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Comparison of Oxidative Metabolism in Starved, Fat-Fed and Carbohydrate-Fed Rats

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1. Rats were starved for 48hr. or fed for 1 week on a high-fat or a high-carbohydrate diet. The effects of these dietary alterations on the rate of production of $^{14}CO_2$ from trace amounts of [U-^{14}C]glucose, [1-^{14}C]palmitate or [1-^{14}C]acetate administered intravenously were studied. 2. The oxidation of [^{14}C]glucose was most rapid in the carbohydrate-fed condition and was decreased significantly and to the same extent after starvation and after feeding with fat. 3. Under all dietary regimes studied the maximum rate of elimination of $^{14}CO_2$ from [^{14}C]palmitate occurred within a few minutes after injection, but considerably more was oxidized after starvation and feeding with fat than after feeding with carbohydrate. 4. Alterations in diet had no effect on the oxidation and high recovery of administered [^{14}C]acetate as $^{14}CO_2$. 5. Graphical analysis showed the presence of several exponential components in the $^{14}CO_2$-elimination curves. 6. In all studies a marked similarity in oxidative pattern was noted between the starved and the fat-fed rat.

Rats on a high-fat diet, on evisceration, maintain higher concentrations of blood glucose and survive twice as long as rats on a high-carbohydrate diet (Roberts, Samuels & Reinecke, 1943–44). Experiments *in vitro* (Masoro, Chaikoff, Chernick & Felts, 1950) have shown that either starvation or the feeding of a high-fat diet leads to lower recoveries of $^{14}CO_2$ when [^{14}C]glucose is oxidized by liver slices. Hansen, Rutter & Samuels (1951) found that glucose uptake is depressed in isolated diaphragms from both starved rats and rats that had received a high-fat diet when compared with the uptake by diaphragms from rats on a high-carbohydrate diet. These findings are compatible with a diabetic-type glucose-tolerance curve found in humans on diets low in carbohydrate (Himsworth, 1935). All of these results suggest that feeding with fat exerts a sparing action on carbohydrate utilization.

On the other hand animals on a high-carbohydrate diet or given a glucose load metabolize more glucose and spare fat. McCalla, Gates & Gordon (1957) found that giving glucose to starved rats decreased substantially the oxidation of injected [^{14}C]palmitate to $^{14}CO_2$. In the intact animal the fat-sparing action of carbohydrate is most prominent on the oxidation of the longer-chain fatty acids (Lossow & Chaikoff, 1955). Masoro & Felts (1958) demonstrated that the addition of glucose to liver slices from fed rats suppressed the oxidation of long-chain fatty acids but not the oxidation of the C_4–C_{10} fatty acids.

No systematic study has yet appeared comparing all of these interrelations *in vivo* with substrates administered intravenously. We have compared the effects of starvation, of a high-fat diet and of a high-carbohydrate diet on the oxidation of trace quantities of [^{14}C]glucose, [^{14}C]palmitate and [^{14}C]acetate injected intravenously into unanaesthetized intact rats. The $^{14}CO_2$-elimination curves were examined by graphical analysis, and the nature of the logarithmic components is discussed.

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MATERIALS AND METHODS

Animals. Male Long–Evans rats (180–210g.) were maintained on a stock diet (Diablo Laboratories, Berkeley, Calif., U.S.A.) and then transferred to individual cages and given a high-carbohydrate or a high-fat diet *ad libitum* for 1 week. The high-carbohydrate diet consisted of: vitamin-free casein, 5%; corn starch, 40%; glucose, 50%; salt mixture, 3% (Hubbell, Mendel & Wakeman, 1937); brewer's yeast, 2%; cod-liver oil, 0·2%. In the high-fat diet, an isocaloric amount of butter fat was substituted for the starch and glucose. Each rat was handled frequently to accustom it to the future experimental procedure. Only those animals that maintained or increased their body weight on the experimental diets were used for study. Another group of rats, designated 'starved', was maintained on the stock diet and all food was withdrawn 48hr. before the experiment. Drinking water was always available.

Preparation of substrates. Solutions of radioactive substrates were prepared to contain approx. 4 μc/ml. [1-^{14}C]Acetate (1·53 mc/m-mole; Research Specialities Co., Richmond, Calif., U.S.A.), [U-^{14}C]glucose (2·9 mc/m-mole; New England Nuclear Corp., Boston, Mass., U.S.A.) and $NaH^{14}CO_3$ (5 mc/m-mole; New England Nuclear Corp.) were all dissolved in 0·9% NaCl solutions. [1-^{14}C]Palmitate (10 mc/m-mole; New England Nuclear Corp.) was combined with bovine serum albumin (Armour Laboratories, Kankakee, Ill., U.S.A.) according to the method of Masoro & Felts (1958).

Experimental procedure. Each rat received, via a tail vein, approx. 2 μc of a trace amount of substrate between 9·00 and 10.00 a.m. Immediately after the injection, each rat was placed in a metabolism chamber constructed from polyethylene bottles of total volume 800 ml. Room air was drawn continuously through the chamber at a rate of 450 ml./min. and bubbled through two CO_2 absorbers connected in series; each absorber contained 50 ml. of 2 N-NaOH. At 5, 15, 30, 60, 90, 120, 150 and 180 min. after injection, the contents of the two absorbers were removed quantitatively and replaced with fresh 2 N-NaOH. The contents and washings were combined and diluted to 200 ml. Samples of the NaOH–carbonate solutions were analysed for radioactivity. After 3 hr. the rats were decapitated and their blood was collected in heparinized tubes.

The results are expressed as percentages of dose expired/min. at 2·5, 10, 22·5, 45, 75, 105, 135 and 165 min., these values being calculated from the amounts of $^{14}CO_2$ collected during each time-interval.

Analytical methods. Radioactive carbonate was estimated by adding 5·0 ml. of the NaOH–carbonate solution and methanol (3 ml.) to the outer compartment of a 50 ml. Erlenmeyer flask fitted with a centre well containing M-Hyamine (Packard Instrument Co. Inc., La Grange, Ill., U.S.A.) in methanol (0·5 ml.). The flask was stoppered with a rubber serum-bottle stopper and 5 ml. of air was withdrawn by a syringe and needle, and 1·0 ml. of N-H_2SO_4 was injected into the outer compartment. After shaking for 1 hr. at room temperature, the contents of the centre well were transferred quantitatively with washings of methanol into a counting vial. The methanol was evaporated under a stream of air and the residue was dissolved in toluene containing 0·4% of 2,5-diphenyloxazole and 0·03% of 1,4-bis-(5-phenyloxazol-2-yl)benzene. The samples were counted in a liquid-scintillation spectrometer (Packard). The counts were corrected for quenching by using an internal standard.

Blood glucose was estimated by a glucose-oxidase method (Glucostat reagent; Worthington Biochemicals Corp., Freehold, N.J., U.S.A.). Plasma free fatty acids were determined by the Dole procedure by using the modification of Trout, Estes & Friedberg (1960).

Statistical tests were carried out by analysis of variance. $P < 0·05$ was taken as the criterion of statistical significance. Variations are shown as the S.E.M.

RESULTS

The rate of expiration and the total recovery of $^{14}CO_2$ in 3 hr. after the intravenous administration of ^{14}C-labelled substrates is shown in Fig. 1. The elimination patterns of $^{14}CO_2$ of each substrate tested were similar in starved and fat-fed rats. However, apart from the results with [^{14}C]acetate, there were marked differences between the patterns found in carbohydrate-fed rats on the one hand and those in starved or fat-fed rats on the other.

[U-^{14}C]*Glucose.* In 3 hr. there was no statistical difference in the mean recoveries of $^{14}CO_2$ between the starved (34·4 ± 0·6%) and fat-fed (36·6 ± 2·6%) groups of rats. In the starved rats the maximum rate of elimination of $^{14}CO_2$ was found at 60 min. after injection of the dose, whereas in the fat-fed rats it was found after 45 min. These results differed significantly from those obtained when [^{14}C]glucose was administered to carbohydrate-fed rats. In these experiments 53·5 ± 2·3% of the dose was recovered and the maximum rate of elimination of $^{14}CO_2$ occurred only 15 min. after injection.

[1-^{14}C]*Palmitate.* Similarities between starved and fat-fed rats were evident in this series of experiments. In 3 hr. there was a mean recovery of administered ^{14}C as $^{14}CO_2$ of 51·4 ± 2·4% in the starved animals and 50·0 ± 4·9% in those on the fat diet; the corresponding value for the carbohydrate-fed rats was only 32·2 ± 2·4%. Under all dietary conditions, the maximum rate of elimination of $^{14}CO_2$ occurred very soon after injection, within 5 min. in the starved and fat-fed animals and within 10 min. in those on the carbohydrate diet.

[1-^{14}C]*Acetate.* Mean recoveries of $^{14}CO_2$ of 79·8 ± 1·6%, 79·0 ± 0·8% and 80·2 ± 0·6% were obtained in 3 hr. from carbohydrate-fed, fat-fed and starved rats respectively. Irrespective of dietary treatment, the maximum rate of expiration of $^{14}CO_2$ occurred within 5 min. of injection.

The values shown on the curves in Fig. 1 were plotted on semilogarithmic co-ordinates in Fig. 2, and the method of graphical analysis of the curves into their components was adopted (Riggs, 1963). Only the descending portion of each curve has been treated in this manner.

In each experiment in which [U-^{14}C]glucose was administered, only a single negative exponential function was evident, which had half-times of 49, 71 and 123 min. in carbohydrate-fed, fat-fed and starved rats respectively. However, the semilogarithmic plots of the results from the rats given [^{14}C]acetate or [^{14}C]palmitate indicated several exponential functions. After the administration of [^{14}C]acetate, two components having half-times of approx. 14 and 70 min. were demonstrated under all dietary conditions. After the administration of [^{14}C]palmitate there were three components in starved and fat-fed rats, but only two in carbohydrate-fed rats. The slowest component had a similar slope on all dietary treatments, ranging from a half-time of 165 min. in carbohydrate-fed animals to 200 min. in those fed with fat. The half-times of the intermediate component found in the fat-fed and starved rats were 21 and

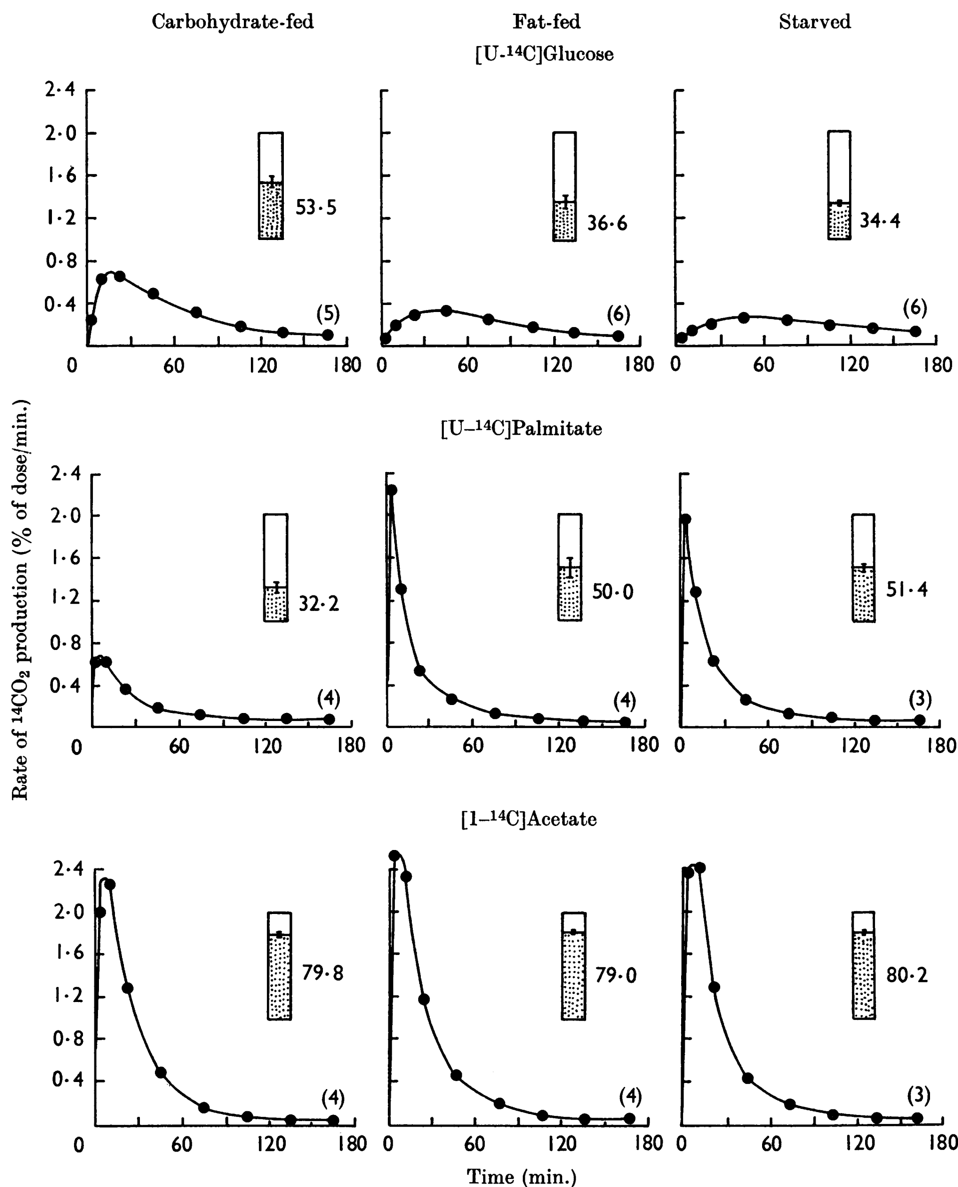

Fig. 1. Rate of elimination of $^{14}CO_2$ from intact rats given ^{14}C-labelled substrates intravenously. The percentage recovery in 3 hr. of radioactivity administered is shown in the stippled rectangle. The number of rats in each group is shown in parenthesis. Variations indicated are the S.E.M.

22·5 min. respectively. The half-times of the initial component were only 6·5 min. in the fat-fed rats, 9·5 min. in the starved rats and 14 min. in the carbohydrate-fed rats.

Graphical analysis of the results obtained from both fed and starved rats given [^{14}C]bicarbonate (Fig. 3) showed three exponential components having half-times of 6, 15 and 62 min. in the fed animals and 6·5, 18·5 and 52·5 min. in starved animals. A separate experiment showed that the half-time of wash-out of $^{14}CO_2$ from the metabolism chamber averaged 2·9 min.

Analysis of the blood samples obtained from the rats after the 3 hr. period of carbon dioxide collec-

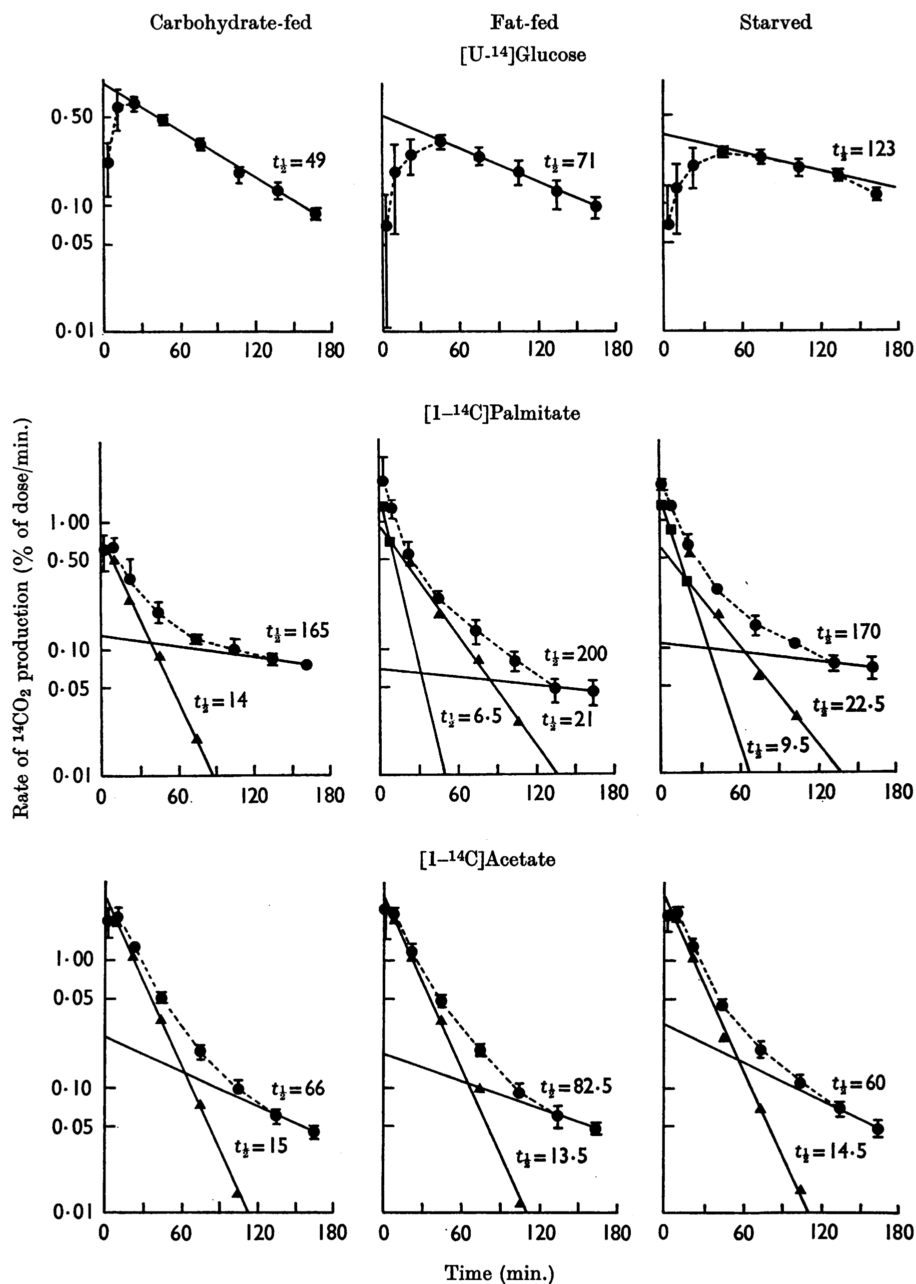

Fig. 2. Rate of elimination of $^{14}CO_2$ from intact rats given ^{14}C-labelled substrates intravenously. The curves are analysed graphically into their exponential components (see the text). The S.E.M. values are indicated by the bars on the experimental curve.

tion gave the following results: Mean plasma free fatty acid concentrations in carbohydrate-fed, fat-fed and starved rats were $0·50 \pm 0·04$, $0·46 \pm 0·03$ and $0·60 \pm 0·03$ m-equiv./l. respectively. The corresponding values for the blood glucose concentrations were $110 \pm 2·1$, $99 \pm 4·3$ and $93 \pm 2·9$ mg./100 ml.

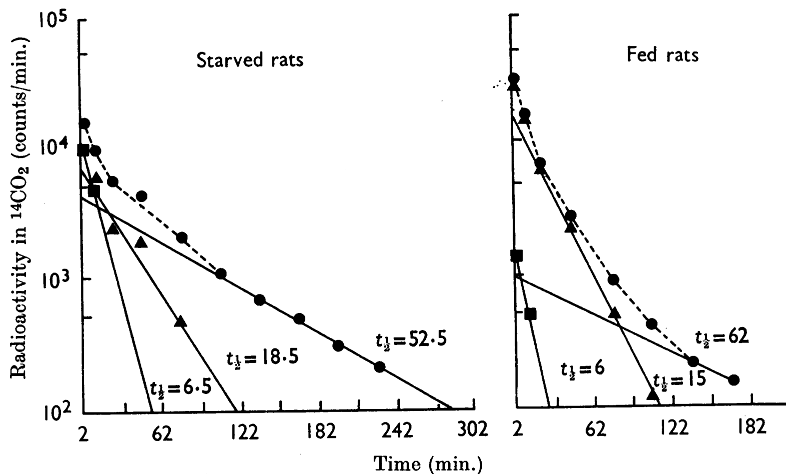

Fig. 3. Rate of elimination of $^{14}CO_2$ from fed or starved rats given a single intravenous injection of $NaH^{14}CO_3$ at zero time. The curves are analysed graphically into their exponential components (see the text). There were two animals in each group.

DISCUSSION

We have compared the effects of a high carbohydrate diet, of a high-fat diet and of starvation on the rates of oxidation of several labelled substrates administered intravenously in the intact rat. When substrates have been administered in unphysiological preparations, or have been given by stomach tube or intraperitoneally, there have always been considerable delays in oxidation compared with the results reported here. In the present study, the rats were conditioned to the experimental procedures, so that no undue alteration in pool size of either glucose or free fatty acids would take place on handling. No attempt was made to follow plasma specific activity for any of the substrates injected, since the turnover times of two of these, acetate and palmitate, are too rapid to be meaningful when related to radioactivity in expired carbon dioxide. Thus we have measured the rate at which $^{14}CO_2$ was expired but not the total production of carbon dioxide.

Rats maintained on the high-carbohydrate diet oxidized the [^{14}C]glucose administered faster than did the starved animals or the animals previously given the high-fat diet. The blood glucose concentration at the end of the experimental period showed little variation between the three groups of rats, indicating a corresponding lack of variation in the size of the extracellular glucose pool. The results of previous work (Mayes, 1962*a*,*b*) indicate that, during the experimental period, the blood glucose concentration probably remained fairly constant in all rats, and that the initial liver glycogen content (with which the label might equilibrate) was probably highest in the carbohydrate-fed rats. The marked decrease in the rate of oxidation of labelled glucose in the starved and fat-fed rats is therefore minimized rather than exaggerated by any changes in pool size. The observation that the maximum rate of elimination was not reached until 15min. in the carbohydrate-fed rats indicates that some time is required for intermediate pools to reach maximum specific activity. This delay was much longer in the starved and the fat-fed rats, implying that the rate of transfer of label through the intermediate pools was depressed. This may have resulted from the inhibition of certain key enzymes, e.g. phosphofructokinase (Randle, Garland, Hales & Newsholme, 1963), involved in glucose oxidation. The delay also implies a much lower oxidative turnover rate of blood glucose in both the starved and the fat-fed rats. Baker, Shipley, Clark, Incefy & Skinner (1961) have discussed the significance of the time of occurrence of maximum $^{14}CO_2$ specific activity. Our data emphasize the similarity in the pattern of oxidation of glucose in the starved and the fat-fed animal.

Starved rats or rats maintained on a high-fat diet oxidized [^{14}C]palmitate more quickly than did carbohydrate-fed animals. As free fatty acids are combined with albumin in the plasma, the pool into which the labelled palmitate was injected was probably confined to the plasma albumin space. The concentrations of plasma free fatty acid were not markedly different among the groups of rats at the end of the 3hr. period in the metabolism

chamber. However, it is likely (see Mayes, 1962*a*) that at the time of injection they were less in the carbohydrate-fed rats, and this would minimize the differences found in the rates of oxidation of [^{14}C]palmitate between the various nutritional states. Unlike the results with [^{14}C]glucose, the maximum rate of production of $^{14}CO_2$ from [^{14}C]-palmitate was reached within a few minutes of injection, implying that plasma free fatty acids have a more rapid access to the oxidative machinery. In the carbohydrate-fed animals maximum activity was not reached as rapidly as in the animals that were starved or given fat. This might be due to a decrease in activity of enzymes concerned in the oxidation of free fatty acids. In addition, a greater esterification of the [^{14}C]palmitate administered could account for the diminished total recovery of $^{14}CO_2$. Thus mechanisms influenced by the dietary state control the rates of oxidation of both glucose and long-chain fatty acids.

No control of the oxidation of [^{14}C]acetate by variations in the diet was encountered. The possible reasons for this are discussed below.

The $^{14}CO_2$-elimination curves from the radioactive substrates have been resolved into their exponential components (Fig. 2). Both the slopes and the number of components may be influenced by the labelling of the plasma bicarbonate pool and by the wash-out characteristics of the metabolism chamber. The very short half-time (2·9min.) recorded for the $^{14}CO_2$ wash-out from the metabolism chamber shows that this would not be a significant factor affecting the interpretation of the $^{14}CO_2$-elimination data from rats. In the experiments where [^{14}C]bicarbonate was injected, 2min. was allowed to elapse before beginning the collection of $^{14}CO_2$ to allow time for equilibration of the label in the circulation (Drury, Wick & Almen, 1956). The number of components and the rate of elimination of $^{14}CO_2$ from all animals given [^{14}C]bicarbonate were similar. Analysis of the $^{14}CO_2$-elimination curves by graphical means led to the construction of three exponential components (Fig. 3), in essential agreement with Shipley, Baker, Incefy & Clark (1959), who used the single-injection procedure, and with Morris & Simpson-Morgan (1963), who used a continuous-infusion technique. In common with the present work, these studies showed an exponential component having a half-time of approx. 6min., which is likely to represent wash-out from the pool of bicarbonate in extracellular fluid (Shipley *et al.* 1959). The curve of intermediate half-time is probably due to elimination of $^{14}CO_2$ once equilibrium has been reached with the intracellular bicarbonate pool. The third component must represent the elimination of ^{14}C from various pools of slow turnover.

It is proposed that, whenever the rate of oxidation of a substrate has a half-time shorter than the time required for the intra- and extra-cellular bicarbonate pools to attain equilibrium, this will result in three main components of $^{14}CO_2$ elimination, owing to a rapid labelling of the extra-cellular bicarbonate pool. If the rate of oxidation is lower, resulting in an initial component of intermediate half-time, only two components will result since adequate time is available for the intra- and extra-cellular bicarbonate pools to equilibrate to form one effective pool. Correspondingly, if the rate of oxidation results in a component having a long half-time, then only one component might be expected as the main bicarbonate pools would be turning over as fast as or faster than that of the substrate undergoing oxidation.

The data of Feller, Strisower & Chaikoff (1950) showed only a single exponential component in the $^{14}CO_2$-elimination curve obtained from the oxidation of [^{14}C]glucose. Our results show also an apparent single exponential component under a wide range of dietary variations. The interrelations between the bicarbonate pools and the glucose pools have been considered by Baker *et al.* (1961).

Graphical analysis of the $^{14}CO_2$-elimination curves from [^{14}C]palmitate yielded three exponential components from the starved and fat-fed rats, but one of these components was not present after feeding with carbohydrate. When [^{14}C]palmitate was administered to fat-fed rats, the initial component had a very short half-time of 6·5min. This indicates that the [^{14}C]palmitate was oxidized almost immediately, producing a pulse labelling of the bicarbonate pool. The fact that the slope of the initial component from the carbohydrate-fed animals was less indicates a delay in oxidation that is shown also by the delay in the time at which the maximum rate of $^{14}CO_2$ evolution occurred (Fig. 1). The possible reasons for the delay have been discussed.

The mechanism whereby carbohydrate spares the oxidation of long-chain fatty acids has been reviewed by Fritz (1961), who concluded that much of the lipid-sparing action of glucose is due to enhanced glyceride formation in adipose and other tissues. The mechanism whereby fatty acids spare the oxidation of glucose has also received attention. Randle *et al.* (1963) have summarized evidence showing that fatty acids diminish phosphorylation of glucose, decrease glycolysis by inhibiting phosphofructokinase and impair pyruvate oxidation in muscle. These explanations could account for our observations on the sparing of glucose oxidation during starvation and after feeding with fat, when the concentrations of free fatty acids are elevated.

The analysis of the $^{14}CO_2$-elimination curves after the injection of [^{14}C]acetate revealed two

exponential components that were not influenced by the dietary state. In the fat-fed and starved rats, the rate at which $^{14}CO_2$ was eliminated fell more slowly immediately after the administration of [^{14}C]acetate than after the administration of [^{14}C]palmitate. An explanation of this may be that the specific activity of [^{14}C]acetate remains high for a longer period after injection owing to its distribution into a fluid compartment considerably larger than the albumin space into which [^{14}C]palmitate would equilibrate. This may obscure any alterations of oxidative patterns caused by changes in the diet. Thus, when trace amounts of acetate are injected, it appears that the rate of evolution of $^{14}CO_2$ does not give any information on metabolic processes that are very rapid, such as the comparative turnover times of the citric acid cycle.

It is difficult to explain the results showing undiminished total oxidation of [^{14}C]acetate in carbohydrate-fed animals. It would be expected that more label would be diverted into lipogenic pathways under these conditions, thereby lowering the recovery of label expired as $^{14}CO_2$. It is possible that the rats were not studied during that period of the feeding cycle when lipogenesis was most active.

The results show how similarities exist in the metabolic patterns of the fat-fed and the starved animal. In both dietary states there is an apparent sparing of [^{14}C]glucose oxidation and an enhancement of the oxidation of [^{14}C]palmitate. Similar exponential components are found in the $^{14}CO_2$-elimination patterns. These findings support the postulate (Felts & Mayes, 1965; Felts, 1965; Mayes & Felts, 1966) that the major pathway of assimilation of dietary fat into the tissues involves the prior hydrolysis of triglyceride to free fatty acid. The similarity in metabolic pattern of the starved and the fat-fed rat is due most probably to free fatty acids being the primary metabolic fuel in both conditions.

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