the ^{14}C infused hourly which was excreted as $^{14}CO_2$ per 10 m-mole of CO_2 in each hour of the infusions in Series 5. Mean results are given for each group of 4 animals together with their standard errors.

Labelled fatty acid	Percentage of ^{14}C infused hourly excreted as $^{14}CO_2$ per 10 m-mole CO_2 expired			
	0-60min.	60-120min.	120-180min.	180-240min.
Palmitic	8.5±0.9	26.8±1.5	34.3±0.8	38.2±0.2
Oleic	10.3±1.2	34.7±2.4	43.4±1.9	47.2±2.3
Linoleic	11.9±1.3	32.3±1.9	38.9±1.6	43.4±1.9

From these figures it can be seen that, in the second, third and fourth hours of the infusions, the oxidation of the palmitic acid was significantly lower than the oxidation of the oleic and linoleic acids. The biological significance of this result is obscure. The absolute amount of palmitic acid infused in these experiments was of the same order as that infused in the previous experiment when 100 mg total esterified fatty acid was infused per hour. The increase in the amount of label excreted in the fourth hour over that in the third hour indicated that the biological half-life of the fatty acids oxidized slowly in these experiments

was of the same order as that in the previous experiments. The equivalent exponential components describing this terminal part of the curves for the different fatty acids were; palmitic, $e^{-0.0010t}$; oleic, $e^{-0.0012t}$; and linoleic, $e^{-0.0012t}$. It would seem from this that the smaller amount of palmitic acid oxidized in these experiments was due to a decrease in the amount of this acid oxidized relatively quickly.

The total amount of radioactivity present in the livers at the end of the infusions, expressed as a percentage of the hourly rate of infusion, was $25.3 \pm 2.7\%$, $13.5 \pm 0.8\%$ and $18.8 \pm 3.5\%$ for the palmitic, oleic and linoleic acid label experiments respectively. The recoveries of labelled fat in the livers of the animals infused with ^{14}C-palmitic acid labelled chylomicrons was significantly higher than in animals infused with oleic acid labelled chylomicrons ($p < 0.01$). This result should be compared with that in the previous series where the recovery of labelled palmitic and oleic acids in the liver were very similar whilst twice as much linoleic acid was recovered in the liver.

CHAPTER FIVE

THE EFFECT OF THE INFUSION OF GLUCOSE ON THE METABOLISM OF CONTINUOUSLY INFUSED CHYLOMICRON FATTY ACIDS

Experiments reported in the literature have shown that animals 'loaded' with carbohydrate oxidize a smaller proportion of a single injection of ^{14}C-labelled fatty acids than do fasted animals. The physiological significance of these experiments is clouded by the extraordinarily large amounts of glucose used. Experiments in which single injections of labelled fatty acids have been given are difficult to interpret and the results presented in Chapter 4 of this thesis have indicated that the oxidation of a single injection of chylomicron fatty acids is not strictly comparable to the oxidation of continuously infused chylomicron fatty acids.

As it is possible to provide a large proportion of the immediate energy requirements of rats with continuously infused chylomicron fatty acids, it was decided to study the effect of glucose on the oxidation of continuously infused chylomicron fatty acids when the glucose was infused simultaneously at rates commensurate with its normal rates of entry into the circulation. Before these experiments could be done, it was necessary to study some of the parameters of normal glucose metabolism in rats.

Experimental procedures

The normal rates of entry of endogenous glucose into the circulation of fasted rats and the effect of exogenous glucose on these rates was studied by isotope dilution using the method of Searle, Strisower & Chaikoff (1956) in which a priming dose of labelled glucose was given to the animal followed by a continuous infusion of labelled glucose. These experiments were done only to get an idea of the order of magnitude of the rates of entry of glucose and consequently they were not as rigorously controlled as the assumptions underlying the calculations would require.

Rats which had been starved for 24 hr and cannulated for continuous intravenous infusions were given a priming dose of generally labelled ^{14}C-glucose followed by a continuous intravenous infusion of the labelled glucose in virtually weightless amounts. The priming dose of glucose was approximately one half of the amount of radioactive glucose to be infused each hour. In experiments in which the effect of exogenous glucose on the rate of entry of endogenous glucose was studied, unlabelled glucose was infused together with the labelled glucose. The rats were anaesthetized with pentobarbitone and bled out after 2 or 3 hr of infusion and the blood glucose isolated as potassium gluconate. The specific activity of the blood glucose was determined

and the rate of entry of endogenous unlabelled glucose carbon was calculated as follows:

Rate of entry of unlabelled glucose carbon

$$= \frac{\text{Rate of infusion of radioactivity as glucose C}}{\text{Specific activity of the blood glucose C}}$$

It was found that the glucose used for these experiments was not radiopure. Although it was of stated 99% radiopurity, not more than 82% of the radioactivity could be recovered as potassium gluconate. This was confirmed by paper chromatography of the glucose with carrier glucose. This glucose had been stored for some time in the dry state before it was used and had apparently undergone some chemical change. The nature of the impurities was not determined (cf. autoradiograph of glucose chromatogram Figure 9) but the results were corrected for the maximum possible error introduced by the impurity, and are given in Table 14.

TABLE 14

The rates of entry of glucose into the blood stream of rats fasted for 24 hr, with and without the infusion of exogenous glucose. Mean results are given for each group together with their standard errors. The number of experiments is indicated in brackets.

Group		Glucose entry rate (mg/100 g B.W./hr) Total	Exogenous	Endogenous
Bled out after 2hr.	(5)	62±5	0	62±5
Bled out after 3hr.	(5)	71±7	0	71±5
Infused with glucose bled out after 3 hr.	(6)	126±8	69±3	57±8

The entry rate calculated when the rats were bled out after 3 hr infusion was not significantly different from that calculated when they were bled out after 2 hr infusion. The endogenous rate of entry of glucose measured when glucose was infused in moderate amounts was only slightly less than when no glucose was infused and this difference was not significant.

The effect of the rate of infusion of glucose on the concentration of glucose in the circulating plasma. A number of rats were starved for 24 hr and then infused with glucose at rates between 25 and 400 mg/hr. Blood samples were collected from an indwelling cannula at the beginning and at the end of the first, second and third hours of these infusions and the concentration of glucose in the plasma was measured. The mean concentration of glucose in the plasma of these animals during the experiment is shown in Figure 34. The mean pre-infusion plasma glucose concentration was 117 $\pm$ 3.6 mg/100 ml. As the rate of infusion of glucose increased, the plasma glucose concentration tended to rise until a concentration of approximately 160 mg/100 ml. was reached. It remained at about this concentration over a range of rates of infusion between 50-200 mg/hr. When the rate of infusion of glucose exceeded 200 mg/hr the plasma glucose concentration increased rapidly with increasing rates of infusion of glucose. In other

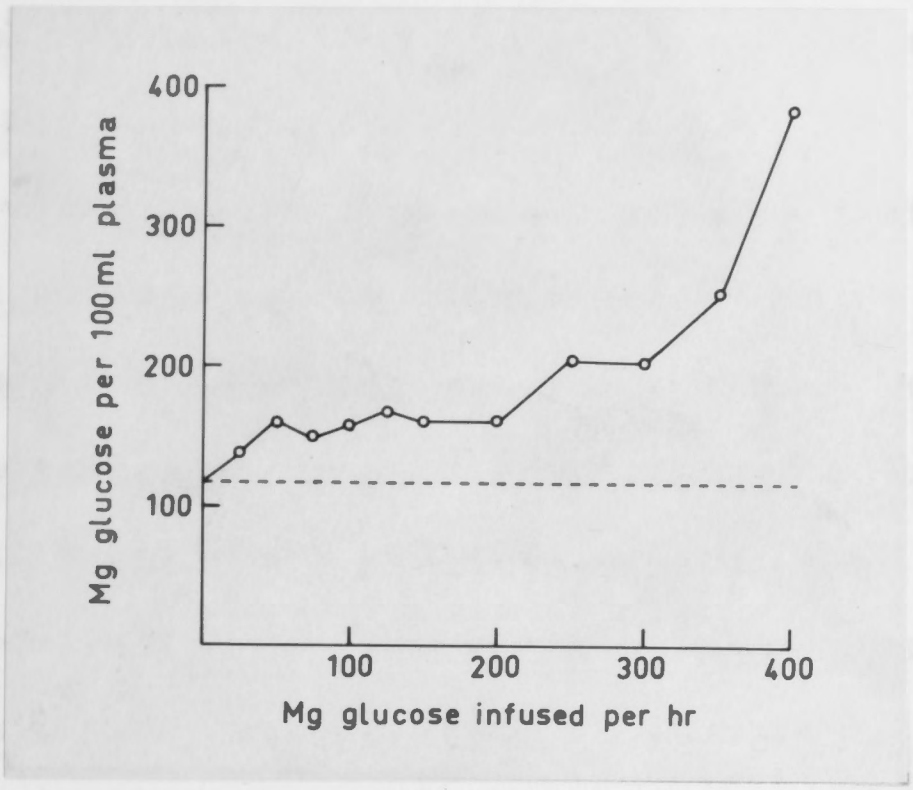


FIGURE 34.

The effect of the intravenous infusion of glucose on the concentration of glucose in the plasma of rats. The concentration of glucose during the infusion is plotted against the rate of infusion of glucose. The concentration of glucose was measured in samples of plasma obtained at the end of the 1st, 2nd and 3rd hours of the infusion, and each point on the graph is the mean of three such measurements from a single rat.

experiments it was observed repeatedly that when rats were infused with moderate amounts of glucose, their plasma glucose level rose during the infusion to this plateau concentration of about 160 mg/100 ml. When an infusion of glucose was given to a rat with a thoracic duct fistula, the glucose concentration in the thoracic duct lymph increased to a similar level and remained constant for a considerable time.

It would appear from these results that the level of plasma glucose can be maintained at about 160 mg/100 ml even though the supply of exogenous glucose varies greatly. It does not seem to be purely coincidental that this concentration is about the same as the level at which the concentration of free intracellular glucose in the liver equals the concentration of glucose in the plasma (Cahill, Ashmore, Searle & Zottu, 1958). The range of rates of infusion of glucose over which this plateau concentration persisted is of the same order as the measured rate of entry of glucose when no glucose was infused. It is possible that once this plateau concentration of glucose was produced, the increased rates of infusion of exogenous glucose were compensated for by decreased rates of entry of endogenous glucose. When the entry of endogenous glucose reached zero, the plasma glucose concentration increased with further increases in the rate of infusion of glucose.

The oxidation of continuously infused ^{14}C-glucose.

Rats which had been starved for 24 hr were infused continuously with generally labelled ^{14}C-glucose. The ^{14}C-glucose was infused either in virtually weightless amounts or together with unlabelled glucose at rates of 100, 200 and 300 mg/hr. The labelled glucose was used soon after it was received from the manufacturer and was stated to be 99% radiopure. The rats were infused for 3 hr and the consumption of oxygen and excretion of $^{14}CO_2$ were recorded continuously during the infusions. The CO_2 expired in successive half-hour intervals during the infusions was measured and the respiratory quotients calculated. Three rats were infused at each rate of infusion of glucose: the infusion of the unlabelled glucose was begun 1 hr before the infusion of the labelled glucose. The amount of $^{14}CO_2$ excreted and O_2 consumed in consecutive 5 min. intervals of the infusions was measured and the rate of excretion of the $^{14}CO_2$ was equated to the oxygen consumption. The pooled excretion curves of $^{14}CO_2$ are shown in Figure 35, for each rate of infusion. It can be seen from this figure that over a range of rates of infusion of glucose from 100 to 300 mg/hr, the patterns of excretion of $^{14}CO_2$ were very similar; when glucose was infused in virtually weightless amounts the pattern was quite different. These $^{14}CO_2$ excretion curves were different from those obtained when ^{14}C-labelled

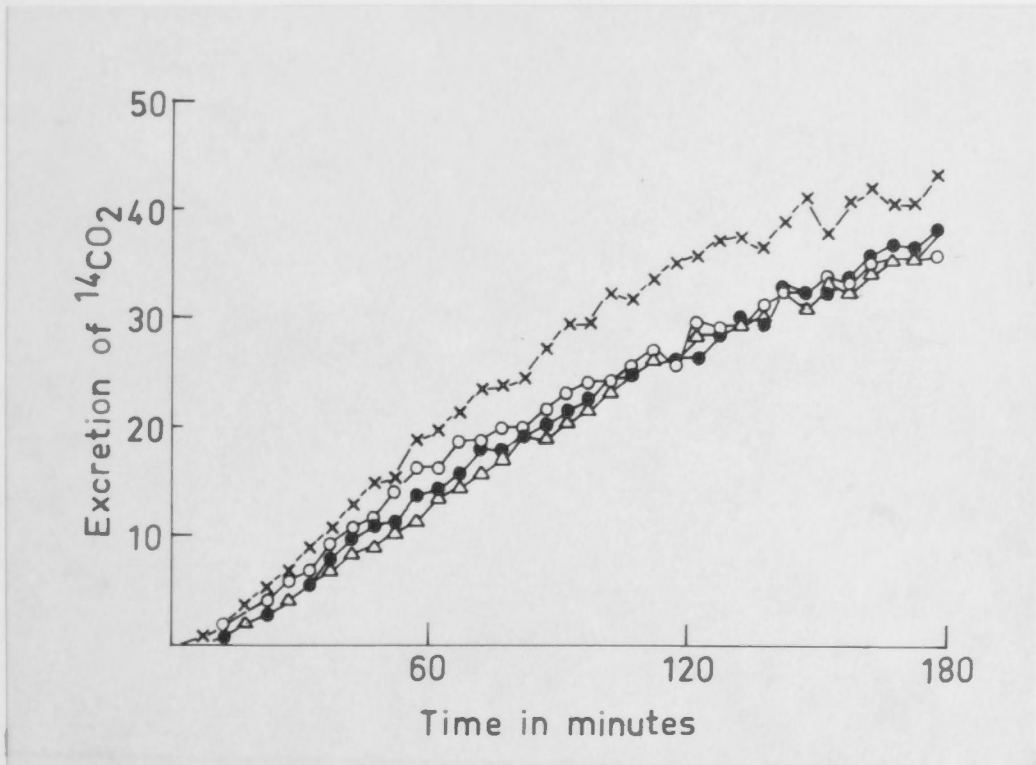


FIGURE 35.

The excretion of $^{14}CO_2$ by rats during the continuous intravenous infusion of generally labelled ^{14}C-glucose. The rate of excretion of radioactivity as $^{14}CO_2$, expressed as a percentage of the rate of infusion of radioactivity and corrected for the simultaneous rate of oxygen consumption, is plotted against time. Glucose was infused at rates of 0 (×), 100 (○), 200 (●) and 300 (△) mg/hr. Each curve is the mean result of 3 rats.

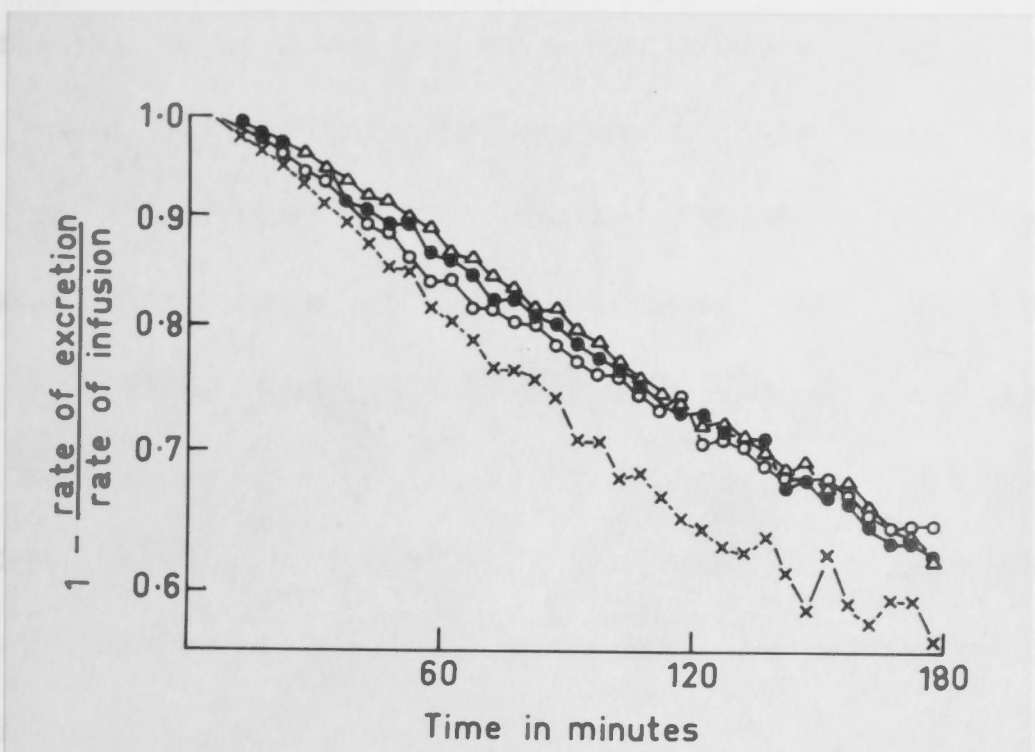


FIGURE 36.

The characteristics of the patterns of excretion of $^{14}CO_2$ by rats infused continuously with generally labelled ^{14}C-glucose. Log(1 - rate of excretion of radioactivity as $^{14}CO_2$/rate of infusion of radioactivity) is plotted against time. Glucose was infused at rates of 0 (×), 100 (○), 200 (●) and 300 (△) mg/hr. Each curve is the mean result of 3 rats.

chylomicrons were infused continuously (cf. Chapter 4) and a kinetic analysis of the curves (Figure 36) showed that, during the three hour infusion, the excretion pattern could be described almost completely by a single rate process with a half-life of 220 minutes when no exogenous glucose was infused, or with a half-life of 250 minutes when glucose was infused at rates of 100-300 mg/hr. The distortion introduced into such excretion curves by the bicarbonate pools would be minimal and would only cause the rate of excretion of the $^{14}CO_2$ to lag behind its formation. Thus it appeared that the glucose carbon was mixing with a very large pool of carbon in the body. These oxidation patterns lead one to question the so-called fraction of glucose, so often referred to, which is oxidized promptly to CO_2.

The effect of the glucose infusions on the respiratory quotient. The mean respiratory quotients for each half hour of the infusions are given in Table 15, for each rate of infusion. From these figures it can be seen that there was no consistent tendency for the R.Q. to rise during the infusions. The increase in R.Q. with the increasing rates of infusion of glucose did not reflect the increased amounts of exogenous glucose being supplied, but more likely the total amount of glucose, exogenous and endogenous, that was available for oxidation. It appeared that when glucose was

infused at rates of 100 and 200 mg/hr, similar quantities of glucose were available for oxidation. With these rates of infusion the plasma glucose concentration would reach the plateau level noted above; at this level more glucose would be available for oxidation than when no glucose was infused but less than when rate of infusion of glucose exceeded the plateau range for plasma glucose.

TABLE 15

The respiratory quotients measured in successive half-hour intervals during the infusion of increasing amounts of glucose.

Glucose Infusion (mg/hr)	Respiratory quotient Half-hour						
	1st	2nd	3rd	4th	5th	6th	Mean
0	0.77	0.77	0.80	0.76	0.73	0.75	0.76
100	0.76	0.81	0.80	0.80	0.77	0.80	0.79
200	0.77	0.77	0.82	0.82	0.79	0.81	0.80
300	0.83	0.79	0.80	0.84	0.84	0.81	0.82

Studies involving the simultaneous infusion of glucose and chylomicrons. An experiment was designed to test the effect of glucose on the metabolism of continuously infused chylomicron fatty acids. The experiment was designed so as to incorporate into it some of the considerations disclosed by the experiments discussed previously and in Chapter 4. These factors

included varying the rate of infusion of glucose and chylomicron fatty acids, and varying the amount of glucose infused before the experiment. The interactions between these factors were also examined. The experimental design and the coded treatments are listed in Table 16.

TABLE 16

The design of the experiment to test the effect of the simultaneous infusion of glucose on the metabolism of continuously infused chylomicron fatty acids.

Treatment combination			
Rate of infusion of glucose (mg/hr)	Duration of glucose preinfusion (hr)	Rate of infusion of chylomicron fatty acids (mg/hr)	Code
0	0	50	001
0	0	100	002
0	1.5	50	011
0	1.5	100	012
0	3	50	021
0	3	100	022
150	0	50	101
150	0	100	102
150	1.5	50	111
150	1.5	100	112
150	3	50	121
150	3	100	122
300	0	50	201
300	0	100	202
300	1.5	50	211
300	1.5	100	212
300	3	50	221
300	3	100	222

The chylomicrons were obtained from rats with thoracic duct fistulae; they were fed olive oil in which

^{14}C-palmitic acid was dissolved. The glucose and chylomicrons were infused through separate indwelling venous cannulae into rats which had been fasted for 24 hr. The glucose was infused for different periods of time (0, 1½ and 3 hr) before the infusion of the chylomicrons was begun and animals which received no glucose were infused with physiological saline; the total rate of infusion of fluid during the simultaneous infusions was 2.8 ml./hr. The labelled chylomicrons were infused for 3 hr and during this time the oxygen consumption and excretion of $^{14}CO_2$ in the animals' expired breath were continuously recorded, and the CO_2 produced in consecutive half-hour intervals was measured. At the end of the experiments, the animals were anaesthetized with pentobarbitone and rapidly bled from the abdominal aorta. The blood was centrifuged immediately. The liver, heart, kidneys, thigh muscles and a sample of adipose tissue were removed in that order and dropped into liquid air. The tissues were stored at -59°C until they were freeze-dried. The glycogen content of the dried liver, heart, muscle and kidney tissues was measured. The lipids were extracted from samples of the dried livers and adipose tissue and their radioactivity measured. The plasma was separated from the centrifuged blood and stored at -4°C until studied further. The concentration of ketone bodies and

unesterified fatty acids in the samples of plasma were measured. The 18 possible treatment combinations were used to make an orthogonal factorial experiment, the results of which could be analyzed by a standard analysis of variance. The individual infusions were performed in random order. The scheme for the analysis of the results and the coded items analyzed are listed in Table 17.

The three rates of infusion of glucose were chosen such that with the lowest, endogenous glucose only was available to the rats and plasma glucose levels of around 100 mg/100 ml would be present. The second rate of infusion of glucose, 150 mg/hr, was chosen such that it lay within the range over which the blood glucose concentration does not change. The highest rate of infusion (300 mg/hr) would be enough to exceed the plateau range of plasma glucose concentration and would probably result in a similar increment in the plasma glucose concentration to that effected by the 150 mg/hr infusion rate. The experiment was thus probably orthogonal not only in respect to the rates of infusion of glucose, but also in respect to the plasma glucose concentrations. The preinfusion times were chosen to test the effect of previously assimilated glucose on the metabolism of the chylomicron fatty acids. The rates at which the chylomicrons were infused were higher

TABLE 17

The scheme for the analysis of variance of the results obtained in the experiment to test the effect of the simultaneous infusion of glucose on the metabolism of continuously infused chylomicron fatty acids. Any nonsignificant items were pooled with the error term to give a new error term with a greater number of degrees of freedom.

Source of variation	Code	Degrees of freedom
Main effects		
Rate of infusion of glucose (G)		2
Linear	100	1
Quadratic	200	1
Duration of preinfusion (I)		2
Linear	010	1
Quadratic	020	1
Rate of infusion of chylomicron fatty acids (F)		1
Linear	001	1
First order interactions		
G x I		4
Linear x Linear	110	1
Quadratic x Linear	210	1
Linear x Quadratic	120	1
Quadratic x Quadratic	220	1
G x F		2
Linear x Linear	101	1
Quadratic x Linear	201	1
I x F		2
Linear x Linear	011	1
Quadratic x Linear	021	1
Second order interactions		
G x I x F	Error	4
	Total	17

than the animals would normally have experienced, but it was shown in Chapter 4 that there is no decrease in the efficiency of oxidation of the chylomicron fatty acids infused over this range, and that the increased amounts of chylomicron fatty acids oxidized appear to be compensated for by a decreased oxidation of endogenous lipid substrates.

The respiratory metabolism of the experimental animals. The oxygen consumption, carbon dioxide production and the respiratory quotients of the individual animals for each half-hour interval of the infusions are listed in Tables 18, 19 and 20. The mean O_2 consumption and CO_2 production in each half-hour interval did not change significantly during the infusions and the variation in these measurements was due entirely to differences between the individual animals. The mean R.Q. for each half-hour interval, however, did change significantly during the infusions and this is indicated by the significance of the 'times' item in the analysis of variance. The significance of this item was due to the low R.Q. in the first half-hour interval of the infusions. When the sums of squares attributable to differences between individual animals were further partitioned, none of the treatment effects were found to be significant.

The R.Q. of individual animals varied appreciably from half-hour to half-hour and such fluctuations have been reported previously (cf. Werthessen, 1937). The mean R.Q. showed a tendency to rise from 0.72 when no glucose was infused to 0.76 when glucose was infused at the rate of 300 mg/hr. Such an increase, if it was significant, would indicate that the rats derived some 14% of their total energy requirements from the oxidation of the infused glucose.

The oxidation of the infused chylomicron fatty acids to CO_2. The major fatty acids present in chylomicrons obtained from rats fed olive oil are palmitic, oleic and linoleic acids (Morris, 1963). As the oxidation of each of these fatty acids when incorporated into chylomicron triglycerides was shown to be very similar in Chapter 4 of this thesis, the oxidation of the ^{14}C-labelled palmitic acid to CO_2 in these experiments was used as an index of the oxidation of all the fatty acids present in the chylomicrons.

The patterns of excretion of $^{14}CO_2$ recorded during the infusion of the labelled chylomicrons are shown in Figure 37, where the results are pooled for each rate of infusion of glucose, and in Figure 38, where the results are pooled for each rate of infusion of chylomicron fatty acids. The rate of excretion of $^{14}CO_2$ was equated to the simultaneous rate of consumption of oxygen

TABLE 18

The volume of O_2 consumed in successive half-hour intervals during the simultaneous infusion of glucose and chylomicrons.

Treatment combination	O_2 consumed (cc. at S.T.P.) Half-hour						
	1st	2nd	3rd	4th	5th	6th	Mean
001	142	160	163	149	153	158	154
002	180	175	182	179	183	192	182
011	192	210	211	212	202	216	207
012	196	196	204	205	203	197	200
021	270	193	234	206	189	184	213
022	157	156	161	167	181	182	167
101	202	206	216	208	222	224	213
102	168	154	159	160	158	160	160
111	161	157	150	161	158	164	159
112	195	196	197	196	200	197	197
121	179	192	179	188	193	190	187
122	176	179	183	179	177	180	179
201	221	220	220	179	167	160	195
202	204	215	210	203	218	203	209
211	258	240	236	231	218	195	230
212	194	215	215	223	219	223	215
221	180	184	183	189	192	188	186
222	165	164	155	161	161	152	160
Mean	191	190	191	189	189	187	189

Analysis of variance

Item	Sum of squares	D.F.	Mean square	V.R.	p
Individuals	53291	17	3135	17.5	<0.001
Times	316	5	63	0.35	>0.20
Error	15234	85	179		
Total	68841	107			

TABLE 19

The volume of CO_2 expired in successive half-hour intervals during the simultaneous infusion of glucose and chylomicrons.

Treatment combination	CO_2 expired (cc. at S.T.P.) Half-hour						
	1st	2nd	3rd	4th	5th	6th	Mean
001	103	131	128	119	113	113	118
002	129	133	139	141	137	144	137
011	134	151	146	170	143	158	150
012	141	143	147	158	181	103	146
021	186	141	165	148	131	144	153
022	82	124	115	101	98	119	107
101	156	159	154	143	166	144	154
102	112	123	119	107	116	118	116
111	141	136	119	141	130	133	133
112	146	153	154	167	117	130	145
121	114	141	131	143	122	135	131
122	126	147	135	148	127	143	138
201	164	185	138	150	130	145	153
202	149	169	175	161	175	179	168
211	151	160	150	148	154	154	153
212	147	159	159	179	158	177	163
221	140	136	159	151	136	145	145
222	132	118	119	123	124	134	125
Mean	136	145	142	144	137	140	141

Analysis of variance

Item	Sum of squares	D.F.	Mean square	V.R.	p
Individuals	28226	17	1660.3	10.2	<0.001
Times	1831	5	366.2	2.25	>0.05
Error	13830	85	162.7		
Total	43887	107			

TABLE 20

The respiratory quotient measured in successive half-hour intervals during the simultaneous infusion of glucose and chylomicrons.

Treatment combination	Respiratory quotient						
	Half-hour						
	1st	2nd	3rd	4th	5th	6th	Mean
001	0.67	0.82	0.78	0.80	0.74	0.72	0.75
002	0.72	0.76	0.77	0.79	0.75	0.75	0.76
011	0.70	0.72	0.70	0.80	0.71	0.73	0.73
012	0.73	0.73	0.72	0.70	0.89	0.52	0.72
021	0.69	0.73	0.71	0.72	0.69	0.78	0.72
022	0.52	0.80	0.72	0.60	0.56	0.66	0.64
101	0.76	0.77	0.72	0.68	0.75	0.64	0.72
102	0.67	0.87	0.75	0.67	0.73	0.74	0.74
111	0.87	0.87	0.79	0.88	0.82	0.81	0.84
121	0.64	0.74	0.73	0.76	0.63	0.71	0.70
122	0.72	0.82	0.74	0.83	0.72	0.79	0.77
201	0.74	0.84	0.63	0.84	0.78	0.91	0.79
202	0.73	0.79	0.84	0.79	0.80	0.88	0.81
211	0.58	0.67	0.65	0.64	0.71	0.79	0.67
212	0.70	0.74	0.74	0.81	0.72	0.79	0.75
221	0.78	0.74	0.87	0.80	0.71	0.77	0.78
222	0.80	0.72	0.77	0.77	0.77	0.88	0.79
Mean	0.71	0.77	0.75	0.76	0.73	0.75	0.74

Analysis of variance

Item	Sum of squares	D.F.	Mean square	V.R.	p
Individuals	0.2296	17	0.0135	3.29	<0.001
Times	0.0499	5	0.0099	2.41	<0.05
Error	0.3504	85	0.0041		
Total	0.6299	107			

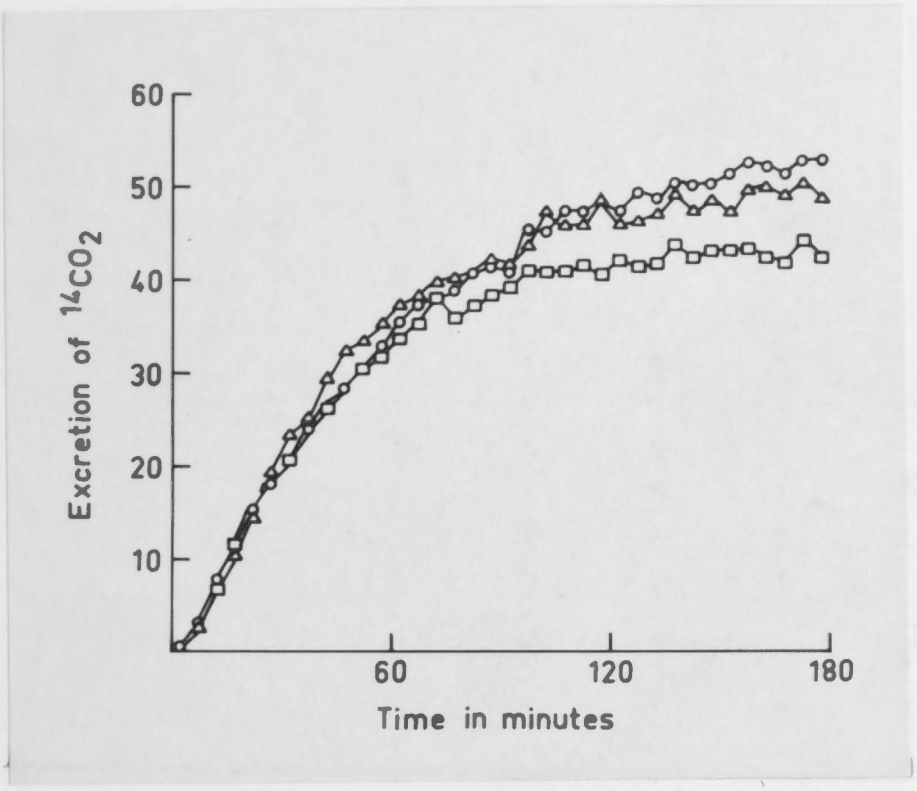


FIGURE 37.

The excretion of $^{14}CO_2$ by rats during the simultaneous intravenous infusion of unlabelled glucose and chylomicrons labelled with ^{14}C-palmitic acid. The rate of excretion of radioactivity as $^{14}CO_2$ expressed as a percentage of the rate of infusion of radioactivity and corrected for the simultaneous rate of oxygen consumption, is plotted against time for the different rates of infusion of glucose. Glucose was infused at rates of 0 (O), 150 (△) and 300 (□) mg/hr, and each curve is the mean result of 6 rats.

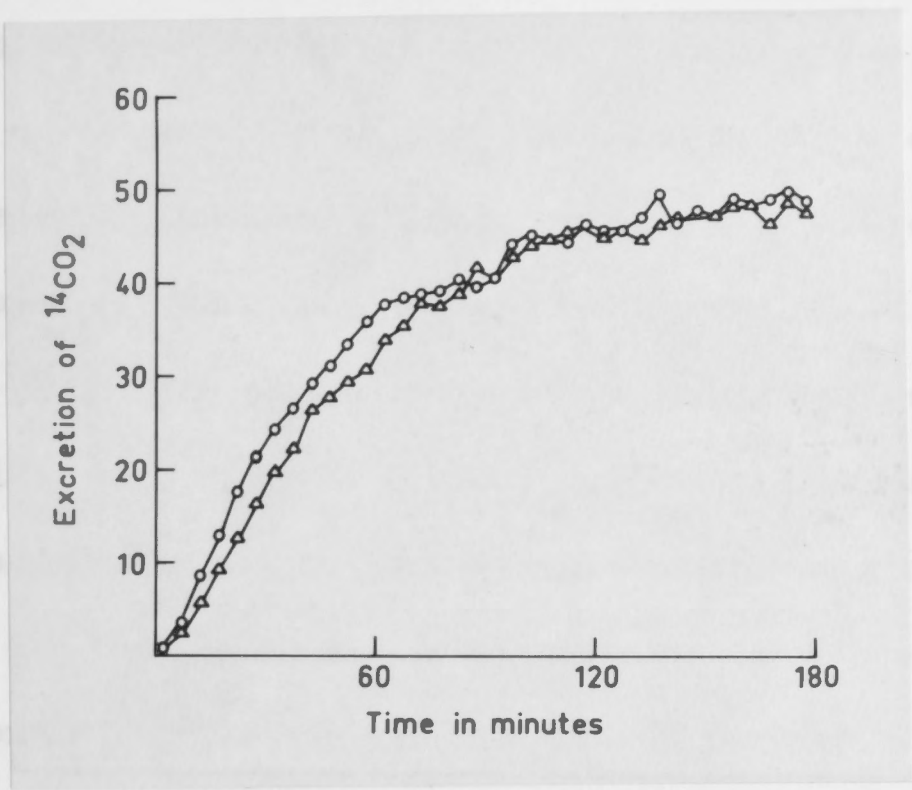


FIGURE 38.

The excretion of $^{14}CO_2$ by rats during the simultaneous infusion of unlabelled glucose and chylomicrons labelled with ^{14}C-palmitic acid. The rate of excretion of radioactivity as $^{14}CO_2$ expressed as a percentage of the rate of infusion of radioactivity and corrected for the simultaneous rate of oxygen consumption, is plotted against time for two rates of infusion of chylomicron fatty acids. Chylomicrons were infused at rates of 50 (O) and 100 (Δ) mg of total esterified fatty acid per hour, and each curve is the mean result of 9 rats.

and for the purposes of comparison all excretion curves were normalized to the mean oxygen consumption. Three features are apparent in these excretion curves. Less of the infused chylomicron fatty acids were oxidized to CO_2 in the third hour of the infusions in which glucose was infused simultaneously; the terminal part of the excretion curves became flatter with increasing rates of infusion of glucose and the initial rate of oxidation of the infused chylomicron fatty acids was slower with the higher rate of infusion of chylomicrons.

By equating the amount of the infused chylomicron fatty acids excreted as $^{14}CO_2$ in the third hour of the infusions to the amount of oxygen consumed, an estimate was made of the proportion of the animals' total energy requirements furnished by the oxidation of the infused chylomicron fatty acids. These data are given in Table 21 and show that the proportion of the infused chylomicron fatty acids oxidized to CO_2 in the third hour of the infusions was not significantly related to the rate of infusion of fat or the duration of the preinfusion of the glucose. The proportion of the energy requirements derived from the oxidation of the chylomicrons was significantly reduced in those experiments in which glucose was infused and this reduction was linearly related to the rate of infusion of the glucose. When no glucose was infused, 27% of the animal's total energy

requirements were provided by the oxidation of the infused chylomicron fatty acids for each 100 mg of chylomicron fatty acids infused per hour, whilst when glucose was infused at rates of 150 and 300 mg/hr the corresponding figures were 25.8% and 22.7% respectively. Thus in experiments in which 300 mg of glucose was infused per hour, the proportion of the animal's energy requirements provided by the oxidation of the infused chylomicron fatty acids in the third hour of the infusions was reduced by about 16%.

TABLE 21

The percentage of the rats' energy requirements provided by the oxidation of the infused chylomicron fatty acids in the third hour of the infusions for each 100 mg fatty acid infused per hour.

Treatment	%	Treatment	%
001	28.2	112	25.1
002	25.8	121	26.4
011	26.6	122	23.2
012	27.7	201	23.2
021	24.2	202	22.9
022	30.1	211	19.0
101	26.4	212	20.5
102	25.7	221	27.2
111	28.2	222	23.6

Analysis of variance

Item	Sum of squares	D.F.	Mean square		V.R.	p
100	57.20	1	57.20	a	11.5	< 0.01
200	3.36	1	3.36		0.68	> 0.2
Error	74,53	15	4.97			
Total	135.09	17				

[a] Mean glucose effect variance ratio 6.09 ($p < 0.05$)

The shapes of the $^{14}CO_2$ excretion curves were tested by comparing the amount of $^{14}CO_2$ excreted in the first hour of the infusions to that excreted in the second hour, and the amount excreted in the second hour to that excreted in the third hour. These comparisons are listed in Table 22. The initial rate at which the excretion of $^{14}CO_2$ increased was related significantly only to the rate at which fat was infused. The factors which influence the initial excretion of the $^{14}CO_2$ are the rapid components associated with the clearance of the chylomicrons from the circulation and the excretion of the $^{14}CO_2$ from the body bicarbonate pools after the oxidation of the chylomicrons. The longer circulating half-life of the chylomicron fatty acids at the higher rate of infusion could therefore account for the significant delay in the initial excretion of $^{14}CO_2$. The rate at which the excretion of $^{14}CO_2$ continued to rise in the third hour of the infusions was related significantly to two factors. The rate was significantly slower for animals which were infused with glucose, and this effect was linearly related to the rate of infusion of glucose. The rate was also significantly slower for animals which were infused with 100 mg fatty acids/hr than for those infused with 50 mg fatty acids/hr when 150 mg glucose/hr was infused in both groups.

Kinetic analysis of the terminal part of the $^{14}CO_2$ excretion curves. The rate at which the excretion of $^{14}CO_2$

TABLE 22

The ratios of the amount of $^{14}CO_2$ excreted in the first hour to the amount excreted in the second hour, and the amount of $^{14}CO_2$ excreted in the second hour to the amount excreted in the third hour. The excretion of $^{14}CO_2$ in each hour was equated to the amount of oxygen consumed.

Treatment	1st/2nd	2nd/3rd	Treatment	1st/2nd	2nd/3rd
001	0.479	0.827	112	0.435	0.877
002	0.469	0.899	121	0.502	0.860
011	0.467	0.879	122	0.408	0.955
012	0.361	0.765	201	0.509	0.957
021	0.492	0.882	202	0.340	0.889
022	0.338	0.759	211	0.493	0.894
101	0.499	0.842	212	0.533	0.951
102	0.382	0.929	221	0.490	0.885
111	0.491	0.843	222	0.423	0.864

Analysis of variance of ratio of 1st hr to 2nd hr

Item	Sum of squares	D.F.	Mean square	V.R.	p
001	0.029849	1	0.029849	14.1	<0.01
Error	0.033911	16	0.002119		
Total	0.063760	17			

Analysis of variance of ratio of 2nd hr to 3rd hr

Item	Sum of squares	D.F.	Mean square	V.R.	p
100	0.015336	1	0.015336) [a]	8.15	<0.05
200	0.000720	1	0.000720)		
201	0.010990	1	0.010990	5.83	<0.05
Error	0.026389	14	0.001885		
Total	0.053435	17			

[a] Mean glucose effect variance ratio 4.26 ($p < 0.05$)

continues to rise during the third hour of continuous infusions of chylomicrons labelled with ^{14}C fatty acids has been shown to be due to the proportion of the chylomicron fatty acids which are oxidized relatively slowly. As the fatty acids handled in this way account for some 70% of the total chylomicron fatty acids, any effect of glucose on their oxidation would be of importance. The excretion curves were plotted as log(1 - rate of excretion of ^{14}C as $^{14}CO_2$/rate of infusion of ^{14}C) and the regressions of these derived curves on time, between 90 and 180 minutes after the beginning of the infusions were calculated. These regression coefficients which are listed in Table 23, are all significantly different from 0.

The regression coefficients for the two rates of fat infusion were not significantly different from each other when glucose was infused at rates of 0 and 300 mg/hr. These calculations indicate that the mechanism by which the oxidation of the chylomicron fatty acids was reduced in the third hour of the infusions was associated with a reduction of the oxidation of the fatty acids which are oxidized relatively slowly. This reduction was associated with an increase in the biological half-life of these fatty acids. The interaction between the rates of infusion of glucose and chylomicron fatty acids suggested that the effect of glucose was in

TABLE 23

The regression of log(1 - rate of excretion of ^{14}C as $^{14}CO_2$/rate of infusion of ^{14}C) on time, measured in minutes, between 90 and 180 minutes of the simultaneous infusion of glucose and ^{14}C-labelled chylomicrons. The standard errors of the regression coefficients are given and the significance of the differences between the regressions indicated. (- , not significant; + $p < 0.02$; ++ $p < 0.001$).

Rate of infusion Glucose (mg/hr)	Rate of infusion Fat (mg/hr)	Regression coefficient		Significance of difference from (2)	(3)	(4)	Equivalent exponential component
0	50	0.00109 ± 0.00012	(1)	-	++	++	$0.70e^{-0.0025t}$
0	100						
150	50	0.00082 ± 0.00016	(2)		+	+	$0.66e^{-0.0019t}$
150	100	0.00035 ± 0.00012	(3)			-	$0.60e^{-0.0008t}$
300	50	0.00030 ± 0.00015	(4)				$0.63e^{-0.0007t}$
300	300						

some way related to the total amount of energy being offered to the tissue cells. It is of interest to note that, if the values of the exponential components which describe the oxidation of the slowly oxidized fatty acids at zero time are indicative of the proportion of the total fatty acids so oxidized, then the increase in the biological half-life of the fatty acids due to the infusion of glucose is accompanied by a decrease in the proportion of the total fatty acids which are oxidized slowly. These interactions are shown in Figure 39, and the corollary of this is that although the overall effect of the glucose was to reduce the oxidation of the chylomicron fatty acids, it might in fact have enhanced the rapid oxidation of the fatty acids.

The concentration of ketone bodies in the plasma. The concentration of ketone bodies, measured as their acetone equivalents, are listed in Table 24. The concentration of ketones in the plasma was reduced in the animals which were infused with glucose. This reduction which was highly significant, was not related linearly to the rate of infusion of glucose. The mean concentration in the plasma of animals which received no glucose was 15.5 mg/100 ml. whilst in animals which were infused with 150 mg glucose/hr and 300 mg glucose/hr, the concentrations were 6.7 and 5.6 mg/100 ml. respectively. These concentrations were not related

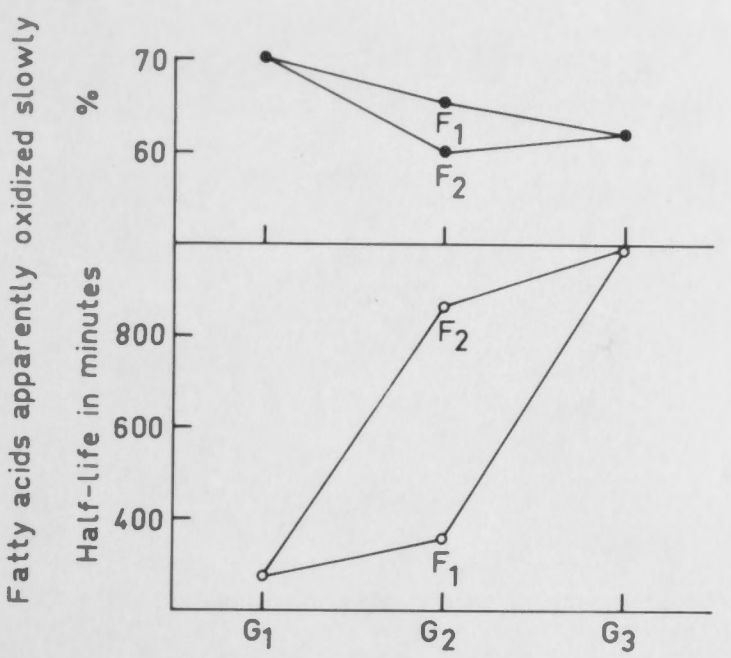


FIGURE 39.

The behaviour of the chylomicron fatty acids which appeared to be oxidized slowly during the simultaneous infusions of glucose and chylomicrons labelled with ^{14}C-palmitic acid. The half-life of the fatty acids in the body before being oxidized, and the proportion of the total infused fatty acids which appeared to be oxidized in this way are shown for the various rates of infusion of glucose and fat. Glucose was infused at rates of 0 (G_1), 150 (G_2) and 300 (G_3) mg/hr and chylomicrons at rates of 50 (F_1) and 100 (F_2) mg total esterified fatty acids per hour.

TABLE 24

The concentration of ketone bodies, measured as their acetone equivalent, in the plasma of the rats at the end of simultaneous infusions of glucose and chylomicrons.

Treatment combination	Concentration (mg/100ml.)	Treatment combination	Concentration (mg/100 ml.)
001	13.7	112	8.4
002	14.9	121	7.1
011	17.2	122	6.0
012	18.4	201	4.7
021	13.2	202	5.7
022	15.4	211	4.4
101	8.5	212	6.1
102	5.0	221	7.3
111	5.4	222	5.5

Analysis of variance

Item	Sum of squares	D.F.	Mean square	V.R.	p
100	291.07	1	291.07	118.3	<0.001
200	58.01	1	58.01	23.6	<0.001
Error	36.96	15	2.46		
Total	386.04	17			

significantly to any other treatment. The plasma ketones were thus reduced to less than 50% of the fasting concentration in those animals which were infused with glucose, even though the oxidation of the infused chylomicron fatty acids to CO_2 was reduced by only about 16%.

The concentration of unesterified fatty acids in the plasma. It was thought that the concentration of unesterified fatty acids in the plasma might reflect the degree of mobilization of endogenous lipids. However none of the treatments had any significant effect of the levels of unesterified fatty acids in the plasma at the end of the experiments. When no glucose was infused the mean plasma concentration was 0.75 μeq/ml., whilst when glucose was infused at rates of 150 mg/hr and 300 mg/hr the concentrations were 0.43 and 0.58 μeq/ml. respectively. None of these differences was significant. The concentrations of unesterified fatty acids are listed in Table 25.

The lipid content of the livers. The lipid content of the livers was significantly reduced from 22.5 mg/100 mg dry weight when no glucose was infused to 19.0 mg/100 dry weight when glucose was infused at the rate of 300 mg/hr. It was thought that this effect might be due to the increased amount of glycogen in the livers of the rats which were infused with glucose.

TABLE 25

The concentration of unesterified fatty acids in the plasma of rats at the end of simultaneous infusions of glucose and chylomicrons.

Treatment	Concentration (μeq/ml.)	Treatment	Concentration (μeq/ml.)
001	0.50	112	0.44
002	0.36	121	0.45
011	0.63	122	0.44
012	1.25	201	0.50
021	0.59	202	0.70
022	1.18	211	0.54
101	0.44	212	0.62
102	0.51	221	0.76
111	0.30	222	0.36

However when the lipid content of the livers was equated to the glycogen free dry weight, although the absolute differences were decreased, the significance of the glucose effect was increased due to a relatively greater decrease in the total variance. The lipid content decreased from 22.7 mg/100 mg glycogen free dry weight when no glucose was infused to 21.1 mg/100 mg glycogen-free dry weight when glucose was infused at the rate of 300 mg/hr. The lipid content of the livers is given in Table 26.

The distribution of the labelled chylomicron fatty acids in the liver and adipose tissue. The amount of radioactivity recovered in the liver total lipids was significantly greater as the rate of infusion of glucose increased. The percentage of the amount of labelled

TABLE 26

The lipid content, expressed as mg/100 mg dry weight and mg/100 mg glycogen-free dry weight, of the livers removed from the rats at the end of simultaneous infusions of glucose and chylomicrons.

Treatment	Lipid content		Treatment	Lipid content	
	Total	Glycogen-free		Total	Glycogen-free
001	22.2	22.5	112	20.2	21.9
002	22.6	22.8	121	20.3	22.4
011	23.3	23.5	122	18.8	20.6
012	22.2	22.3	201	19.2	20.8
021	22.8	22.9	202	19.8	21.7
022	21.7	21.9	211	20.5	22.4
101	18.6	19.5	212	19.4	20.8
102	19.9	21.1	221	17.2	20.5
111	19.9	21.8	222	17.9	20.5

Analysis of variance of liver dry weight lipid

Item	Sum of squares	D.F.	Mean square		V.R.	p
100	36.05	1	36.05	a	10.3	<0.01
200	4.99	1	4.99	a	1.43	>0.20
Error	52.43	15	3.50			
Total	93.47	17				

Analysis of variance of liver glycogen-free dry weight lipid content

Item	Sum of squares	D.F.	Mean square		V.R.	p
100	7.05	1	7.05	b	10.6	<0.01
200	1.78	1	1.78	b	2.67	<0.2
Error	10.01	15	0.667			
Total	18.84	17				

[a]Mean glucose effect variance ratio 5.86 ($p < 0.05$)

[b]Mean glucose effect variance ratio 6.63 ($p < 0.01$)

TABLE 27

The radioactivity of the lipids, expressed as a percentage of the amount of radioactive lipid infused each hour per gram of lipid, extracted from the livers and samples of adipose tissue removed from the rats at the end of simultaneous infusions of glucose and chylomicrons.

Treatment	Lipid radioactivity		Treatment	Lipid radioactivity	
	Liver	Adipose tissue		Liver	Adipose tissue
001	63	0.88	112	82	1.04
002	61	1.04	121	69	1.94
011	67	1.52	122	66	----
012	84	1.33	201	79	3.48
021	61	1.04	202	85	2.02
022	88	1.04	211	90	2.45
101	81	1.49	212	83	2.07
102	64	2.82	221	86	1.36
111	76	6.54	222	93	4.98

Analysis of variance in liver lipid radioactivity

Item	Sum of squares	D.F.	Mean square	V.R.	p
100	705	1	705 } a	9.1	<0.01
200	114	1	114 } a	1.47	>0.2
Error	1161	15	77.4		
Total	1980				

Analysis of variance of adipose tissue lipid radioactivity

As one sample of adipose tissue was lost, only the values obtained with the lowest and highest rates of infusion of glucose were used in this analysis.

Item	Sum of squares	D.F.	Mean square	V.R.	p
100	7.54	1	7.54	8.58	<0.05
Error	8.79	10	0.879		
Total	16.3347	11			

[a] Mean glucose effect variance ratio 5.30 ($p < 0.05$)

fatty acids infused per hour associated with 1 g of liver lipids increased from 71% when no glucose was infused to 88% when glucose was infused at the rate of 300 mg/hr.

Unfortunately one of the samples of adipose tissue was lost and a full analysis of the results could not be carried out. However by comparing the results when no glucose was infused to those when 300 mg glucose/hr was infused, the percentage of the amount of chylomicron fatty acids infused per hour associated with 1 g of adipose tissue lipid was significantly increased from 1.14% to 2.73%. The effect of the glucose appeared to be non-linear as the corresponding mean of the five animals infused with 150 mg glucose/hr was 3.09%.

The measurements of the specific activity of the liver and adipose tissue lipids are given in Table 27.

The effect of the infusions on the tissue glycogen levels. The glycogen content of the livers, muscles, hearts and kidneys removed from the animals at the end of the infusions is listed in Table 28. In all tissues except the kidneys, the level of glycogen was significantly higher in those animals which were infused with glucose, and this effect was related linearly to the rate of infusion. The effect was greatest in the liver where the glycogen concentration increased from 0.87 mg/100 mg dry weight when no glucose was infused to 11.21 mg/100 mg

TABLE 28

The glycogen content, expressed as mg/100 mg dry weight, of the tissues removed from the rats at the end of simultaneous infusions of glucose and chylomicrons.
L = liver, M = muscle, H = heart, K = kidney.

Treatment	Glycogen (L)	(M)	(H)	(K)	Treatment	Glycogen (L)	(M)	(H)	(K)
001	1.27	0.55	0.67	0.37	112	8.18	1.01	1.45	0.40
002	0.92	0.25	1.86	0.33	121	10.33	0.67	1.99	0.12
011	0.84	0.12	1.53	0.59	122	9.35	1.29	1.58	0.15
012	0.51	0.11	0.71	0.33	201	8.87	1.02	2.22	0.27
021	0.57	0.12	0.72	0.21	202	9.76	0.84	0.77	0.25
022	1.09	0.81	1.87	0.36	211	9.49	0.76	2.42	0.24
101	4.65	1.25	1.39	0.75	212	5.93	2.17	2.40	0.51
102	6.02	0.72	2.38	0.44	221	18.95	1.83	1.92	0.15
111	9.54	1.76	2.04	0.24	222	14.28	1.52	2.84	0.38

Analysis of variance of liver glycogen

Item	Sum of squares	D.F.	Mean square	V.R.	p
100	268.314	1	268.314 [a]	26.0	<0.001
200	15.550	1	15.550 [a]	1.52	>0.2
010	44.390	1	44.390	4.32	<0.2
020	8.103	1	8.103	0.79	>0.2
Error	133.330	13	10.256		
Total	469.687	17			

Analysis of variance of muscle glycogen

Item	Sum of squares	D.F.	Mean square	V.R.	p
100	3.1827	1	3.1827 [b]	16.48	<0.01
200	0.3025	1	0.3025 [b]	1.57	>0.2
Error	2.9002	15	0.1933		
Total	6.3854	17			

Analysis of variance of heart glycogen

Item	Sum of squares	D.F.	Mean square	V.R.	p
100	2.2620	1	2.2620 [c]	6.69	<0.05
200	0.0831	1	0.0831 [c]	0.25	>0.2
Error	5.0651	15	0.3377		
Total	7.4102	17			

[a] Mean glucose effect variance ratio 13.84 ($p < 0.001$)

[b] Mean glucose effect variance ratio 9.02 ($p < 0.01$)

[c] Mean glucose effect variance ratio 3.48 ($p < 0.2$)

dry weight when glucose was infused at the highest rate. The muscle and heart glycogen increased from 0.33 mg/100 mg dry weight to 1.36 mg/100 mg dry weight, and from 1.23 mg/100 mg dry weight to 2.10 mg/100 mg dry weight respectively. The glycogen content of the various tissues tended to increase with increasing total periods of glucose infusion, but these differences were not significant.

The oxidation of chylomicron fatty acids at very high rates of infusion of fat. In the experiments just described it was found that when glucose and chylomicrons were infused simultaneously in significant amounts into rats, the glucose did not spare the chylomicron fatty acids to any great extent. This is in contrast to the published results of several groups of workers who suggest that the oxidation of glucose occurs preferentially over fatty acids. The highest rate at which glucose was infused in the preceding experiments was of the same order as the maximal rates at which the rats could absorb glucose from their guts (ca. 360 mg/hr, Cori, 1925) and it was not considered profitable to use higher rates of infusion of glucose. It was decided to test the possibility that the fatty acid sparing effect of the glucose in the experiments of other workers was due, not to a preferential oxidation of the glucose, but instead to a competition for available oxidative pathways between

the radioactive fatty acids and the fatty acids newly synthesized from the large amounts of glucose. A rat was infused with chylomicrons labelled with ^{14}C-palmitic acid at a rate of 278 mg of total esterified fatty acids per hour, and the excretion of $^{14}CO_2$ and consumption of O_2 during the infusion were recorded continuously. This rate of infusion of chylomicron fatty acids was approximately isocaloric with an infusion of 100 mg chylomicron fatty acids per hour together with 300 mg glucose per hour. The pattern of excretion of $^{14}CO_2$ by this rat is shown in Figure 40. The proportion of the rat's energy requirements provided by the oxidation of each 100 mg fatty acids infused per hour in the third hour of this experiment was significantly lower than the overall mean of the preceding series ($p < 0.01$), being only 16.5% as compared with 25.2$\pm$S.D. 2.80%. However when this curve was analyzed kinetically, it was found that the mechanism of this reduction was different from that in the previous experiments when glucose was infused. Whereas with glucose the reduction was due entirely to a decrease in the amount of fatty acids oxidized relatively slowly, in this experiment the kinetics of the slow component in the oxidation were not affected very much (half-life approximately 430 min) and the reduction was almost entirely in the proportion of the infused fatty acids which were oxidized relatively quickly.

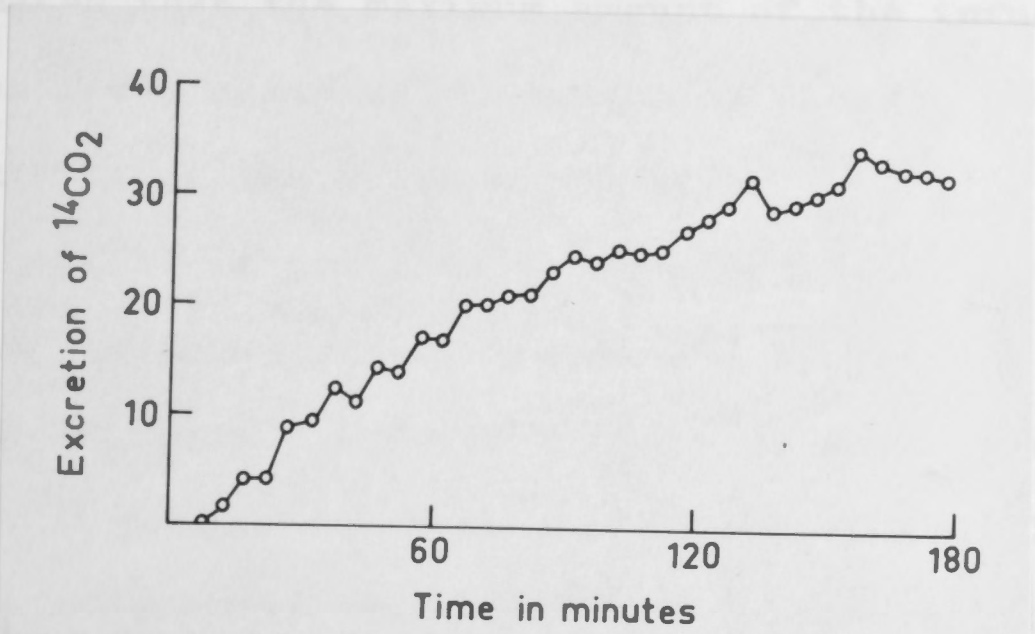


FIGURE 40.

The excretion of $^{14}CO_2$ by a rat when infused with chylomicrons labelled with ^{14}C-palmitic acid at a rate of 278 mg total esterified fatty acid per hour. The rate of excretion of radioactivity, expressed as a percentage of the rate of infusion of radioactivity and corrected for the simultaneous rate of oxygen consumption is plotted against time.

The proportion of the infused fatty acids oxidized relatively quickly was only 12% of those infused. This was the first time the efficiency of oxidation of chylomicron fatty acids was shown to be affected by the rate of infusion. From this result it can be estimated that the maximum amount of the infused chylomicron fatty acids which can be oxidized relatively quickly is of the order of 33 mg/hr.

If it is possible to extrapolate from the results of the previous set of experiments to predict the effect of simultaneously infused glucose on such an excretion curve, it is obvious that the effect of the glucose would be quite dramatic, especially if the excretion curve was compared to those of rats receiving a smaller infusion of fat with no glucose. As the effect of the glucose appeared to be to reduce the amount of fatty acids oxidized relatively slowly, and these fatty acids accounted for some 60% of the total fatty acids oxidized in the third hour of this experiment, the oxidation would be reduced by over 50% in the third hour of the infusion by the simultaneous infusion of glucose.

CHAPTER SIX

THE OXIDATION OF CHYLOMICRON FATTY ACIDS BY THE ISOLATED HEART-LUNG PREPARATION

In the experiments reported in Chapters 4 and 5 of this thesis it was found that intact rats can derive a large proportion of their immediate energy requirements from the oxidation of continuously infused chylomicron fatty acids. From these observations it seemed likely that chylomicron fatty acids might be used by many tissues of the body as a source of energy. Whilst it has been shown that hepatectomized animals are able to utilize chylomicrons, and indirect evidence from many other experiments has suggested that chylomicrons are removed from the circulation by tissues other than the liver, there is no direct evidence that chylomicron fatty acids can be utilized by any isolated organs other than the liver. It has been suggested by some workers that the liver plays a central role in the metabolism of chylomicrons by removing them from the circulation initially, and modifying them before they are used by extrahepatic tissues. In order to investigate this aspect of chylomicron metabolism experiments were done to study the utilization of chylomicron fatty acids by isolated heart-lung preparations from rats.

Chylomicrons are unique amongst the various energy substrates transported in the blood in that they are

relatively large particles and special mechanisms might be involved in their removal from the circulation. In studying the metabolism of chylomicrons by isolated organs it is important, therefore, to maintain the blood perfusate and capillary endothelium in a condition as close to normal as possible. Isolated heart or heart-lung preparations which have been described previously were considered unsuitable for these studies as they involve the use of unphysiological perfusates, or the use of heparin. Heparin-activated clearing factor has been isolated from heart muscle (Korn, 1958) and the use of heparin in the perfusate could lead to intravascular hydrolysis of the chylomicrons and obfuscate the interpretation of the experimental results. The isolated heart-lung preparation which was developed was free from these objections. Blood was circulated through the coronary vessels by the action of the heart. The rat's own unaltered blood was used without any added heparin or other anticoagulants. Before the oxidation of chylomicron fatty acids was studied, a number of preliminary experiments were done to characterize the requirements and properties of the preparation. All of the isolated heart-lung preparations used in the experiments to be reported in this chapter were obtained from rats which had been fed ad libitum up until the time of the experiment.

Characteristics of the heart-lung preparation.

Respiratory gas. In preliminary experiments it was found that if the lungs were ventilated with air or O_2 a complete heart block developed and this led to irreversible ventricular fibrillation with failure of the heart. If the respiratory gas was changed to 5% CO_2 in O_2 before the heart fibrillated, the heart block was abolished and the normal cardiac cycle was restored. When air was used as the respiratory gas, the arterial blood appeared well oxygenated, but the blood in the cardiac veins appeared quite venous. When O_2 or 5% CO_2 in O_2 was used as the respiratory gas, the blood in the cardiac veins appeared to be well oxygenated. So as to ensure that enough O_2 was available, 5% CO_2 in O_2 was used to ventilate the lungs in the metabolism experiments. In experiments in which the expired CO_2 was allowed to accumulate in the apparatus, the partial pressure of the CO_2 in the gas phase rose continually during the experiments. If CO_2 was produced at a rate of about 10 ml./hr the concentration of CO_2 in the gas phase would increase from 5% to about 5.4% in 2 hr. An increase in the concentration of the CO_2 to this level did not appear to affect the hearts.

Times taken for the various procedures. The aorta was clamped between 30-45 seconds after the abdominal aorta was cut. The venous pressure was reduced sufficiently

by this time to prevent the heart being overstrained when the aorta was clamped. The lungs were ventilated within 1 min of cutting the abdominal aorta so that the hearts were anoxic for no longer than 1 min. The infusion of metabolites was begun usually 7-10 minutes after cutting the aorta and the heart and lungs were placed in the organ chamber and warmed to 38°C within 20 min of cutting the abdominal aorta. No attempt was made to control the temperature of the organs before they were put in the organ chamber.

Requirements for fluid. If the infusion of fluid into the preparation was insufficient, characteristic changes appeared in the E.C.G. patterns and the heart usually failed after about 60 min. The actual amount of fluid required was not determined, but it appeared to be between 0.09 ml./hr and 0.175 ml./hr; when fluid was infused at a rate of 0.09 ml./hr changes in the E.C.G. patterns developed, whilst when fluid was infused at a rate of 0.175 ml/hr these changes did not appear. The infusion of fluid at this rate was apparently necessary to maintain the blood volume of the preparation. In one heart in which the characteristic changes in the E.C.G. had developed, the injection of a small volume of fluid promptly restored the E.C.G. patterns to normal. The fluid which was infused in all experiments was bicarbonate-Ringer solution. It was infused at a rate

of 0.175 ml./hr in all the metabolism experiments.

The blood perfusate. Although no anticoagulants were used the blood perfusing the organs did not clot and there was never any gross evidence of cardiac infarction. Although the lungs normally contain large amounts of endogenous heparin, this heparin did not appear to be responsible for the failure of the blood to clot as the blood clotted rapidly when it was bled out from the preparation. The volume of blood which could be obtained from the organs was of the order of 1 ml. In spite of the large rate of infusion of fluid relative to the circulating blood volume, the haematocrit value of the blood after an experiment lasting $2\frac{1}{2}$-3 hr was usually between 30-40%. There was rarely any evidence of haemolysis in the blood after an experiment and there was always a distinct buffy coat on the red cells when the blood was centrifuged.

The viability of the hearts. As the survival of the hearts depended on their own efficient contractions the viability of each preparation was assessed in these terms. If for any reason there was interference with the coronary circulation, the hearts failed rapidly.

Subject to their requirements for CO_2 in the respiratory gas and an infusion of fluid into the circulation, the hearts appeared to remain essentially

unchanged for longer than 3 hr. When the operative procedures had been well worked out it was usually possible to assess the potential viability of the heart as soon as it was put in the organ chamber. It was rare for a heart to fail during an experiment once it had been assessed as suitable for the experiment. When the aortic stump was cut even after a long experiment, blood was pumped out quite forcefully and the heart, deprived of its coronary circulation, failed rapidly. The direction of blood flow through the organs was demonstrated readily by injecting dye into the blood in the right heart; first the lungs became uniformly colored, then the aortic stump, the myocardium and finally the blood in the cardiac veins was colored.

The electrocardiogram. Provided the respiratory gas was suitable and the infusion of fluid and exogenous metabolites were adequate, the E.C.G. patterns recorded from a heart remained essentially unchanged for several hours. The essential characteristics of the normal E.C.G. pattern are shown in Figure 41. These patterns were recorded from the same heart at 30 minute intervals, the first record having been made 30 min after the abdominal aorta was cut and about 10 minutes after the heart and lungs were placed in the organ chamber. The E.C.G. patterns from individual hearts showed minor variations as it was not possible to fix the hearts in

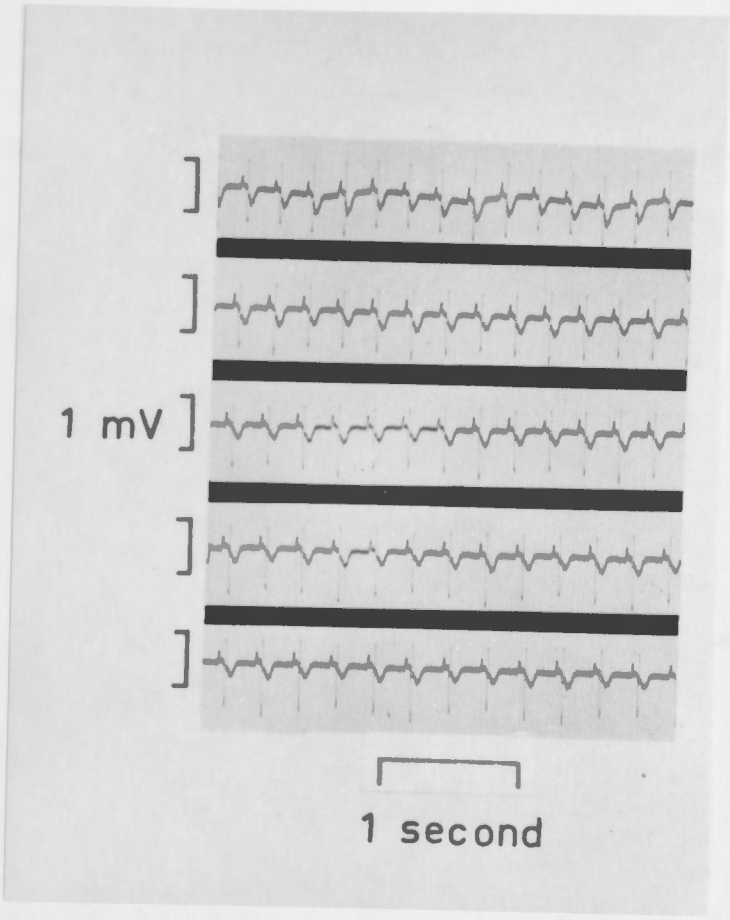


FIGURE 41.

The electrocardiogram recorded at 30 min intervals from a normal isolated heart-lung preparation. The first pattern (top) was recorded 30 minutes after the abdominal aorta was cut and about 10 minutes after the heart and lungs were placed in the organ chamber. This preparation was infused with chylomicrons at a rate of 4 mg of total esterified fatty acids per hour together with 10 mg glucose/hr.

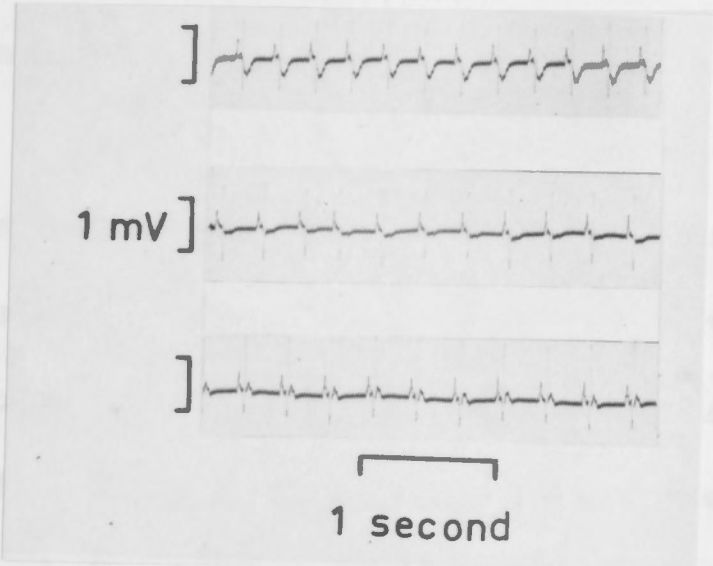


FIGURE 42.

The electrocardiogram recorded at 30 min intervals from a failing isolated heart-lung preparation. The first pattern (top) was recorded 30 min after the abdominal aorta was cut and about 10 min after the heart and lungs were placed in the organ chamber. The heart failed because the infusion cannula was blocked after the first pattern was recorded when the infusion was changed from unlabelled chylomicrons to labelled chylomicrons.

the same position relative to the E.C.G. electrodes. The P wave was almost always upright. The ventricular complex consisted of an initial downward deflection which was followed usually by an upward deflection. The T wave followed immediately and was inverted. The T wave was found to be the best index of incipient changes in the condition of the heart. In hearts that failed, the T wave became flatter and finally upright. These changes in the T wave are shown in Figure 42 where the E.C.G. patterns recorded from a failing heart at 30 min intervals are shown. The heart went into ventricular fibrillation soon after the 3rd pattern shown in Figure 42 was recorded. This heart failed because a blockage occurred in the infusion cannula and the blood volume of the preparation fell.

Requirements of the hearts for exogenous metabolites.

The hearts were able to continue beating for several hours when fluid but no exogenous metabolites was infused. These hearts must have obtained their energy from the oxidation of endogenous energy reserves. However, when the supply of exogenous metabolites was below a certain level, characteristic changes in the T waves of the E.C.G. patterns developed. These changes are shown in Figure 43. The T waves became shorter in duration and smaller in amplitude. They normally remained like this for $2\frac{1}{2}$-3 hr without becoming upright. These changes were

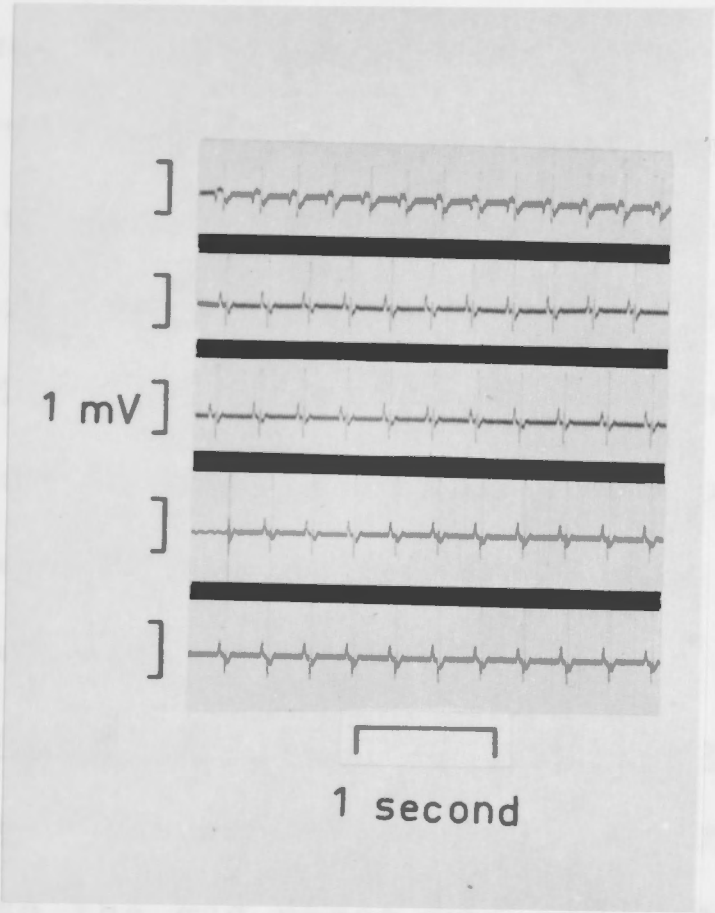


FIGURE 43.

The electrocardiogram recorded at 30 min intervals from an isolated heart-lung preparation which was infused with chylomicrons at a rate of 2 mg of total esterified fatty acids per hour with no glucose. The first pattern (top) was recorded 30 min after the abdominal aorta was cut and about 10 min after the heart and lungs were placed in the organ chamber.

seen in hearts which were infused with chylomicron fatty acids at rates of 2 and 4 mg/hr without glucose. When 10 mg of glucose was infused simultaneously per hour, or when larger amounts of chylomicrons were infused without glucose, these changes did not develop.

Heart rates. The hearts usually contracted between 200 and 250 times a minute, and the rate of contraction of individual hearts tended to decrease slightly during an experiment. These decreases in heart rate were associated with an increase in the interval between contractions and not with an increase in the P-T interval. These heart rates are slower than in intact animals.

The excretion of $^{14}CO_2$ from the blood into the respiratory gas. The excretion of $^{14}CO_2$ by the heart-lung preparation into the gas phase was studied by infusing $NaH^{14}CO_3$ into the circulation. The excretion of $^{14}CO_2$ was recorded continuously, and the amount excreted in 15 min intervals was measured and equated to the amount of $NaH^{14}CO_3$ infused during the interval.

These experiments were done in conjunction with preliminary experiments to assess the ability of the heart and lungs to utilize exogenous metabolites. Unlabelled chylomicron fatty acids were infused at rates of 2, 4, 6 and 8 mg/hr either with or without unlabelled glucose at the rate of 10 mg/hr. The infusion of the metabolites was begun as soon as the inferior vena cava

was cannulated. The simultaneous infusion of $NaH^{14}CO_3$ was begun after the heart and lungs had been in the chamber for about 15 min and the infusion was continued for 2 hr. At the end of the experiment the heart and lungs were removed from the chamber, the aortic stump was cut and the blood was collected. The blood was centrifuged and the plasma was inspected for any haemolysis or cloudiness due to the presence of chylomicron lipid.

The patterns of excretion of $^{14}CO_2$ into the gas phase during the infusions of the $NaH^{14}CO_3$ are shown in Figure 44. In the first experiments the electrolyte solution surrounding the organs was not equilibrated with the gas phase before the experiments, and under these conditions the initial rate of excretion of $^{14}CO_2$ was slow. By the end of 2 hr the rate of excretion of radioactivity as $^{14}CO_2$ into the gas phase had reached only 75-80% of the rate of infusion. In one of these experiments at least 60% of the activity which had been infused but which had not been excreted into the gas phase, was found in the fluid of the organ chamber. The volume of fluid in the organ chamber in these experiments was about 15 ml. When a larger volume of fluid (ca. 100 ml.) was kept in rapid equilibrium with that in the organ chamber, the rate of excretion of $^{14}CO_2$ into the gas phase remained low throughout the experiment. In two other experiments a large volume of

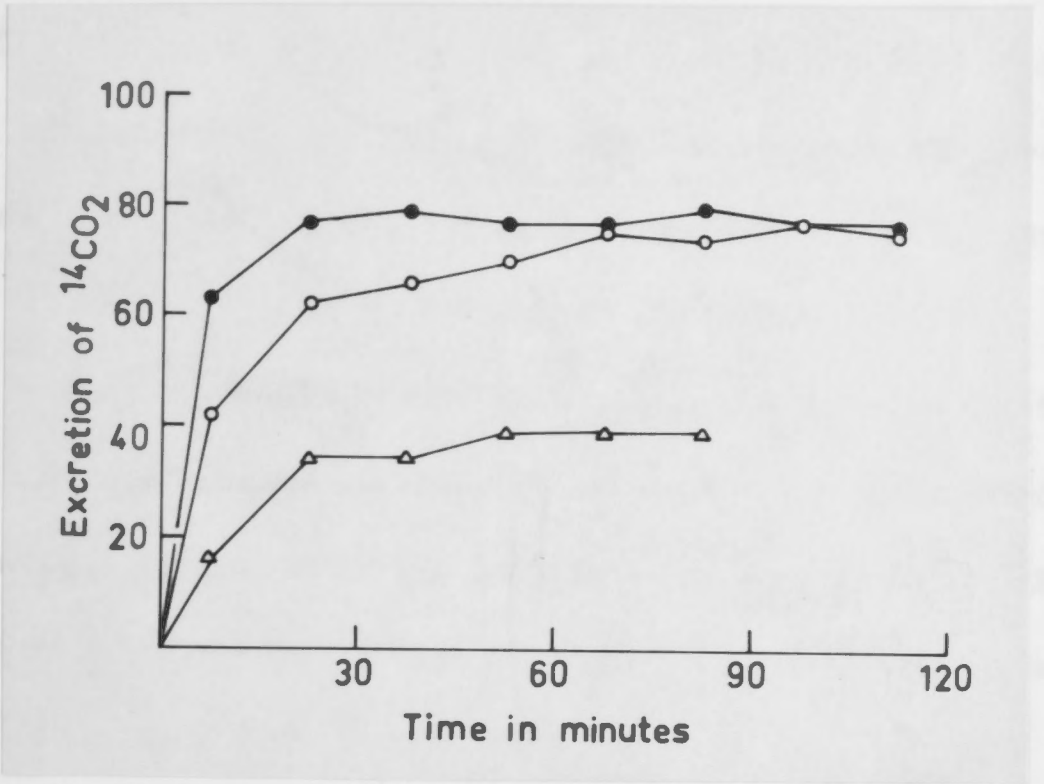


FIGURE 44.

The excretion of $^{14}CO_2$ into the gas phase by the isolated heart-lung preparation during the continuous intravenous infusion of $NaH^{14}CO_3$. The fractional rate of excretion of radioactivity as $^{14}CO_2$, expressed as a percentage of the rate of infusion of radioactivity, is plotted against time.
(O) About 15 ml. of electrolyte solution which had not been equilibrated against the gas phase before the experiments was in contact with the organs. Mean result of 6 experiments.
(△) About 100 ml. of electrolyte solution which had not been equilibrated against the gas phase before the experiments was in contact with the organs. Result of a single experiment.
(●) About 100 ml. of electrolyte solution which had been equilibrated against the gas phase before the experiments was in contact with the organs. Mean result of 2 experiments.

fluid was kept in equilibrium with that in the organ chamber, but it was equilibrated against the gas phase before the experiments. The initial rate of excretion of $^{14}CO_2$ into the gas phase was more rapid than in those experiments in which the fluid was not equilibrated against the gas phase, but it tended to plateau at about 75-80% of the rate of infusion.

These results were taken to indicate that when the fluid in the organ chamber was not equilibrated with the gas phase, there was an initial net movement of CO_2 from the organs into the fluid. When a small volume of fluid was used the concentration of CO_2 in the fluid came into equilibrium with that in the organs during the course of the experiment, but when a much larger volume of fluid was used, net movement of CO_2 into the fluid continued throughout the experiment. When the fluid was equilibrated with the gas phase before the experiment, no net movement of CO_2 into the fluid occurred but it appeared that the CO_2 and $[HCO_3]^-$ were in diffusion equilibrium between the blood and the fluid surrounding the organs, and this equilibrium was such that during a 2 hr experiment, the movement of $^{14}CO_2$ into the fluid of the organ chamber greatly exceeded the movement of $^{14}CO_2$ in the reverse direction. It was concluded from these experiments that in studies with ^{14}C-labelled metabolites, the actual rate of oxidation of

of the metabolites to CO_2 could be up to 30% higher than that indicated by the rate of excretion of $^{14}CO_2$ into the gas phase.

The presence of chylomicron lipid in the plasma. The plasma collected from the hearts at the end of the experiments was milky only in those experiments in which the rate of infusion of chylomicron fatty acids was 6 mg/hr or higher. In hearts which were infused with 4 mg chylomicron fatty acids per hour, there was no milkiness in the plasma, and as more than 8 mg of fatty acids had been infused in the experiments and the total plasma volume of the preparations was only about 0.5 - 0.6 ml., a large proportion of the infused chylomicron fatty acids must have been cleared from the blood.

Glycogen content of the hearts. At the end of the experiments the glycogen content of the hearts was measured. The amounts of glycogen present in the hearts are listed in Table 29.

It can be seen from these results that at the end of 2 hr there was virtually no glycogen in the heart when 2 or 4 mg of chylomicron fatty acids were infused per hour without glucose. However when 6 or 8 mg of chylomicron fatty acids were infused per hour without glucose, there was some glycogen in the heart. It appeared that the infusion of the chylomicron fatty acids

might have spared the oxidation of some of the cardiac glycogen.

TABLE 29

The glycogen content of hearts at the end of experiments in which chylomicrons and glucose were infused in varying amounts into the circulation of the isolated heart-lung preparation.

Rate of infusion of chylomicron fatty acids (mg/hr)	Rate of infusion of glucose (mg/hr)	Glycogen content of heart (mg/100 mg wet wt.)
2	0	0.00
2	10	0.21
4	0	0.00
4	10	0.30
6	0	0.15
6	10	0.40
8	0	0.14
8	10	0.32

The transfer of glucose from the fluid in the organ chamber to the heart and lungs. Because CO_2 and $[HCO_3]^-$ appeared to diffuse readily from the blood to the fluid in the organ chamber, an experiment was done to see if other solutes could pass between the blood and the fluid. One heart-lung preparation was suspended in electrolyte solution which contained generally labelled ^{14}C-glucose together with unlabelled glucose to give an initial concentration of 100 mg/100 ml. The $^{14}CO_2$ which was

excreted into the gas phase in 150 min was equivalent to 3.3 mg of the glucose in the surrounding medium. In a similar experiment in which the heart failed soon after being placed in the organ chamber, the lungs were ventilated for 2¼ hr and in this time $^{14}CO_2$ equivalent to only 0.3 mg of glucose was excreted into the gas phase. It would appear that glucose can diffuse between the blood in the heart-lung preparation and the fluid in the organ chamber. In the previous experiments therefore, when no glucose was infused or added to the fluid surrounding the organs the passage of glucose from the blood to this fluid would have led to a rapid depletion of glucose in the blood perfusing the organs, even if none were used by the organs. Nevertheless, the infusion of adequate amounts of chylomicrons spared the utilization of some of the cardiac glycogen.

The oxidation of chylomicron fatty acids by the isolated heart-lung preparation. A series of experiments was done to study the oxidation of ^{14}C-palmitic acid incorporated into chylomicrons when the chylomicrons were infused directly into the circulation of the isolated heart-lung preparation. The chylomicrons were collected from rats with thoracic duct fistulae which had been fed olive oil. Unlabelled chylomicrons and chylomicrons labelled with ^{14}C-palmitic acid were concentrated by centrifugation and resuspended in

bicarbonate-Ringer solution. The chylomicrons were infused into the isolated heart-lung preparations at rates of 2, 4, 6 and 8 mg of total esterified fatty acids per hour either with no glucose or with glucose at the rate of 10 mg/hr. The infusion of unlabelled chylomicrons was begun as soon as the inferior vena cava was cannulated. When the heart and lungs were in the organ chamber and after the unlabelled chylomicrons had been infused for 35 min, the labelled chylomicrons were substituted for the unlabelled chylomicrons in the infusion mixture. The labelled chylomicrons were infused for 2 hr and the amount of radioactivity which was excreted into the gas phase as $^{14}CO_2$ in consecutive 15 min intervals was measured and equated to the amount of radioactivity infused during the intervals. At the end of each experiment the heart and lungs were removed from the organ chamber and the blood was collected from the aortic stump. The blood was centrifuged and the concentration of total esterified fatty acids in the plasma was measured. Glucose was added to the fluid in the organ chamber to give an initial concentration of 100 mg/100 ml. in all experiments.

The excretion of $^{14}CO_2$ into the gas phase in each 15 min interval of the infusions is listed in Table 30. It can be seen from these figures that there was a significant oxidation of the infused chylomicron fatty

acids in each experiment. The figures listed in Table 30 are the actual measured rates of excretion of $^{14}CO_2$ and from what was found in the preceding experiments it can be concluded that the actual rates of oxidation were 25-30% higher than these figures indicate. There was a considerable variation in the proportion of the infused chylomicron fatty acids which were oxidized, but this was not related to the infusion of glucose. The mean rate of excretion of ^{14}C as $^{14}CO_2$ into the gas phase was 27.4% of the rate of infusion in the final 15 min interval of these experiments both when glucose was infused and when no glucose was infused. However the oxidation of the chylomicron fatty acids did appear to be related to the concentration of esterified fatty acids in the plasma at the end of the experiment. The concentration of esterified fatty acids in the plasma tended to increase with increasing rates of infusion of chylomicron fatty acids, but at any rate of infusion of chylomicron fatty acids, the concentration of esterified fatty acids in the plasma was higher in those experiments in which the oxidation of the chylomicrons was reduced or in which a relative plateau was not attained in the pattern of excretion of $^{14}CO_2$. The concentrations of total esterified fatty acids in the plasma at the end of these experiments are also listed in Table 30.

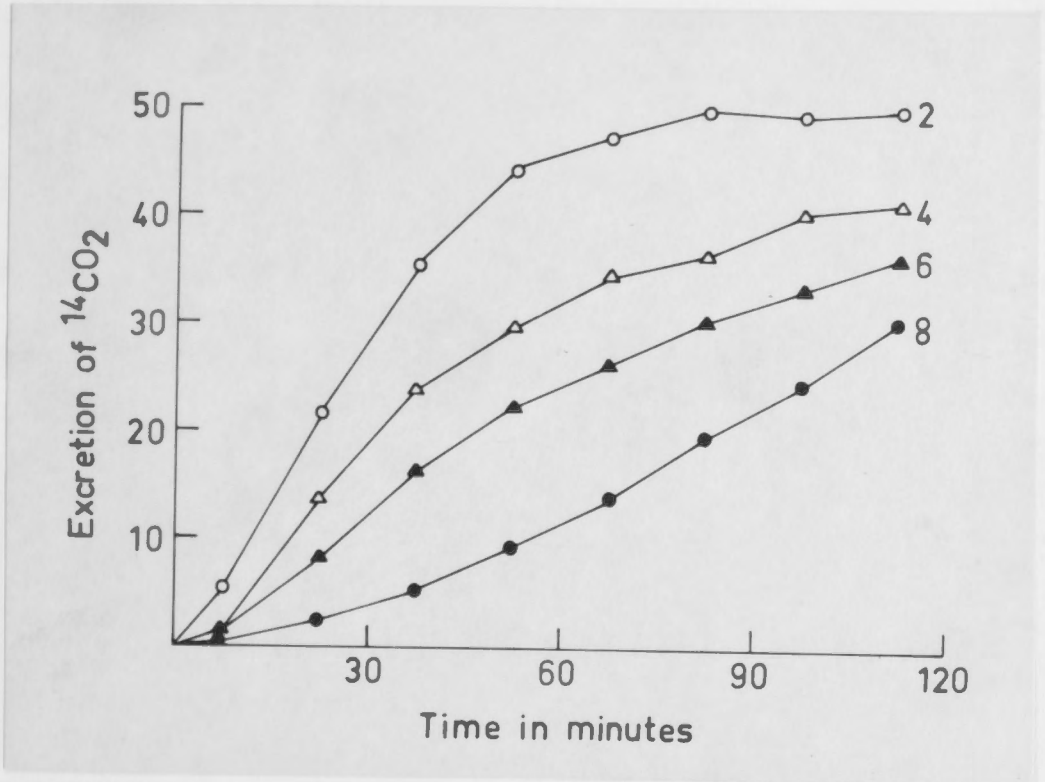


FIGURE 45.

The excretion of $^{14}CO_2$ into the gas phase by the isolated heart-lung preparation during the continuous intravenous infusion of chylomicrons labelled with ^{14}C-palmitic acid. The fractional rate of excretion of radioactivity as $^{14}CO_2$, expressed as a percentage of the rate of infusion of radioactivity, is plotted against time. The rate of infusion of chylomicron fatty acids (mg/hr) is indicated on the figure. Glucose was infused simultaneously at a rate of 10 mg/hr. Each curve is the result of a single experiment.

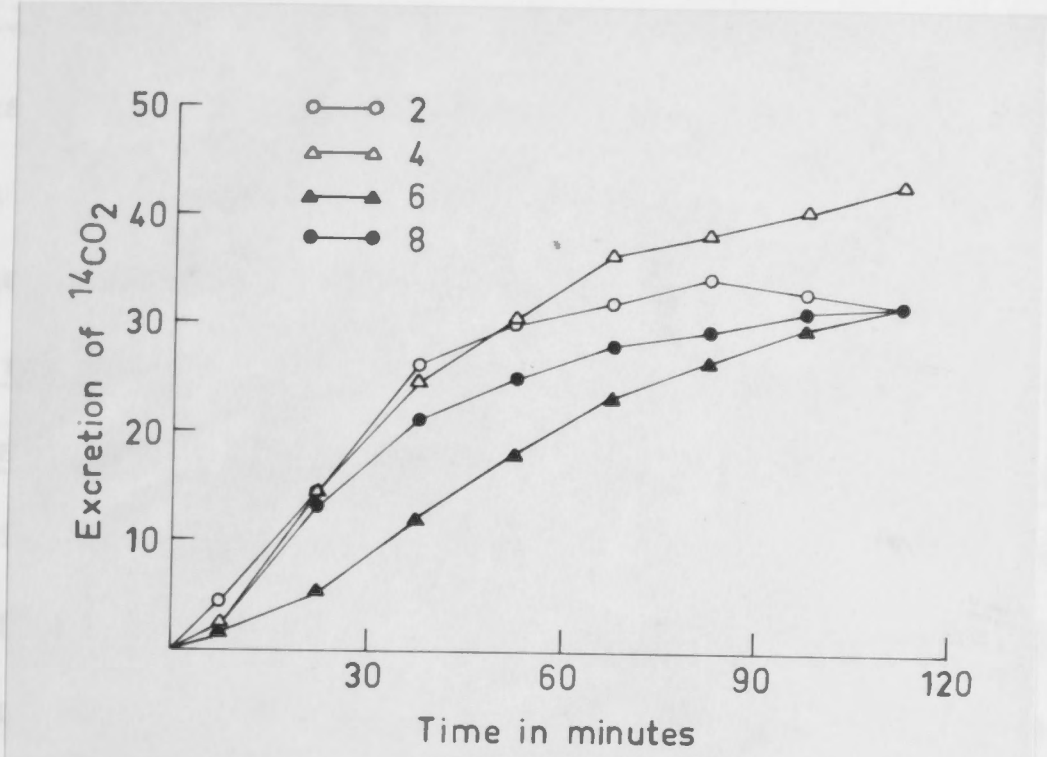


FIGURE 46.

The excretion of $^{14}CO_2$ into the gas phase by the isolated heart-lung preparation during the continuous intravenous infusion of chylomicrons labelled with ^{14}C-palmitic acid. The fractional rate of excretion of radioactivity as $^{14}CO_2$, expressed as a percentage of the rate of infusion of radioactivity is plotted against time. The rate of infusion of chylomicron fatty acids (mg/hr) is indicated on the figure. No glucose was infused in these experiments. Each curve is the result of a single experiment.

The patterns of excretion of $^{14}CO_2$ are shown in Figures 45 and 46 for those experiments in which the oxidation of the chylomicrons proceeded rapidly. These patterns indicate that in the heart-lung preparation, the oxidation of the chylomicron fatty acids followed a similar time course to their oxidation in intact animals. The fatty acids appeared to be partitioned within the organs; a proportion being oxidized rapidly and the remainder more slowly.

Quantitative significance of the oxidation of chylomicron fatty acids by the isolated heart-lung preparation. In some of the experiments when 6 and 8 mg of total esterified fatty acids were infused per hour, the $^{14}CO_2$ excreted into the gas phase in the final 15 min intervals of the experiments was equivalent to the oxidation of more than 2 mg of the infused fatty acids per hour. From the results of previous experiments it was shown that the actual rate of oxidation could be 25-30% higher than indicated by the excretion of $^{14}CO_2$ into the gas phase. It is possible therefore that in two of the experiments in which 8 mg of chylomicron fatty acids were infused per hour, the rate of oxidation of the infused chylomicron fatty acids exceeded 3 mg/hr. Although it was not possible to measure the gaseous metabolism of these isolated heart-lung preparations, the oxidation of 3 mg of fatty acids per hour would

require over 6 cc of oxygen per hour and this would be a considerable proportion of the expected oxygen consumption of the preparations. The concentrations of total esterified fatty acids in the plasma of these preparations when 6 or 8 mg of total esterified fatty acids were infused per hour were of the same order as the levels in the plasma of intact rats when chylomicrons were infused at a rate of 100 mg of fatty acids per hour. From the figures obtained with the isolated heart-lung preparations it is possible that these organs could have accounted for up to 6-10% of the fatty acids oxidized by the intact animals.

The role of heparin activated clearing factor in the removal of chylomicrons from the circulation of the isolated heart-lung preparation. The lipids in the plasma obtained at the end of the previous experiments were assessed qualitatively for the presence of unesterified fatty acids by thin-layer silicic acid chromatography. In all of the samples examined there were appreciable quantities of unesterified fatty acids. It was possible therefore, that the chylomicrons were hydrolyzed before they were cleared from the circulation. There appears to be a close association between the removal and oxidation of chylomicron fatty acids, and their retransport as unesterified fatty acids. Although the retransport of chylomicron fatty acids is not

TABLE 30

The excretion of $^{14}CO_2$ into the gas phase during experiments in which ^{14}C-labelled chylomicrons and glucose were infused into the circulation of the isolated heart-lung preparation. The excretion of $^{14}CO_2$ in consecutive 15 min intervals is given as a percentage of the amount of chylomicrons infused during the interval. The concentration of total esterified fatty acids in the plasma at the end of the experiments is also given.

Rate of infusion		Expt.	Excretion of $^{14}CO_2$ 15 min interval								Plasma
Fatty Acids (mg/hr)	Glucose (mg/hr)	No.	1	2	3	4	5	6	7	8	T.E.F.A. (mg/ml.)
2	10	4	1.3	3.5	8.3	14.8	21.2	26.3	32.8	37.9	3.03
2	10	7	5.4	21.8	35.6	44.3	47.5	50.0	49.5	50.6	2.18
2	0	11	4.5	14.4	26.3	30.1	32.3	34.9	32.7	32.1	1.42
4	10	1	1.9	13.9	24.0	29.8	34.6	36.6	40.4	41.4	2.50
4	10	14	0.5	2.7	5.1	5.5	5.8	6.4	6.4	7.1	4.83
4	10	15	0.0	1.1	2.6	5.0	6.4	8.3	9.9	11.2	6.85
4	0	9	0.0	1.9	3.4	4.8	6.9	9.3	11.5	14.2	4.95
4	0	12	2.6	14.6	24.7	30.7	36.5	38.5	40.5	43.2	1.89
6	10	5	1.6	8.3	16.7	22.4	26.3	40.8	33.4	36.6	3.40
6	0	6	1.6	5.1	11.9	17.9	23.1	26.3	29.8	30.4	4.95
6	0	16	0.6	3.0	8.3	12.0	15.2	18.2	20.7	22.8	4.95
8	10	2	0.5	2.6	5.3	9.4	14.0	19.7	24.5	30.3	8.97
8	10	13	0.2	0.6	1.0	1.7	2.1	2.9	3.2	3.7	9.95
8	0	10	2.9	13.0	21.2	25.0	28.1	29.3	31.5	32.0	4.25
8	0	17	0.5	2.8	6.8	10.8	12.5	14.8	16.0	17.0	10.20
8*	0	18	1.8	6.7	5.5	-	-	-	-	-	0.85

* Rat was given 200 IU heparin intravenously before the heart was removed. This heart failed but it was infused with chylomicrons for a total of 85 minutes before it was bled out.

believed to be of quantitative importance in the overall oxidation of chylomicron fatty acids by intact animals, it is possible that this mechanism might be of importance in the oxidation of chylomicron fatty acids by some tissues.

An attempt was made to promote the rapid intravascular hydrolysis of chylomicrons in the isolated heart-lung preparation. Two experiments were performed in which heparin was injected into the rats before their hearts and lungs were isolated. In both of these experiments the hearts failed and the failures were associated with the appearance of blood in the fluid in the organ chamber. Because of these failures no more of these experiments were attempted. However in one of these experiments, chylomicrons were infused at a rate of 8 mg of total esterified fatty acids per hour for 85 minutes before the heart failed. The concentration of total esterified fatty acids in the plasma obtained from this heart was very much lower than in any of the previous experiments (see Table 30). It would seem therefore that although the heparin activated clearing factor might be responsible for the removal of the chylomicrons from the circulation of the isolated heart-lung preparation, this removal is not due to an intravascular hydrolysis analagous to the post-heparin clearing response.

The presence of demonstrable fat in the heart and lungs.

Specimens of the hearts and lungs were fixed in formol-saline and frozen sections cut from the fixed tissues were stained for fat. Small amounts of fat were visible in phagocytic cells in the lungs and these amounts tended to increase with the higher rates of infusion of fat. There was not histologically demonstrable fat in any of the hearts.

CHAPTER SEVEN

DISCUSSION

The excretion of $^{14}CO_2$ by rats

The complexities of the processes involved in the excretion of CO_2 from the body have been investigated by several groups of workers (cf. Kornberg, Davies & Wood, 1952; Steele, 1955; Drury, Wick & Almen, 1956; Robinson & Coxon, 1957; Shipley, Baker, Incefy & Clark, 1959; Coxon & Robinson, 1959a) and these data have been used in the interpretation of experiments in which the oxidation of various labelled substances injected intravenously was studied (Steele, Altszuler, Wall, Dunn & de Bodo, 1959; Coxon & Robinson, 1959b; Baker, Shipley, Clark, Incefy & Skinner, 1961). In some of these experiments the $^{14}CO_2$ excretion curves have been interpreted in terms of interconnecting pool systems and mathematical models have been fitted to experimental data to give rate constants associated with the transfer of CO_2 between the various pools (Steele, 1955; Shipley, Baker, Incefy & Clark, 1959; Steele, Altszuler, Wall, Dunn & de Bodo, 1959; Baker, Shipley, Clark, Incefy & Skinner, 1961). Workers using this approach have been aware that this form of treatment requires the existence of a non-isotopic steady state within the pool systems for these derived rate constants to have any precise

meaning. Whether this condition is fulfilled in many of the experiments is by no means certain. In the experiments described in this thesis, the animals were trained to the experimental conditions and were placed in the metabolism system about an hour before the experiments were begun. Even under these conditions there were large variations in the $^{14}CO_2$ excretion curves of individual animals due to periods of spontaneous activity. The 'steady state'as applied to the excretion of CO_2 would seem to be a condition which does not pertain in intact unanaesthetized animals. These fluctuations in individual excretion patterns seemed to be randomly distributed with time, and by pooling the results from a number of animals, a comparatively smooth mean excretion curve was obtained which could be analyzed kinetically.

In the experiments reported in this thesis, the results obtained when rats were infused with $NaH^{14}CO_3$ were similar in general form to results which have been published previously. It was surprising that even when the excretion curves were corrected for the lag introduced by the compartments of the metabolism system, almost an hour elapsed before the rate of excretion of the infused label as $^{14}CO_2$ reached 90% of its rate of infusion. When the mean pattern of excretion of $^{14}CO_2$ was analysed, two exponential components with half-lives

of about 7-8 min and 43 min were extracted and a third more rapid component with a half-life of about 3 min was calculated to describe the curve completely. The conversion of the excretion curve to a form which would have been obtained if the $NaH^{14}CO_3$ had been given as a single intravenous injection, enabled a comparison to be made with results published previously for rats (Shipley, Baker, Incefy & Clark, 1959). When this comparison was made, the two patterns although of similar general form, were quite different quantitatively. In the experiments of Shipley, Baker, Incefy & Clark (1959), about half of the injected label was expired in the first 1-2 min following the injection of the $NaH^{14}CO_3$, before the animals were placed in the metabolism system. In view of the fact that an activity such as face cleaning can double the rate of excretion of $^{14}CO_2$, if the animals used by Shipley et al. struggled during the injection or afterwards, the rate of excretion of $^{14}CO_2$ would be increased, and an assumed non-isotopic steady state would not have existed in fact. Similar criticisms can be made of experiments in which rats were alternately placed in the metabolism system and then taken out and the ends of their tails cut off for repeated blood samples; procedures which, according to the authors, the rats resented vigorously. (Baker, Shipley, Clark, Incefy & Skinner, 1961).

In a mathematical treatment of Kornberg's data for $^{14}CO_2$ excretion in cats (Kornberg, Davies & Wood, 1952), Steele (1955) proposed a model comprising a central plasma pool and two peripheral pools which he designated 'soft tissue' and 'bone'. He showed that if $^{14}CO_2$ arising from the oxidation of a labelled substance was released continuously as bicarbonate into one or other of the peripheral pools, the excretion of $^{14}CO_2$ would be different from that obtained by continuously infusing $NaH^{14}CO_3$ into the circulation. Shipley, Baker, Incefy & Clark (1959) also proposed a three pool system to explain their experimental results, but they made the point that, as the carbonic anhydrase activity in tissue cells is low, metabolic CO_2 would diffuse directly into the blood more quickly than it could be converted to bicarbonate. In view of this they derived their results from the assumption that all metabolic CO_2 goes directly into the blood before it is converted into bicarbonate by the carbonic anhydrase in the red blood cells.

In the analysis of the experiments reported in this thesis no attempt has been made to assign any physiological significance to the patterns of excretion of $^{14}CO_2$ in terms of the tissues involved. It has been pointed out previously that such analyses are gross oversimplifications of the complex processes underlying the excretion of the $^{14}CO_2$. Drury, Wick & Almen (1956), in studying the $^{14}CO_2$ content of various organs following

a single injection of labelled bicarbonate found that the amount remaining in the different tissues was related to the fractional rates of perfusion of the tissues. Coxon & Robinson (1959a) have also shown that the specific activities of arterial and venous blood CO_2 equilibrate at different rates in different tissues when $NaH^{14}CO_3$ is injected. There seemed little justification therefore in postulating a pool system which can have no physiological meaning to explain the results. However the excretion curves were analyzed so that some estimate might be made of the exponential components which feature in the excretion of $^{14}CO_2$. These data could then be used in the interpretation of $^{14}CO_2$ excretion patterns during the continuous intravenous infusion of ^{14}C-labelled metabolites. In Appendix III it is shown that the exponential components associated with the oxidation of a ^{14}C-labelled metabolite and with the subsequent excretion of the resulting $^{14}CO_2$ from the bicarbonate pools are preserved in the composite $^{14}CO_2$ excretion curves recorded during such an infusion. It is only the constants by which these exponential components are multiplied that are altered. Thus by knowing the characteristics of the bicarbonate pools, recorded patterns of excretion of $^{14}CO_2$ can be analyzed to provide information concerning the rate at which $^{14}CO_2$ enters the blood. If the assumption of Shipley, Baker, Incefy & Clark (1959) that metabolic CO_2 enters the blood more

rapidly than it can be converted to bicarbonate in the tissue cells is correct, then the rate at which $^{14}CO_2$ enters the blood will be a good approximation of the rate at which it is produced. On this assumption hypothetical rates of formation have been considered and the effect of the bicarbonate pools on the excretion of the $^{14}CO_2$ has been described.

The most rapid component in the excretion curves described in Chapter 3 was determined by arbitrarily fixing the size of the pool of bicarbonate with which the bicarbonate of the blood is in rapid equilibrium. All of the bicarbonate of the extracellular fluid was included (cf. Shipley, Baker, Incefy & Clark, 1959; Coxon & Robinson, 1959a). However if the pool is in fact smaller or larger, the half-life of this component will be shorter or longer respectively, and the derived equation for the fraction of a single intravenous injection of $NaH^{14}CO_3$ remaining in the pool at any time after the injection would be changed accordingly. However the size of this pool is also used in calculating rates of oxidation of labelled substances from recorded patterns of excretion of $^{14}CO_2$ and it can be changed over a wide range without introducing any serious error into calculated rates of oxidation except when these rates of oxidation are rapid.

The metabolism of continuously infused chylomicron fatty acids by intact rats

The oxidation of continuously infused chylomicron fatty acids. When chylomicrons labelled with ^{14}C-palmitic, ^{14}C-oleic or ^{14}C-linoleic acids were obtained from rats fed triolein and infused continuously into the circulation of starved rats, the rates of oxidation of the three labelled fatty acids, as measured by the excretion of $^{14}CO_2$ in the rats' expired breath, were quantitatively similar. In view of this finding the oxidation of each of the labelled fatty acids was used as an index of the oxidation of all the fatty acids present in the chylomicrons. In addition, when these chylomicrons were infused at rates of from 5 to 100 mg total esterified fatty acid per hour, the percentage rates of oxidation of the labelled fatty acids did not change significantly. When chylomicrons with a different fatty acid composition were infused, the oxidation of ^{14}C-oleic and ^{14}C-linoleic acids was similar to the previous experiments. The oxidation of ^{14}C-palmitic acid incorporated into the chylomicrons, however, was reduced when there was a relatively high proportion of palmitic acid in the chylomicrons. It is difficult at this stage to assign any biological significance to this reduced oxidation as this result might have been due to a difference in the quality of the two chylomicron preparations labelled with ^{14}C-palmitic acid. It did not

seem that this difference could be attributed to a change in the physical characteristics of the chylomicrons due to the different fatty acid composition, as the oxidation of the oleic and linoleic acids incorporated into the chylomicrons was not changed.

In each experiment, the rate of excretion of $^{14}CO_2$ increased rapidly until the second hour of the infusion when a relative plateau in the pattern of excretion of $^{14}CO_2$ was attained. This relative plateau then persisted for at least up to the end of the 4th hr of the infusion. By analyzing the patterns of excretion of $^{14}CO_2$, and from a consideration of the dynamics of excretion of $^{14}CO_2$ from the body, it appeared that some 25-30% of the infused fatty acids behaved as though they were taken up into a relatively small pool of fatty acids which had a rapid fractional rate of turnover. The fatty acids which entered this pool were oxidized rapidly and had a mean metabolic half-life, before being oxidized, of about 13-15 min. The remainder of the fatty acids appeared to be taken up into a pool of fatty acids with a slower fractional rate of turnover and a mean metabolic half-life of 9-10 hr. In these experiments it was not possible to decide whether the two proposed pools of fatty acids have a different intracellular distribution or a different tissue distribution. A consideration of the patterns of excretion of $^{14}CO_2$ in this way is an obvious oversimplification of the patterns of oxidation,

but it affords a useful basis for comparisons when interpreting the results of different experiments. Geyer, Matthews & Stare (1949) studied the oxidation of emulsified glyceryl(trilaurin-^{14}C) by several tissues *in vitro*, and they noted that the patterns of the production of $^{14}CO_2$ by the tissues had two phases. Volk, Millington & Weinhouse (1952) demonstrated active pools of endogenous fatty acids in various tissues which were oxidized more rapidly to CO_2 than the total fatty acids present in the tissues. Moreover, these active pools of fatty acids were in equilibrium with all the fatty acids in the tissue, but the rates at which the two pools of fatty acids equilibrated with each other varied in different tissues. It is shown in Appendix IV that the patterns of excretion of $^{14}CO_2$ which occur during the continuous intravenous infusion of a ^{14}C-labelled substrate are comparable to the cumulative patterns of excretion of $^{14}CO_2$ which occur following a single intravenous injection of the ^{14}C-labelled substrate. It would seem, therefore, that a similar partitioning of the chylomicron fatty acids occurred in experiments in which chylomicrons were given as a single intravenous injection (French & Morris, 1958; Bragdon, 1958).

The pool in which the fatty acids were oxidized rapidly in the experiments described in this thesis provided at least 16% of the rats' total energy requirements, as with the highest rates of infusion of chylomicron

fatty acids, some 16% of the total CO_2 produced by the rats was coming from infused fatty acids which were oxidized in this pool. As there appeared to be no decrease in the percentage oxidation of the infused chylomicron fatty acids at the highest rates of infusion, it might be expected that a higher proportion of the total CO_2 production could be provided by chylomicron fatty acids oxidized in this pool. Such a pool of fatty acids could exist in tissues with a high metabolic rate and with a relatively small content of fatty acids, and under conditions of fasting, the supply of fatty acids to this pool would be very dependent on mobilized fat. Metabolically active tissues other than the liver have been found to contain only a small proportion of chylomicron fatty acids when these tissues were examined following a single intravenous injection of labelled chylomicrons (Morris & French, 1958; Bragdon & Gordon, 1958). This has been taken to indicate that these tissues are relatively unimportant in the removal of chylomicrons from the circulation. This may not be true if the turnover of fatty acids in these tissues is rapid.

It is unlikely that the total liver fatty acids are representative of this pool for several reasons. The liver contains a large amount of fatty acids, and although more than 20% of the labelled fatty acids given in a single injection of chylomicrons can be recovered

from the liver soon after the injection (French & Morris, 1958), the rate at which the specific activity of the fatty acids in the liver decreased showed that their half-life was about 6 hr. This would place the liver in the class of tissue which has a relatively large pool of fatty acids with a slow fractional rate of turnover. It is possible, however, that the liver lipids are not metabolically homogenous, and there may be a fraction of lipid in this organ turning over rapidly (cf. Stein & Shapiro, 1959).

It is unlikely that adipose tissue in general represents a part of the slow pool of fatty acids as the amount of chylomicron fat which was recovered in the adipose tissue of fasted rats following a single intravenous injection of chylomicrons was small (Bragdon & Gordon, 1958). However, the amount of injected fatty acids recovered in a small sample of fat may not give a true indication of their distribution in all the adipose tissue. It is possible therefore that a more active component of the fatty acids of adipose tissue may also be included in the pool of fatty acids turning over slowly. From a consideration of the slope of the terminal part of the patterns of excretion of $^{14}CO_2$ and the proportion of the total energy requirements of the rat provided by fatty acids oxidized in this pool, its size can be estimated to lie between 1-2 g of fatty acids. As this pool would contain a large proportion of the

infused chylomicron fatty acids at any time during the infusions, and in view of the results of experiments in which the distribution of single intravenous injections of chylomicron fatty acids were studied, it would seem that this pool of fatty acids is distributed generally throughout the tissues of the body.

The removal of continuously infused chylomicrons from the circulation. The circulating levels of esterified fatty acids in the plasma of rats during the continuous intravenous infusion of chylomicrons could have been predicted from previous studies in which the circulating half-life of chylomicrons was calculated following a single intravenous injection (French & Morris, 1957). In these experiments the circulating half-life increased as increasing amounts of fatty acid were injected. The increase was attributed to a saturation effect occurring in the tissues responsible for the clearance of the chylomicrons from the circulation. In the experiments described in this thesis, this saturation effect was demonstrated by the non-linear relationship between the rate of infusion of chylomicron fatty acids and their concentration in the plasma. However, with the highest rates of infusion of chylomicrons, a relative plateau was obtained in the plasma levels demonstrating that this saturation effect was ephemeral. When the rate of infusion of chylomicrons was increased from 50 to 100 mg total esterified fatty acid per hour, the

circulating half-life of the chylomicrons increased from 4 to 16 min.

The role of the liver in the removal of chylomicrons from the circulation. In most of the experiments described in this thesis, about 20% of the labelled chylomicron fatty acids infused hourly, was recovered in the liver at the end of the experiments. This was only a small proportion of the total amount of radioactivity which had been infused. Moreover, there were differences in the amounts of radioactive fat recovered from the liver when chylomicrons labelled with different fatty acids were infused, and these differences were related to the fatty acid composition of the chylomicrons. When the concentration of linoleic acid in the chylomicrons was low, a greater proportion of labelled linoleic acid was recovered in the liver than was the case with palmitic or oleic acids. However, when the concentrations of linoleic and palmitic acids in the chylomicrons were high, a greater proportion of the infused palmitic acid was recovered in the liver than was the case with linoleic or oleic acids. Nestel, Bezman & Havel (1962) obtained chylomicrons from dogs fed cream labelled with ^{3}H-palmitic acid, and chylomicrons from dogs fed corn oil labelled with ^{14}C-linoleic acid. These were injected simultaneously into dogs, and the results showed that a greater proportion of the injected ^{3}H-palmitic

was retained in the liver than was the case with the ^{14}C-linoleic acid. In these experiments the ^{3}H-palmitic acid was incorporated into chylomicrons with a relatively high content of palmitic acid while the ^{14}C-linoleic acid was incorporated into chylomicrons with a relatively high content of linoleic acid. These differences most likely reflect differences in re-transport of the fatty acids from the liver, rather than differences in the initial uptake of the fatty acids by the liver. Simpson-Morgan & Morris (1962) showed that ^{14}C-palmitic, ^{14}C-oleic and ^{14}C-linoleic acids incorporated into chylomicrons were taken up by the isolated perfused rat liver and oxidized to CO_2 at similar rates, but significantly more of the ^{14}C-linoleic acid was retransported to the perfusate as phospholipid fatty acid than was the case with ^{14}C-palmitic or ^{14}C-oleic acids.

In the experiments reported in this thesis, the proportion of the various labelled fatty acids recovered in the liver was not related to the rate of infusion of total chylomicron fatty acids. The total amount of lipid recovered from the livers was not increased with higher rates of infusion of chylomicrons although increased amounts of the infused chylomicron fatty acids were present in the liver. This meant that the increased uptake of infused lipid was compensated for by a decreased uptake of endogenous lipid. If the liver is

the organ primarily responsible for the initial uptake of chylomicrons from the circulation before the chylomicron fatty acids are retransported and utilized by the other tissues of the body, then the fractional rate of turnover of the liver lipids must have been extremely rapid when large amounts of chylomicron fatty acids were infused. It has been shown previously, that the total liver fatty acids cannot be considered to be turned over sufficiently rapidly to account for this. It does not seem likely therefore that the liver was responsible for the initial uptake of most of the chylomicrons which were infused in these experiments. Nestel, Havel & Bezman (1962) infused chylomicrons labelled with radioactive fatty acids into dogs and measured the flux of the radioactive fatty acids through the fatty acid pool of the liver. They concluded that a large proportion of the infused chylomicrons were removed from the circulation directly by extrahepatic tissues.

The significance of esterified fatty acids in the energy metabolism of rats. It has been difficult to assess the potential of chylomicron fatty acids in providing for the energy requirements of animals from the results of experiments in which single intravenous injections of chylomicrons were given. When chylomicrons are given in this way, comparatively large amounts must

be injected to provide for a significant proportion of the animals' energy requirements. Morris (1958) showed that as the total amount of chylomicron fatty acids injected into rats was increased, the proportion of the injected fatty acids which was oxidized to CO_2 by the rats in the 2 hr following the injection, was decreased. It was anticipated therefore that when chylomicrons were infused continuously, the rate of oxidation of the infused chylomicrons at any time during the infusions, expressed as a fraction of the rate of infusion, would be decreased as the rate of infusion increased. This was not shown to be the case, even when rates of infusion of fatty acids were sufficiently high to suggest that the removal of chylomicrons from the circulation might have been a factor limiting their rate of oxidation. Thus the rats were shown to oxidize chylomicron fatty acids as efficiently when they were infused at rates several times in excess of any they would have experienced, as when they were infused in trace amounts. This suggests that the rats are normally able to oxidize large amounts of esterified fatty acids, and that esterified fatty acids might normally provide for a large proportion of the rats' energy requirements.

As all the animals were starved for the same length of time, their respiratory quotients were assumed to be similar, and the caloric equivalents of their CO_2 would

be comparable. On this basis, it was seen that the proportion of the total calories furnished by the infused chylomicrons increased linearly with the amount of fat infused. As there was not a great change in the total CO_2 produced with increased rates of infusion, there must have been correspondingly less endogenous lipid oxidized at the higher rates of infusion of chylomicrons. It would be reasonable to expect that a higher proportion of the rat's energy requirements could be provided by higher rates of infusion of chylomicrons than those used in these experiments.

The effect of glucose on the oxidation of continuously infused chylomicrons by intact rats

When unlabelled glucose and chylomicrons labelled with ^{14}C-palmitic acid were infused simultaneously at various rates into fasted rats, the oxidation of the ^{14}C-palmitic acid to CO_2 was reduced when compared with experiments in which no glucose was infused, and this reduced oxidation was related linearly to the rate at which glucose was infused. Before the magnitude and nature of this reduction are considered in relation to the experiments of other workers, the design of the experiments in this thesis will be discussed.

The rates of infusion of glucose were chosen after considering the results of other experiments in which glucose was infused at varying rates to measure their effects on the level of glucose in the circulating plasma.

Any effects of glucose on the metabolism of the infused chylomicrons therefore can be related not only to the rate of infusion of glucose, but also to the probable concentration of glucose in the plasma of the rats during the experiments. From a consideration of the experiments in which glucose alone was infused, rates of infusion of glucose of 0, 150 and 300 mg/hr were used. It was found that the infusion of 300 mg glucose/hr increased the concentration of glucose in the plasma to a high level (ca. 200 mg/100 ml); this rate of infusion is of the same order as the rate at which rats of the size used can absorb glucose (cf. Cori, 1925). It was necessary to use this highest rate of infusion of glucose in order to study the effect of the rate of infusion of glucose on the oxidation of the chylomicron fatty acids without confounding the results with interactions between the infused glucose and endogenous glucose. In experiments in which glucose alone was infused at rates below 200 mg/hr, endogenous glucose appeared to be added to the blood by the liver. The rats were shown to be able to oxidize ^{14}C-glucose to CO_2 as efficiently when it was infused at the rate of 300 mg/hr, as when it was infused at lower rates. It was therefore anticipated that the glucose infusions at the highest level would have a pronounced effect on the oxidation of the chylomicron fatty acids.

The oxidation of the chylomicron fatty acids was reduced by only about 16% in the 3rd hr of the experiments in which glucose was infused at a rate of 300 mg/hr, compared with experiments in which no glucose was infused. Moreover, the infusion of glucose at this rate for different periods of time before the infusion of chylomicrons was begun, had no additional effect on the oxidation of the chylomicron fatty acids. The reduction in the oxidation of the infused chylomicron fatty acids was due entirely to an increase in the metabolic half-life of the fraction of the infused fatty acids which appear to be oxidized relatively slowly. This effect seemed to be related to the total rate at which energy-yielding substrate was infused into the rats. The effect of the glucose, if any, on the fatty acids which appear to be oxidized rapidly, was to increase the amount of fatty acids oxidized in this way.

These results are very different to those reported by other workers who claimed that glucose virtually abolished the oxidation of long chain fatty acids in rats (Lossow & Chaikoff, 1955; McCalla, Gates & Gordon, 1957; Bragdon, 1958). These workers showed that the oxidation of ^{14}C-palmitic acid injected in various forms into carbohydrate 'loaded' rats was only about 1/8th of the oxidation measured in fasted rats. In order to compare these results with the results of experiments reported in this thesis, it is necessary to consider the methods

used to 'load' the rats with glucose. Lossow & Chaikoff (1955) force-fed their rats on a liquid diet, rich in glucose, for several days before the experiments. The fasted rats were not fed for the 24 hr before the experiments. The glucose 'loaded' rats were given 12 ml. of the liquid diet, containing about 5 g of glucose, 12 hr before the experiments, and 6 g of glucose as a 50% solution immediately before the experiments. Lossow & Chaikoff (1955) claimed that the rats were trained to tolerate such large single feeds by progressively increasing the volume given in each single feed over a period of a few days. In the course of the work done in connection with this thesis, it was found that rats weighing 220 g were killed invariably by a single feed of 12 ml. of 50% glucose, and death was due to circulatory collapse which resulted from the loss of large amounts of fluid into the guts, even though the rats developed diarrhoea soon after the feed. Before death, the blood of these rats showed a severe reduction in the circulating plasma volume. It is difficult to imagine how a rat, or any other animal, could be trained to tolerate such treatment. The training might have prevented the rats from dying, but it is doubtful if the animals could develop tolerance to the effects of the glucose solution in the gut and so prevent the large volumes of tissue fluid from being extracted into the gut. Lossow &

Chaikoff reported that the fed rats produced only slightly more CO_2 than the fasted rats. If the thesis is correct that these fed rats were not oxidizing long chain fatty acids to any significant extent, then they should have had a R.Q. close to 1. The fasted rats, on the other hand, would have had much lower R.Q.'s. If the metabolic rates of the two groups were comparable, they would have had similar rates of oxygen consumption, and consequently, the fed rats should have produced as much as 40-50% more CO_2 than the fasted rats. As this was not so, it must mean that the fed rats had considerably lower metabolic rates than the fasted rats, and this might well have been due to the results of the feeding. Moreover, it would be expected that fatty acid synthesis would be proceeding rapidly in rats maintained on a high carbohydrate diet, especially if the diet provided more calories than the rats' energy requirements. Yet Lossow & Chaikoff reported that the oxidation of acetate by the two groups of rats was virtually the same. This can only mean that either very little acetate was synthesized into fatty acids, or the fatty acids synthesized from the acetate were oxidized to CO_2. However, as the oxidation of fatty acids was supposed to be inhibited, this would eliminate the possibility that fatty acid synthesis from acetate was occurring. The long chain fatty acids used by Lossow & Chaikoff (1955) were given

as artificial emulsions and this might, in part, have accounted for the pronounced effect of the glucose on their oxidation.

McCalla, Gates & Gordon (1957) and Bragdon (1958) used a different, but nevertheless drastic, method to 'load' rats with glucose. These workers replaced the rats' drinking water with a solution of 10% glucose in 'half-strength physiological saline'. They reported that the rats drank more than 150 ml. of this solution in about 20 hr. The rats therefore drank, in less than a day, a volume of fluid which weighed almost as much as the rats themselves, and consumed 15 g of glucose. In addition to this the rats were force-fed 1 g of glucose 1 hr before the experiments, and 1 g of glucose immediately before the experiments. The rats given this amount of glucose produced almost twice as much CO_2 as the fasted rats (Bragdon, 1958). This indicated that the fed rats either had a high respiratory quotient, or a high metabolic rate. In experiments reported in this thesis, the only way in which the fractional rate of oxidation of fatty acids incorporated into chylomicrons could be reduced to any great extent, was to infuse chylomicrons at a rate which saturated the sites of oxidation of the fatty acids. When this was done, the bulk of the fatty acids which were being oxidized in the 3rd hr of the infusion, were fatty acids which came from the relatively

slowly oxidized pools. Glucose would be expected to decrease greatly the oxidation of the chylomicron fatty acids under these conditions. It is possible therefore, that the large amounts of fatty acids which might have been synthesized from the glucose given to the rats used by McCalla, Gates & Gordon (1957) and Bragdon (1958), were responsible for the pronounced effect of the glucose on the oxidation of long chain fatty acids, and thus the 'sparing effect' demonstrated was one of fat sparing fat rather than glucose sparing the oxidation of the fat.

Whilst the oxidation of the chylomicron fatty acids to CO_2 was reduced only by about 16% when 300 mg of glucose was infused per hour, the glucose infusion reduced the concentration of ketone bodies in the plasma by over 50%. Ketone bodies are produced exclusively by the liver *in vivo* (Chaikoff & Soskin, 1928), and this production has been attributed to a relative deficiency of oxaloacetic acid in the liver. However Kalnitsky & Tapley (1958) found that the concentration of oxaloacetate was not reduced in the livers of rats when they were fasted for 24 hr, even though the concentration of acetoacetate was increased greatly. These findings have been interpreted as indicating that the deficiency of oxaloacetate in the livers of fasted rats is relative only to the increased formation of acetyl-CoA from the breakdown of long chain fatty acids (cf. Fritz, 1961). If this is the case, the

infusion of glucose in the present experiments must have had a greater effect on the oxidation of long chain fatty acids in the liver than in other tissues. However, it was estimated that the livers of these fasted rats were producing glucose at a rate of about 150 mg/hr. Much of this glucose must have been synthesized from amino acids and possibly lactic acid. The synthesis of glucose from these substances requires that they be converted to oxaloacetate which is then converted to phosphopyruvate and then to glucose by a reversal of the glycolytic pathways (Weinman, Strisower & Chaikoff, 1957). Thus the synthesis of these relatively large amounts of glucose would require not only that many of the intermediates in this synthesis were traversing some of the reactions of the tricarboxylic acid cycle, but also that oxaloacetate was being used up continually. This synthesis of glucose from oxaloacetate might contribute to the relative deficiency of oxaloacetate for the synthesis of citrate from acetyl-CoA. Thus the apparent effect of glucose infusion on the oxidation of long chain fatty acids in the liver might be due to the reduced synthesis of endogenous glucose.

More of the infused ^{14}C-palmitic acid was recovered in the lipids extracted from the livers and samples of adipose tissue of rats which had been infused with glucose, than from the livers and adipose tissue of fasted rats.

Bragdon & Gordon (1958) found a greater proportion of injected chylomicron fatty acids in adipose tissue of glucose 'loaded' rats than was the case with fasted rats. However, they found less of the chylomicron fatty acids in the livers of the carbohydrate fed rats. It has been suggested that chylomicrons are hydrolyzed before their fatty acids are taken up into adipose tissue cells (Reiser, Williams & Sorrel, 1960), and that the rate at which fatty acids are released from adipose tissue is controlled by the rate of synthesis of triglycerides in the adipose tissue (Steinberg, Vaughan, & Margolis, 1960). It is possible that increased rates of re-esterification of the chylomicron fatty acids in the adipose tissue of rats infused with glucose could account for the greater amounts recovered from this tissue. The concentrations of unesterified fatty acids in the plasma tended to be lower in the rats which were infused with glucose and this could have been due to a decreased rate of release of fatty acids from adipose tissue. The possibility that the fatty acids of the liver, and a more active component of adipose tissue fatty acids are components of the pool of fatty acids which are turned over slowly in the body, has been discussed previously. The demonstration that more of the infused chylomicron fatty acids could be recovered in these tissues from rats which had been infused with glucose is in agreement with

the observation that less of these fatty acids were oxidized to CO_2. However, the maximum amounts of the total infused chylomicron fatty acids which could be accounted for in these two tissues were considerably less than the total amount of the infused fatty acids which must have been present in the pool of fatty acids which were turning over slowly. Again it must be concluded that these fatty acids which are turning over slowly are distributed generally throughout the tissues of the body, and might include many structural components of the cells. Glucose might therefore restrict the transport and distribution of fatty acids within cells generally, as well as the overall transport of fatty acids between the various tissues of the body.

The respiratory quotients of the rats used in these experiments indicated that the rats were not deriving much of their energy from the oxidation of glucose, thus the rats infused with glucose at a rate of 300 mg/hr and chylomicron fatty acids at a rate of 100 mg/hr were obtaining about 14% and 22% of their energy requirements from the oxidation of glucose and chylomicron fatty acids respectively. The levels of glycogen in the various tissues indicated that the glucose was being stored in preference to being oxidized. However from studies with ^{14}C-glucose, similar rats appeared to be able to oxidize relatively large amounts of these glucose infusions. The possibility therefore arises that the rats might have

been oxidizing the chylomicrons in preference to the glucose. Certainly the experimental results provide no support for the widely held belief that glucose is a preferentially oxidized substrate which spares the oxidation of chylomicron fatty acids.

The metabolism of chylomicron fatty acids by the isolated rat heart-lung preparation

Ballard, Danforth, Naegle & Bing (1960) demonstrated that the heart is able to extract esterified fatty acids from the blood *in vivo* and these fatty acids can account for a large proportion of the total fatty acid uptake by the heart. These workers however, did not determine whether particulate lipid was extracted from the blood. When chylomicrons labelled with ^{14}C-palmitic acid were injected into intact rats, some of the radioactive fatty acids were recovered in the rats' hearts soon after the injection (Bragdon & Gordon, 1958). The chylomicron fatty acids however might not have been removed from the blood initially by the hearts. In the experiments described in this thesis, it was shown that chylomicron fatty acids were taken up directly by the heart-lung preparation, without the participation of any other tissue. The chylomicron fatty acids must have been taken up into the tissues either in the intact chylomicrons, or after the chylomicrons had been hydrolyzed. Although unesterified fatty acids were detected in the blood taken

from these preparations after the infusion of chylomicrons, this is not evidence, in itself, for the intravascular hydrolysis of the chylomicrons. Morris (1963) in experiments with the isolated perfused rat liver, found that when chylomicrons labelled with ^{14}C-palmitic acid were added to the perfusate, there was a large increase in the concentration of unesterified fatty acids in the perfusate during the perfusion, but the specific activity of fatty acids which gave rise to this increase in concentration was less than that of the chylomicron fatty acids. When unesterified ^{14}C-palmitic acid was added to the perfusate, the concentration of unesterified fatty acids in the perfusate did not change significantly during the perfusion, although most of the unesterified ^{14}C-palmitic acid was removed from the perfusate by the liver. Morris concluded that the unesterified fatty acids in the perfusate were in equilibrium with the fatty acids of the liver. Evans, Opie & Shipp (1963) studied the uptake of unesterified ^{14}C-palmitic acid by the isolated saline perfused rat heart and found that the amount of ^{14}C-palmitic acid removed from the perfusate was always greater than the net amount of titratable unesterified fatty acid taken up by the heart. This result can only be explained by the release of unesterified fatty acid into the perfusate by the heart. It is possible therefore that, in the experiments described in this thesis, the

unesterified fatty acids in the blood taken from the isolated heart-lung preparation after the infusion of chylomicrons, came from within the cells of the organs. In an experiment in which heparin was injected into the rat before its heart and lungs were removed, the concentration of total esterified fatty acids in the blood removed from the heart-lung preparation, after it had been infused with a large amount of chylomicrons, was very much lower than in the experiments in which no heparin was injected into the rats. Therefore, if the chylomicrons were hydrolyzed intravascularly in the preceding experiments, this hydrolysis was different from the hydrolysis of the chylomicrons in the experiment in which post-heparin clearing factor was present in the blood.

The oxidation of the chylomicron fatty acids by the isolated heart-lung preparation. When chylomicrons labelled with ^{14}C-palmitic acid were infused into the isolated heart-lung preparations, the ^{14}C-palmitic acid was oxidized rapidly to CO_2. The patterns of excretion of $^{14}CO_2$ by the preparations indicated that there was a partitioning of the fatty acids in the isolated organs similar to that demonstrated in the intact animal; a proportion of the fatty acids were oxidized rapidly, the remainder more slowly. If all of the chylomicron fatty acids were oxidized as rapidly as the ^{14}C-palmitic acid, as was shown to be the case in the intact rat, the oxidation of the chylomicron fatty acids could have

accounted for a considerable proportion of the energy requirements of the heart and lungs. If these results are extrapolated to the intact rats, heart and lungs could have accounted for more than 10% of the chylomicron fatty acids oxidized by the animal. Cruickshank & Kosterlitz (1941) presented the first direct evidence that the mammalian heart oxidized fat. Since then there have been many experiments demonstrating the oxidation of fatty acids by the heart, but many of these experiments have involved the use of short chain fatty acids or unesterified long chain fatty acids bound to serum albumin. Evans, Opie & Shipp (1963) showed that the isolated heart of the rat, perfused with saline, could oxidize ^{14}C-palmitic acid, when it was complexed with serum albumin and added to the perfusate. The rate of oxidation was sufficient to account for 83% of the total CO_2 production of the heart. These workers measured rates of oxidation up to the equivalent of 11 μ-moles of palmitic acid/g wet wt/hr. Gousios, Felts & Havel (1963) showed that the isolated perfused rabbit heart was able to oxidize both unesterified ^{14}C-palmitic acid and ^{14}C-palmitic acid incorporated into the triglycerides of low density lipoproteins. However both the rate of uptake and rate of oxidation of the lipoprotein fatty acids were slower than for unesterified fatty acid. In the experiments described in this thesis, there was a

considerable variation in the fractional rates of oxidation of the infused chylomicron fatty acids, and this might have been due in part to the different overall rates of metabolism of the individual preparations. However the hearts and lungs used in these experiments were obtained from rats which had been fed ad libitum, and the differences in the oxidation of the chylomicron fatty acids might have reflected differences in the nutritional status of the individual rats. It was necessary to ventilate the lungs of these preparations with 5% CO_2 in O_2 and it was therefore impossible to measure their gaseous metabolism. However, the maximum rates of oxidation of chylomicron fatty acids which were measured in these experiments, were of the same order as the maximum rates of oxidation of unesterified ^{14}C-palmitic acid measured by Evans, Opie & Shipp (1963), and it is possible therefore, that esterified fatty acids might be as important in the energy metabolism of the heart as are unesterified fatty acids.

The effect of glucose on the oxidation of chylomicron fatty acids by the isolated heart-lung preparation.

None of the variations in the oxidation of the infused chylomicron fatty acids by individual isolated heart-lung preparations could be attributed to the simultaneous infusion of glucose. There was some evidence that the oxidation of glucose was spared by the infusion of

chylomicrons; glycogenolysis appeared to be reduced when larger amounts of chylomicrons were infused, even though the concentration of glucose in the blood of these preparations must have been very low. Several other investigators have demonstrated that some metabolites spare the oxidation of glucose by the heart. Williamson & Krebs (1961) showed that acetoacetate and β-hydroxybutyrate were oxidized by the isolated perfused rat heart in preference to glucose. Minton & Raben (1962) showed that the oxidation of ^{14}C-glucose by the isolated perfused rat heart was reduced greatly when palmitate or acetoacetate were added to the perfusate in physiological concentrations, whereas the oxidation of palmitate or acetoacetate was not affected by the presence of glucose in the perfusate. It can be concluded that long chain fatty acids are an important source of energy to the heart and these are actually oxidized in preference to glucose.

SUMMARY

1. A system was developed for continuously measuring the excretion of $^{14}CO_2$ in the expired breath of rats during the continuous intravenous infusion of ^{14}C-labelled substances. This system was subsequently modified so that the O_2 consumption of the rats could be measured simultaneously. A method was developed so that the ^{14}C-labelled substances could be assayed for radioactivity

in the same system as was used for the animal experiments; the rate of excretion of radioactivity in the rats' expired breath thus could be compared directly with the rate of infusion of radioactivity.

2. Intact unanaesthetized rats were infused continuously with $NaH^{14}CO_3$ and the excretion of $^{14}CO_2$ was recorded continuously. The recorded patterns of excretion were analyzed so that some measure might be made of the effects of the rats' bicarbonate pools on the excretion of $^{14}CO_2$ produced during the oxidation of ^{14}C-labelled compounds in the rats' bodies. In this analysis the recorded patterns of excretion of $^{14}CO_2$ were resolved into two exponential components with half-lives of 7-8 and 43 min and a third more rapid component with a half-life of 3 min was calculated to describe the curves completely.

3. Chylomicrons labelled with ^{14}C-palmitic, ^{14}C-oleic or ^{14}C-linoleic acids were infused continuously into fasted unanaesthetized rats and the excretion of $^{14}CO_2$ in their expired breath was measured continuously. The oxidation of each of the labelled fatty acids followed a similar time course, and it was concluded that the oxidation of each of the labelled fatty acids could be used as an index of the oxidation of all the fatty acids present in the chylomicrons. The rate of excretion of $^{14}CO_2$ reached a relative plateau in the second hour of

all experiments. With each fatty acid about 40% of the label which was infused hourly was oxidized to CO_2 during the 3rd hr of the infusions.

4. The circulating half-life of the infused chylomicrons increased with increasing rates of infusion of chylomicrons. This was indicated by a non-linear relationship between the rate of infusion of chylomicrons and the concentration of total esterified fatty acids in the plasma during the infusions. The circulating half-life increased from about 4 min when chylomicrons were infused at a rate of 50 mg total esterified fatty acid per hour, to about 16 min when the chylomicrons were infused at a rate of 10 mg of total esterified fatty acid per hour.

5. The proportion of the infused fatty acids which was oxidized to CO_2 did not change when the rate of infusion of chylomicrons was increased from 5-100 mg of total esterified fatty acids per hour. Moreover, the oxidation of the different labelled fatty acids was not affected, in general, by the fatty acid composition of the chylomicrons into which they were incorporated. The rats derived about 25% of their energy requirements from the oxidation of the infused chylomicron fatty acids in the 3rd hr of experiments in which chylomicrons were infused at a rate of 100 mg of total esterified fatty acids per hour.

6. The patterns of excretion of $^{14}CO_2$ indicated that the

infused chylomicron fatty acids were being handled in two general ways. About 25-30% of the fatty acids appeared to enter a small pool of lipid in which the fatty acids had a half-life of 13-15 min before being oxidized. The remainder entered a large pool of fatty acids which had a half-life of about 9-10 hr before being oxidized.

7. Some parameters of glucose metabolism in fasting rats were measured. The rate of entry of endogenous glucose into the blood stream of fasted rats weighing about 200 g was estimated to be about 150 mg/hr. When glucose was infused at rates of 50-200 mg/hr, the rate of entry of endogenous glucose into the blood decreased as the rate of infusion of glucose increased. Throughout this range of rates of infusion of glucose the concentration of glucose in the plasma remained constant. When the rate of infusion of glucose exceeded 200 mg/hr, the concentration of glucose in the plasma increased with increasing rates of infusion of glucose. The oxidation of continuously infused ^{14}C-glucose was different to that of ^{14}C-fatty acids incorporated into chylomicrons, as there was no relatively rapid phase in the oxidation patterns. The glucose was oxidized as efficiently when it was infused at a rate of 300 mg/hr as when it was infused at lower rates.

8. Unlabelled glucose and chylomicrons labelled with

^{14}C-palmitic acid were infused simultaneously at various rates into fasted rats. The oxidation of ^{14}C-palmitic acid was reduced by about 16% in the 3rd hr of these experiments when glucose was infused simultaneously at a rate of 300 mg/hr. The reduced oxidation was due to an increase in the metabolic half-life of the proportion of fatty acids which are oxidized relatively slowly. Increased amounts of the infused radioactivity were found in the lipids of the livers and samples of adipose tissue removed from rats which had been infused with glucose. In experiments in which glucose was infused, the levels of ketone bodies in the plasma of the rats were reduced to a relatively greater extent than the oxidation of the chylomicron fatty acids. The levels of glycogen in the various tissues, and the increase in respiratory quotients of the rats which had been infused with glucose indicated that the infused glucose might have been stored in preference to being oxidized. The glucose certainly was not oxidized in preference to the chylomicron fatty acids.

9. An isolated perfused rat heart-lung preparation was developed. This preparation was autoperfused through its own vessels with the rat's own unaltered blood. No heparin or any other anticoagulants were required. The characteristics of this preparation are described. It was found that continuously infused chylomicrons were removed from the circulation by this preparation and

^{14}C-palmitic acid incorporated into the chylomicrons was oxidized to CO_2. The oxidation of the chylomicron fatty acids was not affected by the simultaneous infusion of glucose, and the infused chylomicron fatty acids appeared to be partitioned within the tissues of the preparation in a manner similar to that described in the intact animal. The oxidation of the chylomicrons could have accounted for a considerable proportion of the energy requirements of the heart and lungs. Similarly it is possible that the heart and lungs accounted for 10% or more of the chylomicron fatty acids oxidized by the intact rat.

ACKNOWLEDGEMENTS

The work reported in this thesis was carried out in the Department of Experimental Pathology, John Curtin School of Medical Research, Australian National University, in part during the tenure of an Australian National University Scholarship, and in part during the tenure of an Australian and New Zealand Life Insurance Medical Research Fellowship. I am indebted to Professor F.C. Courtice for the opportunity to undertake this work and for his encouragement during its prosecution.

My best thanks are due to my supervisor, Dr. B. Morris, for suggesting the topic, for his guidance during the course of the work, and for his constructive criticism during the preparation of the manuscript.

I also thank Professor A.G. Ogston for his assistance and advice in preparing some of the appendices, Mr. E.P. Adams for providing chromatographic reference data, and Mr. B. Thornton for constructing several pieces of apparatus.

I wish finally to thank my wife for preparing the typescript of this thesis.

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APPENDIX I

The effect of instrument characteristics on the recorded trace. The theory of operation of ionization chambers and the vibrating reed electrometer has been fully described (Tolbert, 1956). The current (i) which flows into the electrometer input is proportional to the total amount of radioactivity (X) within the chamber.

$$i = kX \qquad (1)$$

$$= kVx \qquad (2)$$

where (V) is the volume of the chamber and (x) is the concentration of radioactivity in the air in the chamber.

When the amount of radioactivity in the ionization chamber is changing, the electrometer potential (E) does not give a true instantaneous measure of the amount of activity within the chamber due to the electrical characteristics of the electrometer input. However, over a period, an exact measure of the amount of radioactivity which flows into or out of the chamber may be determined. Assuming instantaneous and complete mixing within the chamber, the amount of radioactivity (Y) which leaves the chamber in any interval $(t_1 - t_2)$ is given by

$$Y = \int_{v_1}^{v_2} x.dv \qquad (3)$$

where (v) is the volume of air which has flowed through

the chamber. If the flow rate of air is constant (ϕ), the amount of radioactivity which has left the chamber is given by

$$Y = \phi \int_{t_1}^{t_2} x.dt$$

From (2),

$$Y = \frac{\phi}{kV} \int_{t_1}^{t_2} i.dt \qquad (4)$$

The current which enters the electrometer flows partly into the electrometer input capacitance (C) and partly through the high resistance leak (R) so that

$$i = \frac{E}{R} + C\frac{dE}{dt} \qquad (5)$$

Integrating (5) with respect to time,

$$\int_{t_1}^{t_2} i.dt = \int_{t_1}^{t_2} \frac{E}{R}.dt + C \int_{t_1}^{t_2} dE$$

$$Y = \frac{\phi}{kVR}\left[\int_{t_1}^{t_2} E.dt + CR\left[E_2 - E_1\right]\right] \qquad (6)$$

The term, $CR\left[E_2 - E_1\right]$ distorts the linear relationship between the amount of radioactivity in the chamber and the electrometer scale deflection when the amount of radioactivity in the chamber is changing. However, its value becomes zero when the integration is performed

between limits such that the initial and final scale deflections are equal. Under these conditions, the integral of (E) with respect to time is exactly proportional to the integral of (i) and hence the integral of (X) with respect to time. It is not necessary to calibrate the instrument in absolute terms and for comparative purposes the amount of radioactivity which leaves the ionization chamber can be measured as the product of the area under the recorded curve and the flow rate of air.

In the experiments reported in this thesis, ^{14}C-labelled substances have been infused continuously into rats and it was anticipated that the rate at which the animals excreted ^{14}C as $^{14}CO_2$ would tend towards the rate of infusion of ^{14}C as a multi-exponential function of time. It will be shown in Appendix II that, under these conditions, the amount of $^{14}CO_2$ in the ionization chamber at any time during the infusion will be a multi-exponential function of time. It was considered important to determine the effects of the response time of the electrometer when operated with a high resistance leak on the record of the amount of activity in the chamber. If the amount of $^{14}CO_2$ in the chamber (X), measured as the equilibrium potential it would produce on the electrometer input at any time, t seconds, is given by

$$X = \sum_i A_i(1 - e^{-B_i t}) \qquad (7)$$

The current (i) originating in the ionization chamber would be given by

$$i = \frac{X}{R} \quad \text{(where R is the high resistance leak in ohms)} \qquad (8)$$

By substituting for (i) in equation (5) the electrometer potential (E) at any time, t seconds, during the infusion would be given by

$$E = \sum_i A_i \left(1 - \frac{1}{1-CRB_i} e^{-B_i t} + \frac{RCB_i}{1-RCB_i} e^{-t/RC}\right) \qquad (9)$$

The value of C is about 10^{-11} farads and for a 10^{12} ohm resistance leak RC becomes 10, and the term $e^{-t/RC}$ would decay to 0.01 in 69 seconds. The constants by which the other exponential components are multiplied in equation (7) would be increased according to equation (9) and for a component with a half-life of 10 minutes (B = .00115) the constant by which it was multiplied would be changed by about 1%. For components with longer half-lives the error would be smaller still. It was considered that this error could be disregarded.

APPENDIX II

Relationship between the excretion of $^{14}CO_2$ by the experimental animal and its detection in the ionization chamber. Because of their finite size in relation to the rate of flow of air through them, the metabolism cage and the ionization chamber introduce a distortion into the recorded $^{14}CO_2$ excretion pattern. However, if the recorded curve can be analyzed as a sum of exponential components, the effect of the system can be determined and the true $^{14}CO_2$ excretion pattern calculated.

If, $\frac{dx}{dt}$ = the rate at which $^{14}CO_2$ is excreted by the animal,

X = the amount of $^{14}CO_2$ in the metabolism cage,

Y = the amount of $^{14}CO_2$ in the ionization chamber,

V_1 = the volume of the metabolism cage,

V_2 = the volume of the ionization chamber,

and ϕ = the flow rate of air through the system,

then

$$\frac{dX}{dt} = \frac{dx}{dt} - k_1X \qquad \left(k_1 = \frac{\phi}{V_1} \right) \qquad (10)$$

and

$$\frac{dY}{dt} = k_1X - k_2Y \qquad \left(k_2 = \frac{\phi}{V_2} \right) \qquad (11)$$

If, when ^{14}C is infused at a constant rate (I) as a ^{14}C-labelled metabolite, the rate of excretion of ^{14}C $(\frac{dx}{dt})$ as $^{14}CO_2$ at any time (t) after the commencement of the infusion can be described in the following way,

$$\frac{dx}{dt} = I \sum_i A_i(1 - e^{-B_i t}) \quad (A_i \text{ and } B_i \text{ are constants, } \sum_i A_i = 1) \qquad (12)$$

then, by substitution in equations (10 & 11), it can be shown that the rate at which ^{14}C as $^{14}CO_2$ leaves the ionization chamber (k_2Y) is given by the following equation,

$$k_2Y = I \sum_i A_i \left[1 - \frac{k_1k_2}{(k_1-B_i)(k_2-B_i)} e^{-B_i t} - \frac{k_2B_i}{(B_i-k_1)(k_2-k_1)} e^{-k_1 t} - \frac{B_ik_1}{(B_i-k_2)(k_1-k_2)} e^{-k_2 t} \right] \qquad (13)$$

Thus it can be seen that an exponential component is introduced into the recorded excretion curve by each compartment in the metabolism system. The half-lives of these components are too short to be found in an analysis of the curves. However they affect the exponential components associated with the excretion of the $^{14}CO_2$ by changing the constants by which they are multiplied. The extent to which these components are affected depends on their half-lives. The longer these are in relation to the components due to the compartments in the system then the smaller will be the effect of the system on them.

APPENDIX III

The effect of the bicarbonate pools on the excretion of metabolically produced $^{14}CO_2$. It has been shown that the specific activity of the expired CO_2 is equal to the specific activity of the blood CO_2 (Kornberg, Davies & Wood, 1952; Robinson and Coxon, 1957; Coxon and Robinson, 1959a), and that the blood bicarbonate is in rapid equilibrium with a larger pool of bicarbonate which could include all of the extracellular bicarbonate (Coxon and Robinson, 1959a; Shipley, Baker, Incefy & Clark, 1959). These authors have also shown that following the administration of a single intravenous injection of $NaH^{14}CO_3$, the fraction of the administered dose of bicarbonate which remains in the pool of bicarbonate which is rapid equilibrium with the blood is a multi-exponential function of time.

F_t = The fraction of the administered dose present in the extracellular fluid at time (t)

$$= \sum_i A_i e^{-B_i t} \qquad A_i \text{ and } B_i \text{ constants}, \quad \sum_i A_i = 1 \qquad (14)$$

If, when ^{14}C is infused as a ^{14}C-labelled metabolite at a constant rate (I) into

experimental animals, the amount of ^{14}C (dX_t) which appears in the extracellular fluid as $[H^{14}CO_3]^-$ between times (t) and (t + dt) is given by

$$dX_t = Idt\left(1 - \sum_j a_j e^{-b_j t}\right) \qquad a_j \text{ and } b_j \text{ constants, } \sum_j a_j = 1 \tag{15}$$

then, from (14), at any time (T) later than (t) this amount will have decayed according to

$$dX_T = dX_t \sum_i A_i e^{-B_i(T-t)} \tag{16}$$

$\int_0^T dX_T$ = The total amount of the infused ^{14}C present in the extracellular fluid as $[H^{14}CO_3]^-$ at time (T)

$$= \int_0^T \sum_i A_i e^{-B_i(T-t)} . dX_t$$

$$= I\int_0^T \sum_i A_i e^{-B_i(T-t)} \left[1 - \sum_j a_j e^{-b_j t}\right] dt \qquad \text{(from 15)} \tag{17}$$

$$= I \sum K_{(i,j)T} \tag{18}$$

If $B_i \neq b_j$, then

$$\sum K_{(i,j)T} = \sum_i \left[\frac{A_i}{B_i} - \left[\frac{A_i}{B_i} - \sum_j \left(\frac{A_i a_j}{(B_i - b_j)} \right) \right] e^{-B_i T} - \sum_j \left(\frac{A_i a_j}{(B_i - b_j)} \right) e^{-b_j T} \right] \quad (19)$$

If $B_i = b_j$, then

$$K_{(i,j)T} = \frac{A_i}{B_i} - \left[\frac{A_i}{B_i} + A_i a_j T \right] e^{-B_i T} \quad (20)$$

The rate of excretion of ^{14}C as $^{14}CO_2 = \Theta I \sum K_{(i,j)T}$

where $\Theta = \dfrac{\text{Rate of excretion of } CO_2}{\text{Amount of } CO_2 \text{ present in the extracellular fluid}}$

$$\frac{\text{Rate of excretion of } ^{14}C \text{ as } ^{14}CO_2}{\text{Rate of infusion of } ^{14}C} = \Theta \sum K_{(i,j)T} \quad (21)$$

If ^{14}C is continuously infused as $\left[H^{14}CO_3\right]^-$ then $b_1 = b_2 = \ldots = b_j = \infty$

and $$\frac{\text{Rate of excretion of } {}^{14}C \text{ as } {}^{14}CO_2}{\text{Rate of infusion of } {}^{14}C \text{ as } [H^{14}CO_3]^-} = \Theta \sum_i \frac{A_i}{B_i}\left[1 - e^{-B_i T}\right] \qquad (22)$$

If (22) is differentiated with respect to time, then at any time (T) during the course of the infusion,

$$\frac{d}{dT}\left[\frac{\text{Rate of excretion of } {}^{14}C \text{ as } {}^{14}CO_2}{\text{Rate of infusion of } {}^{14}C \text{ as } [H^{14}CO_3]^-}\right] = \Theta \sum_i A_i e^{-B_i T}$$

$$\text{(from 14)} \qquad = \Theta F_T \qquad (23)$$

Thus it can be seen from the above argument that if the excretion of $^{14}CO_2$ by experimental animals under standard experimental conditions can be expressed as a multi-exponential function of time, then such functions obtained by the use of $NaH^{14}CO_3$ can be used in interpreting the results obtained with ^{14}C-labelled metabolites. This is possible without postulating any pool system for explaining the behaviour of the $NaH^{14}CO_3$. It can be seen that the exponential components of equation (14) describing the behaviour of the $^{14}CO_2$ together with the exponential components associated with the oxidation of the labelled compound in equation (15) are preserved in the composite excretion curve. It is only the constants by which they are multiplied that are altered in the composite excretion curve.

APPENDIX IV

Patterns of excretion of $^{14}CO_2$ following a single intravenous injection and during a continuous intravenous infusion of a ^{14}C-labelled substance.

If the fraction of the ^{14}C administered in a single intravenous injection of a ^{14}C-labelled substance (f_t) which is present as $[H^{14}CO_3]^-$ in the pool of $[HCO_3]^-$ from which CO_2 is expired, at any time (t) after the injection is given by:

$$f_t = \sum_i A_i e^{-B_i t} \quad , \quad \sum_i A_i = 1 \qquad (24)$$

the rate at which ^{14}C is excreted as $^{14}CO_2$ (R) will be given by

$$R = \phi f_t \qquad (25)$$

where ϕ is the fractional rate of excretion of the $[HCO_3]^-$ in this pool as CO_2.

The total fraction of the injected ^{14}C (F_T) excreted as $^{14}CO_2$ at any time (T) following the injection will be given by

$$F_T = \int_0^T R.dt \qquad (26)$$

$$= \phi \sum_i \frac{A_i}{B_i}(1 - e^{-B_i T}) \qquad (27)$$

If during a continuous intravenous injection of a ^{14}C-labelled substance, the amount of ^{14}C-labelled substance which enters the circulation in any small

interval of time (t + dt) is given by dX, and this is metabolized in the same way as the single injection, then at any time (T) later than (t) the amount of this ^{14}C (dx) which is present as $[H^{14}CO_3]^-$ in the pool of $[HCO_3]^-$ from which CO_2 is expired will be given by

$$dx = dX \sum_i A_i e^{-B_i(T-t)} \quad \text{from (24)} \tag{28}$$

$$\int_0^T dx = \text{the total amount of the infused } ^{14}C \text{ present in this pool as } [H^{14}CO_3]^- \text{ at any time (T) during the infusion} \tag{29}$$

$$= \int_0^T \sum_i A_i e^{-B_i(T-t)} . dX \tag{30}$$

$$= \frac{dX}{dt} \int_0^T \sum_i A_i e^{-B_i(T-t)} . dt \tag{31}$$

The rate of excretion of ^{14}C as $^{14}CO_2$ at any time (T)

$$= \phi \frac{dX}{dt} \sum_i \frac{A_i}{B_i}(1 - e^{-B_i T}) \quad \text{from (25)} \tag{32}$$

$$\frac{\text{Rate of excretion of } ^{14}C \text{ as } ^{14}CO_2}{\text{Rate of infusion of } ^{14}C} = \phi \sum_i \frac{A_i}{B_i}(1 - e^{-B_i T}) \tag{33}$$

From equations (27) and (33) it can be seen that if the ^{14}C-labelled substance is metabolized in the same way during a continuous infusion as it is after a single injection, the rate of excretion of ^{14}C as $^{14}CO_2$, expressed as a fraction of the rate of infusion of ^{14}C, during the infusion should follow an identical time course to the fraction of the total injection of ^{14}C excreted as $^{14}CO_2$ following the injection.