# Studies on Lipogenesis *in vivo*

## EFFECT OF DIETARY FAT OR STARVATION ON CONVERSION OF [$^{14}$C]GLUCOSE INTO FAT AND TURNOVER OF NEWLY SYNTHESIZED FAT

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1. Lipogenesis was studied *in vivo* by giving mice 250mg. meals of [U-$^{14}$C]glucose and measuring the disposition and incorporation of label. About 48% of the $^{14}$C dose was eliminated as $^{14}CO_2$ in the first 2hr. At 60min. after administration, 1·0, 1·9 and 11·9% of the dose was recovered as liver glycogen, liver fatty acid and carcass fatty acid respectively. Of the [$^{14}$C]glucose converted into fat in the epididymal pads about 90% was present as glyceride fatty acid and 10% as glyceride glycerol. 2. Hepatic synthesis of fatty acid was depressed by dietary fat to a much greater extent than was synthesis outside the liver. Both feeding with fat and starvation decreased the proportion of the label taken up by adipose tissue present as fat (triglyceride) and increased the proportion of triglyceride label present as glyceride glycerol. These results are consistent with the hypothesis that the primary action of both these conditions in decreasing fat synthesis is to inhibit synthesis of fatty acids. 3. Turnover of body fat labelled *in vivo* from [U-$^{14}$C]glucose was estimated from the decline in radioactivity measured over the first 24hr. of the experiment. The half-life of liver and extrahepatic fatty acids (excluding epididymal fat) was 16hr. and 3 days respectively. In contrast, no measurable decrease in radioactivity of the fatty acids of epididymal fat was observed for 7 days after administration of the [U-$^{14}$C]glucose.

Lipogenesis has been the subject of a considerable number of investigations in recent years and much is known about the biochemical mechanisms involved in its regulation (Masoro, 1962). In earlier years the liver was considered to be the major, if not exclusive, site in the body for fat synthesis. Wertheimer & Shapiro (1948) called attention to the high metabolic activity of adipose tissue and emphasized that such tissue is not merely an inert storage depot for fat synthesized elsewhere, but rather actively synthesizes fat. The metabolism of adipose tissue has been reviewed by Jeanrenaud (1961) and Vaughan (1961).

In spite of considerable progress it is still not understood how cessation of food intake depresses lipogenesis or how re-feeding stimulates it. The relative contribution of the liver as compared with extrahepatic tissues in the synthesis of fatty acids and cholesterol is not yet adequately defined. In addition, conflicting data have been reported as to the rate of turnover of body fat.

The results of some investigations relating to these questions are reported in this paper. Mice (or rats) were dosed by stomach tube with meals of [U-$^{14}$C]glucose and incorporation of $^{14}$C into fatty acids and cholesterol in liver and extrahepatic tissues was measured. The glucose meal would be expected to result in insulin secretion in physiological amounts and thus plasma concentrations of glucose and insulin optimum for lipogenesis would be present. These conditions should also decrease lipolysis, fatty acid degradation and glycogenolysis, and thus minimize the problem of differential dilution of $^{14}$C with endogenously produced unlabelled acetyl-CoA. Use of [U-$^{14}$C]glucose instead of [1-$^{14}$C]acetate offers the additional advantage that activation of acetate should not be rate-limiting.

## METHODS

*Maintenance of animals.* For the mouse experiments, young adult male mice weighing 20–30g. obtained from the Merck, Sharp and Dohme colony were used. The weight range for any single experiment was kept less than 4g. Unless otherwise noted, the mice had been maintained for 1–2 weeks on a low-fat–high-glucose diet (diet 1 or 2; Table 1). During the earliest experiments animals were maintained on diet 1, which contained 0·25% of corn oil to provide a dietary source of linoleic acid. Later the corn oil was increased to 1% (diet 2) to provide a higher dose of this essential fatty acid. When the dose of dietary fat was studied, diets 2–5 (Table 1) or Purina Laboratory Chow containing 5% of fat was given for 7 days. These purified

Table 1. *Composition of experimental diets*

Labco casein and Celluflour were obtained from the Borden Co., New York, N.Y., U.S.A., and the Chicago Dietetic Supply House, Chicago, Ill., U.S.A., respectively. Glucose was added as Cerelose and was obtained from Merck and Co. Inc., Rahway, N.J., U.S.A. Mazola corn oil was used. In addition a complete vitamin mix was added to each diet to furnish the following micronutrients/100g. of food: thiamine, 1·0μg.; riboflavine, 2·0μg.; pyridoxine, 1·0μg.; calcium pantothenate, 10·0μg.; nicotinamide, 10·0μg.; inositol, 5·0μg.; choline, 100·0μg.; *p*-aminobenzoic acid, 30·0μg.; biotin, 0·05μg.; folic acid, 0·2μg.; α-tocopherol, 14·2μg.; vitamin $B_{12}$ triturate (0·1% trituration with mannitol), 10·0μg.; calciferol, 300 i.u.; vitamin A palmitate, 1600 i.u.

| | Composition (%) | | | | |
|---|---|---|---|---|---|
| Ingredient | Diet 1 | Diet 2 | Diet 3 | Diet 4 | Diet 5 |
| Casein (Labco) | 20·0 | 20·0 | 21·0 | 23·0 | 26·0 |
| Salts U.S.P. no. 2 | 4·0 | 4·0 | 4·0 | 4·0 | 4·0 |
| Glucose | 70·8 | 70·0 | 65·0 | 58·0 | 45·0 |
| Celluflour | 5·0 | 5·0 | 5·0 | 5·0 | 5·0 |
| Corn oil | 0·25 | 1·0 | 5·0 | 10·0 | 20·0 |

diets ranged from 1 to 20% of corn oil with protein (casein) kept constant at 20% of total caloric intake. For the experiments with rats 90–125g. male Charles River CD rats maintained on Purina Laboratory Chow were used. All animals were supplied with food and water *ad libitum* and housed in individual screen-bottomed cages in an air-conditioned room maintained at approx. 24°.

*Dosing and bleeding, and preparation of tissues.* The [U-$^{14}$C]glucose used had a specific activity of 16mc/m-mole and was obtained from New England Nuclear Corp., Boston, Mass., U.S.A. In most experiments, it was added to 50% (w/v) glucose to give 1 or 5μc/ml. and 0·5ml. was given orally to each mouse. In some experiments, the [U-$^{14}$C]-glucose was given by intraperitoneal injection without dilution with unlabelled glucose. In all experiments food was removed from the cages at the time the [U-$^{14}$C]glucose was given except when the [U-$^{14}$C]glucose was added to the diet. Unless otherwise noted, the incorporation rates were determined 60min. after [U-$^{14}$C]glucose was given. The mice were bled unanaesthetized by direct heart puncture or killed by cervical fracture. Livers were removed quickly and weighed, and 1g. portions immediately frozen or placed in 2ml. of 6N-KOH and saponified. Epididymal fat pads were dissected and frozen, and the remainder of the carcasses was saponified by refluxing for 6hr. in 3N-KOH in 50% (v/v) ethanol after standing overnight in aq. 6N-KOH. In some experiments the carcasses included the epididymal fat pads. This detail is specified in each Table.

*Respiratory studies.* The disposition of the [U-$^{14}$C]glucose during a period of 8hr. after oral administration was determined in all-glass rat metabolism cages purchased from Delmar Scientific Co., Maywood, Ill., U.S.A. (Roth, Leifer & Hogness, 1948). Four mice were placed in each chamber and kept separate by stainless-steel wire-mesh dividers. Water but not food was supplied in the chamber. The respiratory $^{14}CO_2$ was collected from air drawn through the chamber by bubbling through 1M-Hyamine-10X (Packard Instrument Co., Downers Grove, Ill., U.S.A.). Total respiratory $CO_2$ was determined by titration of the Hyamine with N-HCl, with phenolphthalein as the indicator.

*Analytical methods.* Plasma glucose was determined by the Technicon Autoanalyzer modification of the method of Hoffman (1937). The specific activity of the circulating glucose was determined by thin-layer chromatography on silica gel G (E. Merck A.-G., Darmstadt, Germany) obtained from Brinkman Instruments, Westbury, N.Y., U.S.A. A 0·1ml. sample of plasma was mixed with 1·9ml. of pyridine, centrifuged and 0·40ml. of this pyridine extract applied to the plates with the aid of a hair-drier. The plates (250μ thick) were developed with the solvent system ethyl acetate–propan-2-ol–pyridine–water (12:5:1:2, by vol.) to a standard distance of 14cm. Typical mobilities under these conditions were: glycerol, 8·0cm.; glucose, 5·1cm.; lactate, 0·7cm.; alanine, 0·7cm.; glutamic acid, 0cm. The plates were cut up, scraped and counted in a mixture of 3ml. of water and 17ml. of DCN phosphor (see below). The specific activity of the plasma glucose was calculated by dividing the radioactivity in counts/min. found as glucose in 0·1ml. of plasma by the mg. of glucose in this volume of plasma as determined chemically. This is referred to in this paper as the specific activity of plasma glucose. Similarly the specific activities of fatty acid and cholesterol fractions were calculated by dividing the counts/min. in the fraction by the weight of the fraction determined gravimetrically.

For isolation of glycogen, fatty acids and cholesterol, 1g. portions of liver were heated in a boiling-water bath in 2ml. of 6N-KOH for 1hr., 3·2ml. of ethanol was added and the extracts were refluxed for an additional 1hr. To precipitate glycogen by a modification of the method of Good, Kramer & Somogyi (1933), 0·3ml. of 30% (w/v) KI was added per tube. Use of ethanol-soluble KI prevented salt contamination in the ethanol precipitations. The glycogen was removed by centrifugation and purified by two reprecipitations from water. The final precipitates were dissolved in water and transferred to tared stainless-steel planchets and dried to thin transparent films with an infrared heat lamp.

Cholesterol was isolated from the non-saponifiable fraction by the digitonide method of Sperry & Webb (1950). After subsequent acidification, fatty acids were extracted with light petroleum (b.p. 30–60°), transferred to tared glass scintillation-counting vials and the ether was removed by evaporation. Carcass cholesterol and fatty acids were isolated in a similar manner from the carcass digests prepared as described above.

The distribution of $^{14}$C activity in the epididymal fat pads was determined as follows. By using a VirTis homogenizer, pads were homogenized directly in toluene–

liquifluor phosphor (described below) and transferred to plastic counting vials for subsequent counting. In some experiments the pads were homogenized in 40ml. portions of hexane. Samples (5ml.) were immediately transferred to counting vials and the remainder was washed with 10ml. portions of water. Samples (5ml.) of the washed hexane phase were transferred to counting vials for subsequent counting. Samples (20ml.) were transferred to tared screw-capped tubes (20mm. × 125mm.) and evaporated to dryness. The residues were hydrolysed by boiling in 0·5N-KOH in 90% (v/v) ethanol for 1hr. After saponification, digests were diluted 1:1 with water and fatty acids were extracted as described above. Since adipose-tissue lipids are reported to be 99% triglycerides (Jeanrenaud, 1961), glyceride glycerol radioactivity was determined as the difference between total lipid radioactivity and that recovered as fatty acid. The validity of the procedure of determining glyceride glycerol by difference in this manner has been established by Fain (1964).

*Counting procedures.* Except for glycogen, all samples were counted for radioactivity with a Packard Tri-Carb liquid scintillation-counting system, model 314EX, at tap 4. DCN phosphor [1,4-dioxan, 3000ml.; Cellosolve, 600ml.; naphthalene, 180g.; 2,5-diphenyloxazole, 36g.; 1,4-bis-(5-phenyloxazol-2-yl)benzene, 1·8g.] was used for [$^{14}$C]-glucose standards, extracts of urine and faeces and dilutions of plasma. The 1,4-dioxan and naphthalene were obtained from Matheson, Coleman and Bell, East Rutherford, N.J., U.S.A. Cellosolve was obtained as Karl Fisher's Reagent Diluent from Merck and Co. Inc., Rahway, N.J., U.S.A. The 2,5-diphenyloxazole and 1,4-bis-(5-phenyloxazol-2-yl)-benzene were obtained from the Packard Instrument Co., Downers Grove, Ill., U.S.A. For fat pads, fatty acids and cholesterol digitonides toluene–liquifluor phosphor (TLF phosphor) was used (42ml. of liquifluor/l. of toluene). Liquifluor concentrate obtained from T. M. Pilot Chemicals Inc., Watertown, Mass., U.S.A., at this dilution furnished 4g. of 2,5-diphenyloxazole and 50mg. of 1,4-bis-(5-phenyl-oxazol-2-yl)benzene/l. of toluene. The cholesterol digitonides were solubilized in 1ml. of Hyamine before adding the TLF phosphor according to the procedure of Shapiro & Kritchevsky (1963).

Glycogen was counted, corrected to infinite thinness, on 1¼in. stainless-steel planchets by using a Nuclear–Chicago Corp. gas-flow counting system, model 4306. All counts, both from gas-flow and liquid-scintillation counting, were corrected to the efficiency of [$^{14}$C]palmitic acid in pure toluene–liquifluor phosphor counted at tap 4 and thus are directly comparable. The counts obtained from gas-flow counting were thus corrected by plating the [$^{14}$C]palmitic acid standard at infinite thinness and comparing with the counts obtained by liquid-scintillation counting.

Essentially no quenching was observed in counting fat-pad extracts or fatty acids obtained from fat pads after saponification. The presence of 1ml. of Hyamine in the TLF phosphor when cholesterol digitonides were counted caused approx. 40% fewer counts owing to quenching. Liver and carcass fatty acids were counted with 90% and 60–80% efficiencies respectively, based on [$^{14}$C]palmitic acid in TLF phosphor.

Table 2. *Disposition of [U-$^{14}$C]glucose meal given to mice*

Experimental details are given in the text. The values given are the averages of eight mice, four in each of two chambers. Each mouse was given 250mg. of [U-$^{14}$C]glucose (0·5$\mu$c) orally immediately before being placed in the chamber. The mice had been maintained on diet 2 (Table 1), but were not fed while in the chamber.

| Sample | Period of collection (hr.) | $^{14}$C recovery (% of dose) |
|---|---|---|
| Respiratory $CO_2$ | 0–1 | 24·5 |
| Respiratory $CO_2$ | 1–2 | 23·0 |
| Respiratory $CO_2$ | 2–3 | 9·8 |
| Respiratory $CO_2$ | 3–5 | 7·1 |
| Respiratory $CO_2$ | 5–8 | 7·4 |
| Urine | 0–8 | 1·0 |
| Faeces | 0–8 | <0·1 |
| Total body fatty acid | | 9·0 |
| Total $^{14}$C recovery | | 81·9 |

## RESULTS

*Fate of [U-$^{14}$C]glucose meal.* The gross dispcsition of the 250mg. [U-$^{14}$C]glucose meal for 8hr. after administration is shown in Table 2. Approx. 48% of the dose was eliminated as $^{14}CO_2$ in the first 2hr. Only 1% was excreted in the urine over the entire 8hr. period. The slight amount of label in faeces was most likely a result of contamination with urine. A total of 81·9% of the label given was recovered as excretory products and body fatty acids.

The mice when placed in the respiratory chamber showed exploratory behaviour for the first hour, but then settled down and went to sleep for the remainder of the period. This was reflected in a 50% decrease in the rate of total carbon dioxide excretion starting in the second hour as compared with the first. Thus it does not appear likely that excessive sympathetic activity played a part in this experiment although this point was not directly investigated.

Table 3 presents the labelling changes in liver glycogen and fatty acid and carcass fatty acids for up to 2hr. after oral administration to mice of 250mg. of [U-$^{14}$C]glucose. The peak in plasma glucose concentration was recorded at 10min. and returned to normal in 1hr., with its specific activity remaining relatively constant over this time-period. The peak in liver glycogen labelling was observed at 40min. and in fatty acids at 60min. Incorporation of $^{14}$C activity into carcass fatty acid was linear for the first 60min. and at 2hr. was only slightly higher than at 1hr. Labelling in the epididymal fat pad paralleled the labelling in total carcass fatty acid. Of the total radioactivity present in the epididymal fat pads, 80–90% was present as fat and of this 90–95% was present as glyceride fatty acid and 5–10% as glyceride

Table 3. *Time-course of labelling after a 250 mg. [U-$^{14}$C]glucose meal given to mice*

Experimental details are given in the text. Each mouse was given 250 mg. of [U-$^{14}$C]glucose (0·5 $\mu$c) orally and killed at the indicated time. Food was removed from the cages during the interval between dosing and killing. There were eight mice/group and the previous diet had been diet 2 (Table 1). In this experiment carcass refers to the body with both the liver and epididymal fat pads removed. Where appropriate, results are given as means ± S.E.M. N.D., Not determined.

| | Plasma glucose | | | | |
|---|---|---|---|---|---|
| Time (min.) | (mg./100 ml.) | (counts/min./mg.) | Liver glycogen (counts/min./liver) | Liver fatty acids (counts/min./liver) | Carcass fatty acids (counts/min./mouse) |
| 0 | 158 ± 12 | — | — | — | — |
| 10 | 282 ± 30 | 1960 | 610 ± 170 | 2360 ± 535 | 10200 |
| 20 | 249 ± 20 | 2160 | 1080 ± 240 | 2130 ± 270 | 19400 |
| 30 | 234 ± 11 | 2130 | 2170 ± 500 | 6560 ± 1080 | 31400 |
| 40 | 217 ± 12 | 1940 | 8500 ± 1860 | 6160 ± 1100 | 41100 |
| 60 | 149 ± 16 | 2040 | 5080 ± 1360 | 9690 ± 440 | 60500 |
| 120 | 126 ± 9 | N.D. | 3030 ± 814 | 9290 ± 1570 | 74200 |

| | Epididymal fat pads | | | |
|---|---|---|---|---|
| Time (min.) | (counts/min./pad) | (% of pad $^{14}$C as fat) | (% of fat $^{14}$C as fatty acid) | (% of fat $^{14}$C as glycerol) |
| 10 | 333 ± 98 | 80 | 90 | 10 |
| 20 | 730 ± 100 | 81 | 95 | 5 |
| 30 | 1080 ± 140 | 85 | 93 | 7 |
| 40 | 1160 ± 120 | 82 | 94 | 6 |
| 60 | 1830 ± 320 | 92 | 95 | 5 |
| 120 | 2140 ± 150 | 90 | 91 | 9 |

glycerol. At 60 min. after administration of the [U-$^{14}$C]glucose meal, 1·0, 1·9 and 11·9% of the dose was recovered as liver glycogen, liver fatty acid and carcass fatty acid respectively.

In the period immediately after ingestion of a glucose meal, one might expect some of the labelled fatty acid synthesized in the liver to be transported to extrahepatic tissues. An experiment was carried out with rats to determine the magnitude of the fatty acid flux in plasma and the results are shown in Table 4. At 30 and 60 min. after oral administration of 1000 mg. of [U-$^{14}$C]glucose (10 $\mu$c), rats were bled by direct heart puncture and the $^{14}$C content of the fatty acids of liver, plasma and carcass was ascertained. Plasma fatty acids, maximal in $^{14}$C content after 30 min., at this time had a specific activity approx. 1% of the [U-$^{14}$C]glucose given. In view of the large amount of unlabelled fat pre-existing in the carcass and the fact that the specific activity of fatty acids in the carcass after 60 min. was approximately as high as that in the plasma, it appears probable that most of the labelled carcass fat was synthesized *in situ* and not transferred from the liver. However, with the specific activity of fatty acids in the plasma higher than those in the liver or carcass at 30 min., it is likely that labelled fatty acids were moving from the liver to the depots and contributing to some extent to the content of

Table 4. *Fatty acid labelling in plasma, liver and carcass of rats after a [U-$^{14}$C]glucose meal*

Experimental details are given in the text. Each rat was given 1000 mg. of [U-$^{14}$C]glucose (10 $\mu$c) orally and killed at the indicated time with food removed from the cages for this interval. There were six rats/group with an average weight of 108 g. (92–124 g.) and the previous diet had been Purina Laboratory Chow.

| | At 30 min. | At 60 min. |
|---|---|---|
| Liver | | |
| % of fatty acid* | 2·7 | 2·8 |
| Counts/min./liver | 1490 | 3490 |
| Counts/min./100 mg. of fatty acid | 1140 | 2600 |
| Plasma | | |
| Fatty acid (mg./100 ml.) | 118 | 160 |
| Counts/min./100 mg. of fatty acid | 3180 | 2190 |
| Carcass | | |
| % of fatty acid† | 5·2 | 3·6 |
| Counts/min./carcass | 31030 | 70500 |
| Counts/min./100 mg. of fatty acid | 598 | 1900 |

* Wt. (g.) of total fatty acid (after saponification)/100 g. fresh wt. of liver.

† Wt. (g.) of total fatty acid (after saponification)/100 g. (body wt.−liver wt.).

labelled fatty acids in the carcass. Apparently the fatty acids newly synthesized in the liver and secreted into the plasma did not completely mix with the total liver pool of unlabelled fatty acids.

*Effect of dietary fat.* Table 5 presents the results of an experiment in which the effect on lipogenesis of 1–20% of corn oil in the diet was studied. Although incorporation of [U-$^{14}$C]glucose into carcass fatty acid in mice given 20% of corn oil in the diet was approx. 60% of that in mice given 1% of corn oil in the diet, hepatic fatty acid synthesis was depressed considerably more than this, the rate in mice given 20% of corn oil in the diet being only 14% of that in mice given 1% of corn oil in the diet. Results showing the incorporation of [U-$^{14}$C]glucose into the epididymal fat pads in this experiment are shown in Table 6. The total uptake of $^{14}$C by the pad was higher on diets containing 20% than 10% of corn oil and, in fact, was as high as on diets containing 5% of fat. However, at the higher fat dose a greater proportion of the counts in the pad was present as water-soluble intermediates and also, of the activity present as fat, a greater proportion was found as glyceride glycerol. The decreases in incorporation of [$^{14}$C]glucose into fatty acids in epididymal fat as shown in the last column of Table 6 paralleled that found for the remainder of the carcass as shown in Table 5.

Table 5. *Effect of dietary fat on lipogenesis in mice*

Experimental details are given in the text. Each mouse was given 250mg. of [U-$^{14}$C]glucose (2·5$\mu$c) orally and killed 60min. afterwards with food removed from the cages for this interval. There were eight mice/group. The diets used are specified in Table 1. In this experiment carcass refers to the body with both liver and epididymal fat pads removed. The values for carcass are the means of two results from subgroups of four animals each. Where appropriate, results are given as means ± S.E.M.

| Diet ... ... ... ... | 1% of corn oil | 5% of corn oil | 10% of corn oil | 20% of corn oil | Purina Chow |
|---|---|---|---|---|---|
| 7-day weight gain (g.) | 6·4±0·3 | 5·6±0·4 | 6·2±0·5 | 6·0±0·5 | 4·8±0·4 |
| Plasma glucose (mg./100ml.) | | | | | |
| At 10min. | 367±42 | 326±23 | 331±42 | 248±14 | 217±8 |
| At 60min. | 149±10 | 142±10 | 151±12 | 219±29 | 183±7 |
| Liver fatty acid | | | | | |
| %* | 3·2 | 2·6 | 2·7 | 3·2 | 2·8 |
| Counts/min./g. of liver | 14600±1700 | 10700±800 | 5280±690 | 2130±260 | 4680±400 |
| Liver cholesterol | | | | | |
| %* | 0·32 | 0·22 | 0·21 | 0·24 | 0·21 |
| Counts/min./g. of liver | 70±16 | 41±6 | 42±7 | 58±12 | 140±24 |
| Carcass fatty acid | | | | | |
| %† | 8·7 | 7·1 | 7·6 | 8·6 | 5·4 |
| Counts/min./g. of carcass | 13300 | 12700 | 8540 | 8160 | 6640 |
| Carcass cholesterol | | | | | |
| %† | 0·29 | 0·30 | 0·27 | 0·28 | 0·28 |
| Counts/min./g. of carcass | 74 | 76 | 57 | 53 | 72 |

* Wt. (g.) of total fatty acid (or cholesterol) after saponification/100g. liver wt.

† Wt. (g.) of total fatty acid (or cholesterol) after saponification/100g. (body wt.—liver wt.—wt. of epididymal fat pads).

Table 6. *Effect of dietary fat on incorporation of [U-$^{14}$C]glucose into epididymal fat pads of mice*

Experimental details are given in the text. Each mouse was given 250mg. of [U-$^{14}$C]glucose (2·5$\mu$c) orally and killed 60min. afterwards with food removed from the cages for this interval. There were eight mice/group. The diets used are specified in Table 1. Where appropriate, results are given as means ± S.E.M.

| | | Incorporation of [$^{14}$C]glucose at 60min. | | | | |
|---|---|---|---|---|---|---|
| Diet | Wt. of pad (mg.) | (total counts/min./pad) | (% of pad $^{14}$C as fat) | (counts/min./pad as fat) | (% of fat $^{14}$C as glycerol) | (counts/min./pad as fatty acid) |
| 1% of corn oil | 131±12 | 8190±460 | 75 | 6140 | 12 | 5400 |
| 5% of corn oil | 117±6 | 6490±270 | 80 | 5190 | 16 | 4460 |
| 10% of corn oil | 136±8 | 3980±730 | 70 | 2790 | 20 | 2230 |
| 20% of corn oil | 142±4 | 6210±310 | 44 | 2730 | 24 | 2070 |
| Purina Chow | 84±2 | 2310±170 | 76 | 1760 | 28 | 1270 |

Table 7. *Effect of starvation or feeding on the turnover of epididymal fat pads labelled* in vivo *in mice*

Experimental details are given in the text. Each mouse was given 250mg. of unlabelled glucose orally followed 20min. later by 0·3$\mu$c of [U-$^{14}$C]glucose (16mc/m-mole) intraperitoneally. There were 16 mice in each group and the previous diet had been diet 1 (Table 1). For the groups listed under 'Fed' food (diet 1 in Table 1) was replaced in the cages 6hr. after [U-$^{14}$C]glucose administration. Where appropriate, results are given as means ± S.E.M.

| | Starved | | | Fed | | |
|---|---|---|---|---|---|---|
| | | Incorporation | | | Incorporation | |
| Time after [$^{14}$C]glucose (hr.) | Wt. of pad (mg.) | (counts/min./ pad) | (counts/min./ 100mg.) | Wt. of pad (mg.) | (counts/min./ pad) | (counts/min./ 100mg.) |
| 1 | 183±12 | 1590±140 | 870 | — | — | — |
| 2 | 154±11 | 1370±110 | 890 | — | — | — |
| 4 | 165±13 | 1330±150 | 800 | — | — | — |
| 6 | 152±10 | 1390±130 | 910 | — | — | — |
| 24 | 94± 8 | 820±90 | 870 | 156±8 | 1430±140 | 920 |
| 48 | 42± 6 | 160±33 | 380 | 195±15 | 1730±210 | 890 |
| 72 | 13± 3 | 18±8 | 140 | 201±14 | 1540±140 | 770 |

Neither carcass nor liver cholesterol synthesis was affected significantly by the dose of dietary fat.

Results for mice fed on Purina Laboratory Chow are included for comparison because many biochemical measurements *in vitro* are made on animals fed on such natural diets. The weight gain of these mice was less than that on any of the purified diets and their carcasses contained less fat, as shown by the percentage of total carcass fatty acid and the weight of the epididymal fat pads. The incorporation of label into both liver and carcass fatty acid was considerably lower than that in mice fed on diets containing 5% of corn oil, an equivalent fat dose. Also, the proportion of counts in glyceride glycerol was higher. Synthesis of extrahepatic cholesterol appeared comparable, but the liver cholesterol synthesis rate was twice that of mice fed on purified diets. This would be consistent with the known greater bile acid excretion of animals fed on natural as compared with purified diets (Portman, Mann & Wysocki, 1955).

*Turnover studies.* A 3-day experiment was carried out in which the turnover of epididymal fat labelled *in vivo* from [U-$^{14}$C]glucose was determined under conditions of feeding and starving. Unlabelled glucose (250mg.) was given orally to mice and 20min. later a tracer of [U-$^{14}$C]glucose (0·3$\mu$c) was injected intraperitoneally. As shown in Table 7, the epididymal fat was mobilized during starvation at such a rate that after 72hr. virtually all the labelled as well as unlabelled fat was gone from this depot. After the first 24hr. of food deprivation about half the labelled fat was gone but the specific activity (counts/min./mg. wet wt. of pad) was not depressed, suggesting that there was little preferential mobilization of recently synthesized fat. In mice starved for 6hr. after being given [$^{14}$C]glucose and then fed for the next

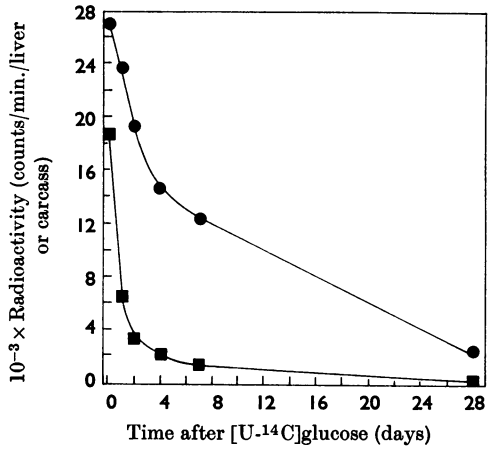


Fig. 1. Turnover of carcass and liver fatty acids in mice. The fatty acids were labelled from [U-$^{14}$C]glucose *in vivo*. Carcass fatty acid (after saponification) includes all the body fatty acid except that in the epididymal fat pads and the liver. The mice, maintained on diet 1 (Table 1), were each given 250mg. of unlabelled glucose orally followed 20min. later by 0·3$\mu$c of [U-$^{14}$C]glucose intraperitoneally. There were 16 mice/group and they were killed at the indicated times after [U-$^{14}$C]glucose administration. Further details are given in the text. ●, Carcass fatty acid; ■, liver fatty acid.

66hr. the $^{14}$C content of the epididymal fat was as high 72hr. after the [$^{14}$C]glucose dose as it was after 1hr. Therefore under these conditions there was no measurable turnover of epididymal fat.

A second experiment was run in which body fat was labelled as in the previous experiment, and the decline in radioactivity of the fatty acids of liver, epididymal fat and the remainder of the carcass

was determined for the following 28 days. The decline of radioactivity in liver and carcass fatty acids is shown in Fig. 1. If one estimates turnover from the decline for the first day, the half-life for liver and carcass fatty acids was 16hr. and 3 days respectively. These may be compared with turnover times of $2\frac{1}{2}$ days and 5–6 days for liver and carcass fatty acids respectively estimated by Stetten & Grail (1943) from the decline in deuterium content of fatty acids labelled *in vivo* from $D_2O$. Stetten & Grail (1943) point out that some of the fatty acids in liver would have been mobilized from the depots under conditions of their experiment and therefore suggest that the actual turnover of fatty acids in the liver is more likely to be a matter of hours than of days. Turnover of fat in the epididymal pads of the mouse presents a different picture, as shown by the results in Table 8. The peak in labelling in the fat pads was observed at 1 day but probably occurred at 2–3hr. (this was not measured) and did not measurably decline for the next 6 days. After an additional 3 weeks, the $^{14}C$ content of the epididymal pads declined to about one-third of the peak value. During the 28-day period essentially all the radioactivity in the fat pad was present as fat and a gradually decreasing percentage of this was found as glyceride glycerol.

*Effect of starvation.* An experiment was carried out to study the influence of food deprivation on lipogenesis *in vivo*. Groups of mice, fed or starved for 18hr., were given either tracer doses of 2$\mu$c of [U-$^{14}C$]glucose (16mc/m-mole) intraperitoneally or 250mg. of [U-$^{14}C$]glucose (2·5$\mu$c) orally. The mice were bled at intervals of 10, 30 and 60min. after dosing and the concentration and specific activity of the circulating glucose were determined. The incorporation and distribution of $^{14}C$ in the epididymal fat pads was measured at 60min. only. The results are shown in Table 9. In the presence or absence of a glucose meal previously starved animals showed 82% and 93% decreases respectively in the incorporation of [$^{14}C$]glucose into epididymal fat pad compared with previously fed animals. In the absence of the meal, because of lowered plasma glucose concentrations and impaired

Table 8. *Distribution of $^{14}C$ into epididymal fat pads of mice*

Experimental details are given in the text. Mice were maintained on diet 1 (Table 1) for 1 week before and 4 weeks after being given 250mg. of unlabelled glucose orally followed 20min. later by 0·3$\mu$c of [U-$^{14}C$]glucose intraperitoneally. Food was removed from the cages only for 1hr. after dosing. There were 16 mice/group. Where appropriate, results are given as means ±S.E.M.

| Time after [$^{14}C$]glucose | Incorporation (counts min./pad) | (% of fat-pad $^{14}C$ as fat) | (% of fat $^{14}C$ as glycerol) |
|---|---|---|---|
| 1hr. | 1360±180 | 99 | 21 |
| 1 day | 1830±200 | 100 | 19 |
| 2 days | 1670±120 | 99 | 17 |
| 4 days | 1500±170 | 95 | 15 |
| 7 days | 1900±180 | 97 | 13 |
| 28 days | 600±90 | 96 | 10 |

Table 9. *Effect of starvation on incorporation of [U-$^{14}C$]glucose into epididymal fat in mice*

Experimental details are given in the text. The mice (eight/group) either were given diet 2 (Table 1) until the time of the experiment or were starved for 18hr. For each nutritional state either 2$\mu$c of [U-$^{14}C$]glucose (16mc/m-mole) was given intraperitoneally or 250mg. of [U-$^{14}C$]glucose (2·5$\mu$c) was given orally. Food was removed from the cages (if present) for the period between dosing and killing. Where appropriate, results are given as means ±S.E.M.

| | Trace of [U-$^{14}C$]glucose (2$\mu$c) intraperitoneally | | 250mg. of [U-$^{14}C$]glucose (2·5$\mu$c) orally | |
|---|---|---|---|---|
| | Fed | Starved | Fed | Starved |
| Plasma glucose (mg./100ml.) | | | | |
| At 0min. | 163±9 | 84±4 | | |
| At 10min. | 173±6 | 115±7 | 338±5 | 504±45 |
| At 30min. | 174±13 | 119±4 | 272±13 | 513±44 |
| At 60min. | 164±2 | 125±6 | 190±8 | 404±34 |
| Plasma glucose (counts/min./mg.) | | | | |
| At 10min. | 41100 | 103800 | 7250 | 7390 |
| At 30min. | 23300 | 61600 | 8870 | 8480 |
| At 60min. | 8100 | 32200 | 7430 | 9400 |
| Epididymal fat pads (at 60min.) | | | | |
| Counts/min./pad | 16320±2880 | 1210±160 | 7620±1370 | 1350±250 |
| % of pad $^{14}C$ as fat | 92±2 | 58±2 | 88±1 | 70±4 |
| % of fat $^{14}C$ as glycerol | 27±6 | 97±1 | 10±1 | 34±6 |

glucose utilization, the specific activity of the plasma glucose was 2–4 times as high in starved as in fed mice, and so the actual decrease in incorporation of [$^{14}$C]glucose into the pads was 98%. In the starved mice the proportion of the radioactivity present as fat fell from 92 to 58% and the proportion of counts in this fat, essentially triglyceride, present as glyceride glycerol increased from 27 to 97%. Therefore the actual decrease in fatty acid synthesis during starvation was over 99%. After the glucose meal the elevation in plasma glucose concentration was higher and the return to normal delayed in starved as compared with fed mice, a manifestation of the glucose intolerance of starvation-diabetes (Keys, Brozek, Henschel, Mickelsen & Taylor, 1950). However, the specific activities of the circulating glucose were similar in fed and starved mice after the 250mg. [$^{14}$C]glucose meal and the incorporation rates may be directly compared. The distribution of radioactivity in the epididymal fat pads was changed in the same manner in this case as in the absence of the meal, although the change was less marked.

## DISCUSSION

Most studies on the regulation of lipogenesis (see Fritz, 1961; Jeanrenaud, 1961; Masoro, 1962; Vaughan, 1961) reported have been performed with either systems *in vitro* or an incubation technique '*in vitro–in vivo*' (Stein & Stein, 1962). Favarger and his colleagues have published a series of papers summarized by Favarger (1964) in which aspects of lipogenesis have been studied in the mouse *in vivo* by using [1-$^{14}$C]acetate and [U-$^{14}$C]glucose as precursors.

The results in Table 3 indicate that when mice were given 250mg. meals of [U-$^{14}$C]glucose the maximum observed incorporation of $^{14}$C into liver glycogen (40min.), liver fatty acids (1hr.) and carcass fatty acids (2hr.) was 1·6, 1·9 and 14·5% respectively. If one assumes that C-3 and C-4 of glucose are lost in the conversion into fatty acid (C-1 is also lost to the extent that the hexose monophosphate shunt is operating), then one can calculate that 25% of the molecules of glucose ingested were converted into fatty acids. Stetten & Boxer (1944) from studies on the incorporation of deuterium into body fat and glycogen of rats fed on a diet containing 85% of glucose and 6% of casein estimated that approx. 30 and 3% of ingested glucose was converted into fatty acids and glycogen respectively.

With the diet containing 20% of corn oil, where close to 40% of the calories were supplied by fat, 13% of the glucose molecules ingested were directed towards fatty acid synthesis. Thus even with a high fat intake lipogenesis would appear to be an important, although not major, pathway for the disposal of ingested carbohydrate. Recently others have also concluded, from studies *in vivo* in which conversion of orally given [U-$^{14}$C]glucose into fatty acid has been studied in rats, that lipogenesis is not a major pathway of [U-$^{14}$C]glucose metabolism. Lequin & Steyn-Parvé (1962) found that 0·5–2·3% of absorbed [$^{14}$C]glucose was converted into fatty acids. Patkin & Masoro (1964) recovered up to 4·7% of absorbed [U-$^{14}$C]glucose as fatty acid in rats 6hr. after a single oral dose. De Freitas & Depocas (1965) have reported that 4% of [U-$^{14}$C]-glucose taken up by rat tissues *in vivo* was converted into fatty acid. Masoro, Chaikoff & Dauben (1949) had previously reported that mice fed on a high-carbohydrate diet converted 10·6% of ingested carbohydrate into fatty acid during a 24hr. feeding period. As the authors stated, this was a minimal estimate since turnover was neglected. Our results, in which 16% of the [U-$^{14}$C]glucose given orally to mice on a diet containing 1% of corn oil was converted into fatty acid, appear comparable with these data of Masoro *et al.* (1949).

As shown in Table 3, incorporation of glucose label into fatty acids in liver is as rapid as into liver glycogen. Fatty acid and glycogen synthesis occur simultaneously after a meal and it is clear that glycogen is not a required precursor for fatty acid synthesis. These results also suggest that the liver can be an important site of fatty acid synthesis. At 10min. after the [U-$^{14}$C]glucose meal, approx. 20% of the total labelled fatty acid was found in the liver. At this early time-period no labelled fat would be transported to the liver from the carcass. At 60min. after the meal more than 10% of the labelled fatty acids were still found in the liver. The conclusion that the liver can be an important site of fatty acid synthesis is at variance with that of Favarger & Gerlach (1958). These workers concluded that the incorporation of glucose into hepatic fatty acids was of relatively little importance and that hepatic metabolism is not oriented towards lipid synthesis. However, our results are consistent with those of Windmueller (1964), who found 12% of the total labelled fatty acid in the liver 8hr. after rats were given tritiated water.

After 8hr. in the metabolism chamber under starvation conditions, approx. 9% of the administered label given was still to be found in the fatty acids of liver and carcass combined (Table 2). The results in Table 3 suggest that after starvation for 8hr. the label left in liver glycogen would be negligible. Since 81% of the $^{14}$C given was recovered as $^{14}CO_2$ and fatty acids, the remaining 19% were probably present as glyceride glycerol (and derivatives), carcass glycogen (primarily muscle), glycolytic and oxidative intermediates and amino acids. Under the conditions of ingestion of 250mg. of [$^{14}$C]glucose, 9 times as much label was found in

fatty acid as in glyceride glycerol. This is not far from the distribution of carbon between these two moieties and may indicate an integrated control mechanism for directing the metabolism of glucose to both fatty acids and the L-α-glycerophosphate necessary for their esterification. This picture is different from the results obtained in experiments *in vitro* with both rat and human adipose tissue (Jeanrenaud, 1961; Hirsch & Goldrick, 1964). In these studies more of the glucose taken up by adipose tissue was converted into glyceride glycerol than into glyceride fatty acid.

The decrease in lipogenesis resulting from feeding with fat is well known (Masoro, 1962). Hill, Linazasoro, Chevallier & Chaikoff (1958) showed a measurable decrease in the conversion *in vitro* of acetate into fatty acid in liver with as little as 2·5% of corn oil in the diet. They also showed that this inhibition in hepatic lipogenesis takes place as early as 1hr. after a single dose of corn oil (Hill, Webster, Linazasoro & Chaikoff, 1960). The point of inhibition in fatty acid synthesis caused by feeding with fat has been suggested by Bortz, Abraham & Chaikoff (1963) to be the step at which acetyl-CoA is carboxylated under the influence of acetyl-CoA carboxylase. The effect of dietary fat on lipogenesis in adipose tissue has not been as widely studied. Hausberger & Milstein (1955) showed a decrease in the conversion *in vitro* of [$^{14}$C]glucose into fatty acids in adipose tissue of rats fed on diets containing 13–60% of fat. Di Giorgio, Bonanno & Hegsted (1962), by measuring carbon dioxide evolution from epididymal fat pads *in vitro*, found lower lipogenesis on diets containing 25% as compared with 5% of fat. They also observed differences in the magnitude of the effect depending on the type of dietary fat given. As pointed out above, the results in Table 5 show that lipogenesis in liver is depressed to a much greater extent by dietary fat than is lipogenesis in adipose tissue. The fact that fatty acid synthesis in adipose tissue continued at a good rate in spite of a relatively high fat intake may contribute to the obesity that develops in rats given high-fat diets (Mickelsen, Takahashi & Craig, 1955).

Our results on synthesis and turnover of fatty acids labelled *in vivo* from [$^{14}$C]glucose demonstrate that liver fatty acids both gain and lose the label more rapidly than fatty acids in the carcass; in short, they confirm that liver fat turns over more rapidly than carcass fat (Stetten & Grail, 1943). Starvation resulted in essentially complete mobilization of fat from the epididymal fat pads in 3 days. However, under non-starvation conditions the difference in turnover between fatty acids in the epididymal fat and the remainder of the extrahepatic fat is noteworthy. Reed, Yamaguchi, Anderson & Mendel (1930), in making a study of factors affecting the distribution of adipose tissue in the rat, found that in the male epididymal fat increased from 4 to 12% of total body fat as the rats increased from 25 to 92 days of age. We found that epididymal fat increased from 10 to 14% of the total body fat during the 28-day turnover experiment. Perhaps this increase is related in some way to the sexual maturing of the animals and, if so, is accomplished more through decreased lipolysis in epididymal fat than increased lipogenesis. Gorin & Shafrir (1963), from lipolysis studies *in vitro*, have estimated that the fatty acids of rat epididymal fat pads have a half-life of 141 days. Our results suggest that this slow turnover of epididymal fat may not be typical of the rest of the extrahepatic fatty acids.

The decrease in fatty acid synthesis in both liver and extrahepatic sites as a result of starvation is well known (Masoro, 1962; Fritz, 1961). Our results (Table 9) show that, whether [U-$^{14}$C]glucose was given as a tracer dose or as a 250mg. meal, incorporation of $^{14}$C into glyceride fatty acid was depressed more than that into glyceride glycerol. Also, the percentage of the $^{14}$C label in adipose tissue present as fat decreased after starvation. In both these effects, food deprivation gave results similar to those found when high doses of dietary fat were given. Bortz & Lynen (1963*a*) have shown that acetyl-CoA carboxylase is inhibited by long-chain acyl-CoA derivatives and that the concentration of such derivatives is elevated in the livers of starved rats (Bortz & Lynen, 1963*b*). Our results are consistent with this mechanism, but they appear to be at variance with the concept that in starvation there is a deficiency of L-α-glycerophosphate for the esterification of newly synthesized fatty acids (Tzur, Tal & Shapiro, 1964). More results on the effects of starvation and re-feeding on fatty acid and cholesterol synthesis *in vivo* are presented in the next paper (Jansen, Zanetti & Hutchison, 1966).

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