# Studies on Lipogenesis *in vivo*

## EFFECTS OF STARVATION AND RE-FEEDING, AND STUDIES ON CHOLESTEROL SYNTHESIS

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1. Studies *in vivo* have been carried out on hepatic and extrahepatic cholesterol synthesis and also on the effects of starvation and re-feeding on both cholesterol and fatty acid synthesis. 2. In rats and mice fed on a stock diet, extrahepatic tissues accounted for about 4 times as much newly synthesized cholesterol as did the liver. The liver appeared to be somewhat more important in the rat than the mouse. Feeding with cholesterol greatly decreased and cholestyramine greatly increased hepatic cholesterol synthesis without much effect on extrahepatic synthesis. 3. Mice starved for up to 7hr. did not lose any of the ability to convert a [U-$^{14}$C]glucose meal into fat, whereas 18hr. of starvation resulted in an 80% loss of fatty acid synthesis in liver and carcass, an 80% loss in liver cholesterol synthesis and a 65% decrease in carcass cholesterol synthesis; 18hr. of food deprivation also decreased the proportion of counts in epididymal fat pads present as fat and increased the proportion present as glyceride glycerol. 4. Re-feeding for up to 7hr. restored fatty acid synthesis from a [U-$^{14}$C]glucose meal to about 50% of the values for non-starved mice but had no effect on hepatic cholesterol synthesis. The altered distribution of counts in the epididymal fat pads caused by starvation was restored to normal after feeding for 1hr.

In the preceding paper (Jansen, Hutchison & Zanetti, 1966), it was reported that starvation caused a decrease in fatty acid synthesis from [U-$^{14}$C]glucose in mice *in vivo*. A decrease in lipogenesis in the starved animal, particularly in the liver, has been established, as has been the repair of lipogenesis associated with re-feeding (Fritz, 1961; Masoro, 1962). However, much remains to be learned about the dynamics of these processes and how they are regulated *in vivo*. Similarly, the inhibition in cholesterol synthesis caused by prolonged starvation is well known (Van Bruggen, Hutchens, Claycomb, Cathey & West, 1952). The dominant role of the liver in cholesterol synthesis appears to be accepted by many workers in the field today (Kritchevsky, 1958), although the evidence is not conclusive. The capacity of extrahepatic tissues to synthesize cholesterol *in vitro* has been amply documented (Kritchevsky, 1958; Cook, 1958). Studies *in vivo* with [$^{14}$C]acetate in rats have shown that more than three-quarters of all the label present as cholesterol in short-term experiments was found outside the liver (Cockburn & Van Bruggen, 1959). Taylor, Patton, Yogi & Cox (1960), from experiments in which hepatic cholesterol synthesis was suppressed by dietary cholesterol, have estimated that 65–75% of serum cholesterol in humans is contributed by tissues other than liver. This estimate was based on the assumption that hepatic cholesterol synthesis was completely suppressed by feeding with cholesterol.

We have studied cholesterol synthesis from [U-$^{14}$C]glucose and [1-$^{14}$C]acetate *in vivo* in rats and from [U-$^{14}$C]glucose in mice, and have found extrahepatic cholesterol synthesis to be dominant in both species, particularly the mouse. The time-sequence of changes in fatty acid, triglyceride and cholesterol synthesis during starvation and re-feeding are also presented.

## MATERIALS AND METHODS

Young adult male mice from the Merck, Sharp and Dohme colony, derived from the Institute of Cancer Research (ICR) strain, were maintained on a purified low-fat–high-glucose diet (diet 2 in Table 1 of Jansen *et al.* 1966) for 1–2 weeks before each experiment unless otherwise noted. At the time they were used, the mice weighed 20–35g., but within individual experiments the weight range was kept less than 4g. For the rat experiments, 90–125g. male Charles River CD rats maintained on Purina Laboratory Chow were used. In all experiments, the 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°. In one experiment, cholesterol or cholestyramine (Tennent

*et al.* 1960) was added to the diet in the amounts shown in Table 4 and fed to male mice weighing approx. 20g. for 7 days. At this time each mouse was given 250mg. of [U-$^{14}$C]glucose (2·5$\mu$c) orally and killed 60 min. later. The starvation–re-feeding experiment was carried out as follows. The mice were divided into 12 groups (eight mice/group) the day before the experiment. Six groups were fed overnight and six starved for 18hr. The animals fed overnight were starved for periods of 1–7hr. as shown in Table 5. Similarly, animals starved overnight were re-fed the 70%-glucose diet for 1–7hr. At the appropriate time, as shown in Table 5, food was removed from the cage (if present) and each mouse given 250mg. of [U-$^{14}$C]glucose (2·5$\mu$c) orally and the $^{14}$C incorporation determined 60min. later.

The techniques used in dosing, bleeding, preparing and analysing tissues and assaying for radioactivity were those described by Jansen *et al.* (1966). The [U-$^{14}$C]glucose (16mc/m-mole) and [1-$^{14}$C]acetate (> 25mc/m-mole) were obtained from New England Nuclear Corp., Boston, Mass., U.S.A.

## RESULTS

*Synthesis of cholesterol in liver and extrahepatic tissues.* In the first experiment the incorporation into fatty acids and cholesterol 60 min. after administration of a [U-$^{14}$C]glucose meal given to rats or mice was determined. The rats were each given 1000mg. of [U-$^{14}$C]glucose (10$\mu$c) and the mice 250mg. of [U-$^{14}$C]glucose (2·5$\mu$c) orally. For this experiment both species had been maintained on Purina Laboratory Chow. As shown in Table 1, twice as much $^{14}$C was incorporated into fatty acids in both livers and extrahepatic tissues in mice as in rats in spite of receiving only one-fourth as much [U-$^{14}$C]glucose. The specific activities of fatty acids in both liver and carcass in mice at 60min. after administration of [U-$^{14}$C]glucose were 4–6 times those in rats at a similar time-interval. However, of the total of newly synthesized fatty acids a similar percentage was found in the liver in both species, namely 6–7%. The point of importance is the proportion, not the absolute amount, of newly synthesized fatty acid or cholesterol found in the liver. When the diet was Purina Laboratory Chow, 14 and 25% of the total labelled cholesterol in the body was found in the livers of mice and rats respectively. In Table 2, the labelling in cholesterol and fatty acids in rats after a meal of [U-$^{14}$C]glucose is compared with that resulting from a subcutaneous dose of a trace amount of [1-$^{14}$C]acetate. As expected, the labelling from acetate was considerably higher than that from glucose. Nevertheless, the proportion of the newly synthesized cholesterol found outside the liver was closely similar for either precursor. In this experiment, a second group of rats was killed at 30min. after the [U-$^{14}$C]glucose meal. In Table 3, labelling of cholesterol in plasma, liver and carcass are shown for time-intervals of 30 and 60min. after the meal. The increase in [$^{14}$C]cholesterol as the time after administration of

Table 1. *Cholesterol and fatty acid synthesis from [U-$^{14}$C]glucose in rats and mice*

Experimental details are given in the text. The animals had been maintained on Purina Laboratory Chow. [U-$^{14}$C]-Glucose was given orally [1000mg. (10$\mu$c)/100g. body wt.] and the animals were killed 60min. later. The average body weight of the rats was 100g. (93–107g.) and that of the mice was 25g. (23–27g.) with eight mice or six rats/group. Food was removed from the cages during the interval between dosing and killing.

| | Mice | Rats |
|---|---|---|
| Liver fatty acid | | |
| %* | 2·81 | 2·81 |
| Counts/min./g. of liver | 4680 | 1200 |
| Counts/min./liver | 9520 | 5610 |
| Counts/min./100mg. of fatty acid | 16700 | 4250 |
| Carcass fatty acid | | |
| %† | 5·37 | 3·36 |
| Counts/min./g. of carcass | 6640 | 752 |
| Counts/min./carcass | 162000 | 71500 |
| Counts/min./100mg. of fatty acid | 12400 | 2110 |
| % of fatty acid $^{14}$C in liver | 6 | 7 |
| Liver cholesterol | | |
| %* | 0·21 | 0·23 |
| Counts/min./g. of liver | 140 | 158 |
| Counts/min./liver | 285 | 738 |
| Counts/min./100mg. of cholesterol | 6670 | 6740 |
| Carcass cholesterol | | |
| %† | 0·28 | 0·21 |
| Counts/min./g. of carcass | 72 | 23 |
| Counts/min./carcass | 1760 | 2220 |
| Counts/min./100mg. of cholesterol | 2570 | 1060 |
| % of cholesterol $^{14}$C in liver | 14 | 25 |

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

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

Table 2. *Comparison of lipogenesis from [1-$^{14}$C]acetate and [U-$^{14}$C]glucose in rats*

Experimental details are given in the text. The rats (six/group), with an average weight of 107g. (90–125g.), had been maintained on Purina Laboratory Chow. [U-$^{14}$C]-Glucose (1000mg.) was given to each rat orally or a tracer dose of [1-$^{14}$C]acetate (> 25mc/m-mole) was given subcutaneously. In either case the $^{14}$C dose was 10$\mu$c and the rats were killed 60min. after dosing. Food was withheld for the 1hr. after dosing.

| | [U-$^{14}$C]Glucose meal | Trace of [1-$^{14}$C]acetate |
|---|---|---|
| Cholesterol | | |
| Counts/min./liver | 739 | 20500 |
| Counts/min./carcass | 1880 | 58000 |
| % of label in liver | 28 | 26 |
| Fatty acid | | |
| Counts/min./liver | 3490 | 48400 |
| Counts/min./carcass | 70500 | 1660000 |
| % of label in liver | 5 | 3 |

Table 3. *Cholesterol synthesis and transport in rats*

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

| | At 30min. | At 60min. |
|---|---|---|
| Plasma | | |
| Cholesterol | | |
| mg./100ml. | 58 | 58 |
| Counts/min./100mg. of cholesterol | 603 | 1000 |
| Liver | | |
| Cholesterol | | |
| %* | 0·23 | 0·21 |
| Counts/min./liver | 266 | 739 |
| Counts/min./100mg. of cholesterol | 2390 | 7330 |
| Carcass | | |
| Cholesterol | | |
| %† | 0·23 | 0·22 |
| Counts/min./carcass | 660 | 1880 |
| Counts/min./100mg. of cholesterol | 283 | 830 |
| % of cholesterol $^{14}$C in liver | 29 | 28 |

* Wt. (g.) of cholesterol (after saponification)/100g. fresh wt. of liver.

† Wt. (g.) of cholesterol (after saponification)/100g. (body wt.−liver wt.).

[U-$^{14}$C]glucose was increased from 30 to 60min. was similar in liver to that in the extrahepatic tissues. At both time-intervals the order of specific activities for the [$^{14}$C]cholesterol was: liver > plasma > carcass.

*Effect of feeding with cholesterol or cholestyramine.* The above results made it appear possible that some cholesterol synthesized in the liver was transported in the plasma and deposited in extrahepatic tissues. As discussed below, an estimate of the dilution of such newly synthesized cholesterol with unlabelled cholesterol suggests that most of the labelled cholesterol present in the extrahepatic tissues must have been synthesized *in situ*. The results of the following experiment strongly support this contention. Groups of mice were fed for 7 days on the unsupplemented 70%-glucose diet or this diet with either 1% of cholesterol or 1% of cholestyramine added. Cholestyramine is an anion-exchange resin that has been shown to bind bile acids in the intestine, thus increasing their excretion (Tennent *et al.* 1960). The [U-$^{14}$C]glucose was administered to some of the animals as a 250mg. meal given by stomach tube, with the animals killed 60min. later. The [U-$^{14}$C]glucose was added to the diet of other mice for the last 24hr. of the feeding period at a dose level of 0·2$\mu$c/g. of diet. There were no significant differences in food consumption or weight gain

Table 4. *Effect of dietary cholesterol or cholestyramine on cholesterol and fatty acid synthesis in mice*

Experimental details are given in the text. Cholesterol or cholestyramine was added to the basal diet (diet 2 in Table 1 of Jansen *et al.* 1966) at 1% and fed to groups of mice (eight/group) for 7 days. In part A, each mouse was given 250mg. of [U-$^{14}$C]glucose (2·5$\mu$c) orally and killed 60min. later. In part B [U-$^{14}$C]-glucose was added to the diet for the last 24hr. of the feeding period at 0·2$\mu$c/g. of diet. In part B, the average dose of [U-$^{14}$C]glucose was 1$\mu$c with no significant differences in food consumption observed among the three groups.

| Diet | Liver cholesterol | | Carcass cholesterol | | Liver fatty acid | | Carcass fatty acid | |
|---|---|---|---|---|---|---|---|---|
| | (%*) | (counts/min./liver) | (%†) | (counts/min./mouse) | (%*) | (counts/min./liver) | (%†) | (counts/min./mouse) |
| A: [$^{14}$C]glucose given as 250mg. meal | | | | | | | | |
| Basal | 0·34 | 134 | 0·24 | 1260 | 4·0 | 26400 | 8·1 | 322000 |
| Basal+1% of cholesterol | 0·94 | 43 | 0·30 | 1360 | 6·4 | 33000 | 8·9 | 333000 |
| Basal+1% of cholestyramine | 0·46 | 918 | 0·27 | 1810 | 4·4 | 21600 | 6·8 | 336000 |
| B: [$^{14}$C]glucose added to diet | | | | | | | | |
| Basal | 0·48 | 144 | 0·26 | 1260 | 5·3 | 18200 | 8·6 | 235000 |
| Basal+1% of cholesterol | 0·95 | 48 | 0·32 | 1210 | 5·2 | 12500 | 8·4 | 183000 |
| Basal+1% of cholestyramine | 0·47 | 418 | 0·24 | 1590 | 5·0 | 13400 | 7·5 | 179000 |

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

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

Table 5. *Effects of starvation and re-feeding on lipogenesis in epididymal fat in mice*

Experimental details are given in the text. Groups 1–6, previously given diet 2 (Table 1 of Jansen *et al.* 1966), were starved 0–7 hr. as shown below. At the appropriate times the mice (eight/group) were each given 250 mg. of [U-$^{14}$C]glucose (2·5 $\mu$c) orally and killed 60 min. later. Groups 7–12, previously given diet 2 (Table 1 of Jansen *et al.* 1966), were starved overnight (18 hr.) and then re-fed on the same diet for 0–7 hr. At the appropriate times the mice (eight/group) were each given 250 mg. of [U-$^{14}$C]glucose (2·5 $\mu$c) orally and killed 60 min. later. Food was removed from the cage at time of dosing. In this experiment carcass refers to the body with both liver and epididymal fat pads removed. Where appropriate, results are given as means ± S.E.M.

| Group | Time starved (hr.) | Wt. of pads (mg.) | Incorporation (counts/min./pad) (not washed) | Incorporation (counts/min./pad) (washed*) | Incorporation (% of pad $^{14}$C as fat) | Incorporation (% of fat $^{14}$C as fatty acid) | Incorporation (% of fat $^{14}$C as glycerol) |
|---|---|---|---|---|---|---|---|
| 1 | 0 | 342 ± 31 | 8240 ± 590 | 7290 ± 500 | 88·8 ± 1·3 | 90·5 ± 0·5 | 9·5 |
| 2 | 1 | 377 ± 60 | 11830 ± 1410 | 10680 ± 1310 | 90·3 ± 1·2 | 89·0 ± 1·2 | 11·0 |
| 3 | 2 | 314 ± 18 | 7320 ± 1040 | 6410 ± 1060 | 85·6 ± 2·3 | 87·3 ± 1·0 | 12·7 |
| 4 | 3 | 318 ± 29 | 10420 ± 1170 | 9210 ± 1050 | 88·1 ± 1·5 | 89·6 ± 1·2 | 10·4 |
| 5 | 5 | 356 ± 34 | 9500 ± 580 | 8260 ± 510 | 87·0 ± 0·8 | 91·2 ± 0·7 | 8·6 |
| 6 | 7 | 309 ± 32 | 8100 ± 1060 | 7070 ± 940 | 87·0 ± 1·1 | 90·4 ± 0·7 | 9·6 |
| 7 | 18 | 194 ± 27 | 1490 ± 210 | 1010 ± 180 | 66·3 ± 4·0 | 61·5 ± 5·4 | 38·5 |
| | Time re-fed (hr.) | | | | | | |
| 7 | 0 | 194 ± 27 | 1490 ± 210 | 1010 ± 180 | 66·3 ± 4·0 | 61·5 ± 5·4 | 38·5 |
| 8 | 1 | 173 ± 28 | 2570 ± 390 | 2220 ± 350 | 86·3 ± 1·8 | 88·6 ± 1·4 | 11·4 |
| 9 | 2 | 212 ± 38 | 3030 ± 430 | 2610 ± 390 | 86·3 ± 1·4 | 88·5 ± 1·7 | 11·5 |
| 10 | 3 | 196 ± 37 | 4910 ± 680 | 4460 ± 640 | 90·4 ± 1·0 | 90·1 ± 2·2 | 9·9 |
| 11 | 5 | 174 ± 14 | 4140 ± 1000 | 3770 ± 960 | 89·4 ± 1·9 | 89·0 ± 2·5 | 11·0 |
| 12 | 7 | 218 ± 25 | 4140 ± 770 | 3650 ± 710 | 87·2 ± 1·0 | 88·3 ± 1·7 | 11·7 |

* Hexane extract of fat pads washed with water.

among the groups and the average intake of $^{14}$C was 1 $\mu$c/mouse. The results are shown in Table 4. Feeding with cholesterol resulted in an average decrease in incorporation of $^{14}$C into liver cholesterol of 68% whether the [$^{14}$C]glucose was given in a 250 mg. meal or given in the diet over a 24 hr. period. In contrast with these results, incorporation of $^{14}$C into cholesterol in extrahepatic tissues was not affected by feeding with cholesterol. Under these latter conditions 96–97% of the total of newly synthesized cholesterol was found outside the liver. Feeding with cholestyramine resulted in a sevenfold increase in incorporation into liver cholesterol from the meal and a threefold increase when the [$^{14}$C]glucose was added to the diet. An increased hepatic cholesterol synthesis rate during feeding with cholestyramine has been shown by Huff, Gilfillan & Hunt (1963). The fact that the increase was less when the labelled glucose was given over a 24 hr. period would be expected from the greater cholesterol turnover rate during feeding with cholestyramine. The increase in [$^{14}$C]cholesterol content of extrahepatic tissues during cholestyramine treatment is relatively small and it is not possible on the basis of this experiment to say what proportion of the increase was caused by an increased synthesis rate and how much as a result of transfer of labelled cholesterol from the liver.

Feeding with cholesterol or cholestyramine did not cause any appreciable changes in incorporation of [U-$^{14}$C]glucose into fatty acids in either liver or extrahepatic tissues (Table 4).

*Effects of starvation and re-feeding.* The results of an experiment in which starvation and re-feeding have been studied *in vivo* are shown in Tables 5 and 6. At the appropriate times, as listed in the Tables, each mouse was given a meal of [U-$^{14}$C]glucose by stomach tube (eight mice/group), and uptake and conversion into triglycerides in adipose tissue and conversion into fatty acids and cholesterol in liver and carcass were determined at 60 min. after the [U-$^{14}$C]glucose was given. Starvation for 18 hr. resulted in a 45% decrease in fat-pad weight (Table 5) and more than an 80% decrease in uptake of [$^{14}$C]glucose by the epididymal fat pad (group 7 versus group 1). In the starved mice, only 66% of the counts present in the fat pads were present as fat and 38% of these essentially triglyceride counts were present as glyceride glycerol. In non-starved mice the corresponding values were 89 and 10% respectively. The 18 hr. starvation period decreased incorporation into both liver cholesterol and liver fatty acid (total after saponification) by approx. 80% (Table 6). During this overnight starvation, the liver fatty acids increased from 4·07 to 6·71% and liver cholesterol from 0·41 to 0·51%. The effect of starvation on incorporation of [U-$^{14}$C]glucose into

Table 6. *Effect of starvation and re-feeding on lipogenesis in liver and carcass in mice*

Experimental details are given in the text and in Table 5. Where appropriate, results are given as means ± S.E.M.

| Group | Time starved (hr.) | Liver fatty acid (%*) | Liver fatty acid (counts/min./g. of liver) | Liver fatty acid (counts/min./ liver) | Liver cholesterol (%*) | Liver cholesterol (counts/min./g. of liver) | Liver cholesterol (counts/min./ liver) | Carcass fatty acid (counts/min.) | Carcass cholesterol (counts/min.) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 4·07 ± 0·31 | 21200 ± 1900 | 46200 | 0·41 ± 0·04 | 94 ± 35 | 205 | 369000 ± 21000 | 1510 ± 110 |
| 2 | 1 | 5·08 ± 0·49 | 21500 ± 2600 | 46500 | 0·42 ± 0·05 | 66 ± 12 | 143 | 432000 ± 42000 | 1690 ± 210 |
| 3 | 2 | 4·73 ± 0·21 | 24300 ± 3130 | 52900 | 0·37 ± 0·03 | 73 ± 15 | 158 | 308000 ± 29000 | 1760 ± 120 |
| 4 | 3 | 5·52 ± 0·60 | 23700 ± 2560 | 53000 | 0·39 ± 0·04 | 75 ± 11 | 168 | 420000 ± 66000 | 1840 ± 150 |
| 5 | 5 | 6·20 ± 0·45 | 26400 ± 2700 | 53800 | 0·37 ± 0·03 | 50 ± 11 | 102 | 434000 ± 38000 | 1800 ± 170 |
| 6 | 7 | 5·63 ± 0·40 | 28100 ± 4300 | 52700 | 0·38 ± 0·04 | 80 ± 35 | 150 | 330000 ± 33000 | 1810 ± 70 |
| 7 | 18 | 6·71 ± 0·77 | 3510 ± 640 | 5340 | 0·51 ± 0·06 | 20 ± 12 | 32 | 80300 ± 8000 | 538 ± 20 |
| | Time re-fed (hr.) | | | | | | | | |
| 7 | 0 | 6·71 ± 0·77 | 3510 ± 640 | 5340 | 0·51 ± 0·06 | 20 ± 12 | 32 | 80300 ± 8000 | 538 ± 20 |
| 8 | 1 | 6·37 ± 0·90 | 8930 ± 1660 | 15000 | 0·60 ± 0·06 | 27 ± 11 | 45 | 112000 ± 6000 | 547 ± 55 |
| 9 | 2 | 7·09 ± 1·07 | 7540 ± 1310 | 12900 | 0·54 ± 0·03 | 9 ± 8 | 15 | 134000 ± 10000 | 560 ± 52 |
| 10 | 3 | 6·10 ± 1·07 | 5580 ± 640 | 9940 | 0·45 ± 0·02 | 27 ± 15 | 48 | 186000 ± 21000 | 813 ± 100 |
| 11 | 5 | 4·39 ± 0·64 | 5160 ± 1230 | 9740 | 0·40 ± 0·04 | 17 ± 7 | 32 | 176000 ± 42000 | 772 ± 93 |
| 12 | 7 | 4·11 ± 0·35 | 8600 ± 1030 | 17900 | 0·43 ± 0·03 | 21 ± 11 | 44 | 174000 ± 33000 | 735 ± 90 |

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

fatty acids and cholesterol in extrahepatic tissues (exclusive of epididymal fat) is also shown in Table 6. Incorporation into fatty acids and cholesterol was depressed by 80 and 65% respectively by 18hr. of starvation. Our results on the decrease in liver of fatty acid synthesis from [U-$^{14}$C]glucose *in vivo* after 18hr. of starvation are similar to the reported decrease *in vitro* after 24hr. of starvation in rats (Masoro, Chaikoff, Chernick & Felts, 1950).

In spite of these rather large decreases in the ability to convert a [$^{14}$C]glucose meal into fat, 7hr. of food deprivation did not decrease either the uptake or distribution of counts in adipose tissue (Table 5), nor did it result in a diminution in the conversion of the meal of [U-$^{14}$C]glucose into fatty acid in either liver or extrahepatic tissues (group 6 versus group 1). The 7hr. starvation period did not change the percentage of liver cholesterol although the percentage of fat (as fatty acid) increased from 4·07 to 5·63% in the liver.

The effects of re-feeding on uptake and distribution of counts in the epididymal fat pads of mice are shown in Table 5. The diet used for re-feeding was the same 70%-glucose diet used before starvation. Re-feeding for 7hr. restored glucose uptake and conversion into fat by the fat pad to approximately half that for non-starved mice (group 12 versus group 1). All of this increase took place in the first 3hr. of re-feeding. The altered distribution of counts in the pads of the mice starved for 18hr., i.e. the proportion of total pad counts present as lipid and the proportion of these lipid counts present as glyceride glycerol, was restored to normal with only 1hr. of re-feeding. As shown in Table 6, 7hr. of re-feeding restored fatty acid synthesis in the liver to about 40% of the non-starved control value, with all of the increase taking place within the first hour of re-feeding (group 12 versus group 1). The effect of re-feeding on carcass fatty acid synthesis closely paralleled that found for the epididymal fat pad with the synthesis rate after 7hr. of re-feeding again only half that on non-starved mice. All of this increase took place in the first 3hr. of re-feeding. However, 18–24hr. of re-feeding is required for a complete recovery in lipogenetic capacity (G. R. Jansen, M. E. Zanetti & C. F. Hutchison, unpublished work).

The effects of re-feeding on cholesterol synthesis are also shown in Table 6. Re-feeding for up to 7hr. did not increase hepatic cholesterol synthesis. Outside the liver, the cholesterol synthesis rate was increased by about 40% by feeding for 3–7hr., again with all the increase taking place within the first 3hr. of re-feeding. Here also the values after 7hr. of re-feeding are only half that observed for extrahepatic cholesterol synthesis in non-starved mice. In view of these results showing that after 7hr. of re-feeding the capacity for lipogenesis had only recovered to about 50% of normal, it is noteworthy that 7hr. of re-feeding restored both the percentage cholesterol and percentage total fatty acid in the liver to normal.

## DISCUSSION

The proportion of [$^{14}$C]cholesterol synthesized *in vivo* from [U-$^{14}$C]glucose and found in the liver of mice was similar whether the label was given as a 250mg. meal and the animals were killed 60min. later or fed *ad libitum* over a 24hr. feeding period. Also, the proportion synthesized from a tracer dose of [1-$^{14}$C]acetate and found in the liver of the rat was similar to that obtained with [U-$^{14}$C]glucose as precursor. These results suggest that approx. 85 and 70–75% of cholesterol newly synthesized from carbohydrate is made in extrahepatic tissues of mice and rats respectively. The results obtained with acetate may be criticized on the grounds that the specific activity of the [$^{14}$C]acetyl-CoA precursor was not known and might have been different in liver from that in other tissues because of differences in the dilution of the label with endogenous unlabelled acetate or acetyl-CoA, or a difference in the capacity to activate the acetate. In the 24hr. feeding experiment, an uncertain proportion of the [$^{14}$C]cholesterol found outside the liver was synthesized in the liver and transported to extrahepatic sites. These objections, however, have less force in the experiment where the [$^{14}$C]glucose was given as a 250mg. meal. The concentrations and specific activities of plasma glucose for 60min. after the meal have been presented by Jansen *et al.* (1966). Under these conditions, where lipolysis, glycogenolysis and fatty acid degradation would be suppressed, it is a reasonable supposition that the specific activity of the [$^{14}$C]acetyl-CoA would be related to that of the [U-$^{14}$C]glucose in plasma and would be comparable in extrahepatic tissues and the liver. There remains the question how much [$^{14}$C]cholesterol synthesized in the liver could be transported in the plasma to extrahepatic tissues in the 60min. after dosing and before death. Several considerations suggest that this would represent a minor fraction of the total. First, the specific activity of the [$^{14}$C]glucose in the plasma was about 1000 times that of circulating [$^{14}$C]cholesterol. Secondly, because of the large amount of unlabelled cholesterol outside the liver, plasma [$^{14}$C]cholesterol would of necessity be diluted to a specific activity much less than that actually found for extrahepatic cholesterol. Finally, the experiment involving feeding with cholesterol and cholestyramine provides the strongest supporting evidence for the important role of extrahepatic tissues in cholesterol synthesis. Feeding with 1% of cholesterol in the diet depressed liver cholesterol synthesis by 68% but did not affect carcass cholesterol

synthesis at all. The lack of effect of feeding with cholesterol on synthesis of cholesterol outside the liver has been shown by Gould, Taylor, Hagerman, Warner & Campbell (1953). Cholestyramine in the diet increased the liver cholesterol synthesis rate by 700%, but the [$^{14}$C]cholesterol content of extrahepatic tissues only increased by 50%. These results strongly suggest that in rats and mice 70–85% of newly synthesized cholesterol is synthesized in extrahepatic tissues. An experiment reported by Morris, Chaikoff, Felts, Abraham & Fansah (1957) had shown that in rats fed on a 2%-cholesterol diet as much as 25% of the serum cholesterol could be contributed by endogenous synthesis. However, on the basis of this work, it is not possible to delineate the relative roles of liver and extrahepatic tissues in cholesterol synthesis.

Starvation for 18hr. in mice did not depress extrahepatic cholesterol synthesis as much as synthesis in the liver. In this respect cholesterol synthesis differed from fatty acid synthesis where the decrease caused by starvation was similar in both compartments. In previous studies (Jansen *et al.* 1966) as well as those reported in this paper, fatty acid and cholesterol synthesis rates in both liver and extrahepatic tissues have been altered by various dietary conditions. In none was a reciprocal relationship between fatty acid and cholesterol synthesis observed, as has been suggested on the basis of studies *in vitro* (Foster & Bloom, 1963).

The fact that the ability to convert a glucose meal into fatty acids was not depressed by 7hr. of starvation is noteworthy: 7hr. in a species that can only survive for about 4 days without food is almost 10% of the total survival time. However, our results do not mean that under these conditions there was no decrease in the amount of fat synthesized in the absence of the glucose meal. A period of starvation such as this might be expected to decrease the plasma glucose concentration and hence insulin concentration and also increase the concentration of plasma free fatty acids. All of these changes would be expected to decrease the synthesis of fat in the absence of added glucose and insulin.

Mice, being nocturnal animals, consume perhaps three-quarters of their daily food at night. It is likely that starvation for 7 hr. at night would have a more pronounced effect than 7hr. during the day. At night, starvation would be exacerbated by a considerably higher level of energy expenditure due to increased locomotor activity which would make the caloric deficit more severe. The fact that starvation for 3–7hr. increased liver fatty acid by almost 50% suggests that even in the daytime such short periods of food deprivation cause a significant alteration in metabolism. However, we have not directly investigated the normal food consumption habits of mice feeding *ad libitum* through the day.

The decrease in lipogenesis caused by starvation has been ascribed variously to (*a*) alteration in carbohydrate metabolism, particularly the activity of the hexose monophosphate shunt (Masri, Lyon & Chaikoff, 1952), (*b*) inhibitory factors (Korchak & Masoro, 1963), (*c*) lack of stimulators (Catravas, 1963), (*d*) lack of $\alpha$-glycerophosphate receptor (Tzur, Tal & Shapiro, 1964), (*e*) loss of activity of citrate-cleavage enzyme (Abraham, Kopelovich & Chaikoff, 1964) and (*f*) loss of activity of acetyl-CoA carboxylase caused by high concentrations of fatty acyl-CoA derivatives (Bortz & Lynen, 1963*a*,*b*).

We have found that re-feeding for 7hr. after 18hr. of starvation resulted in the restoration of the lipogenetic capacity to only about half that found in non-starved mice. All of the increase took place in the first 1–3hr. of re-feeding with no additional increase occurring during the next 4hr. The altered distribution of counts in the epididymal fat pads was restored to normal within the first hour. It may be that the increases in lipogenetic capacity observed in the first 3hr. were related to regulation at the level of enzymic activity whereas further increases that require more than 7hr. of re-feeding (Tepperman & Tepperman, 1961) involve synthesis of enzyme protein, or cofactors such as the acyl-carrier protein described by Majerus, Alberts & Vagelos (1964) and Wakil, Pugh & Sauer (1964).

In the present experiments, biological variation has been minimized by keeping the starting weight of the mice for each experiment within a 4g. range. For example, in the starvation–re-feeding experiment the coefficient of variation for body weight for a non-starved group was 5%. The coefficient of variation for fat-pad weights for this group was 28%. In a group of eight mice with body weight within a 0·5g. range (coefficient of variation 0·8%) epididymal fat pads varied in weight from 50 to 106mg. (coefficient of variation 20%). The variance for fat-pad weights between animals was 5 times that found for the two separate pads in the same animal. We have also found that the incorporation of [U-$^{14}$C]glucose into the fat pads is usually closely similar for the two pads from the same animal. This variability undoubtedly has both genetic and environmental components. Nevertheless, these large differences in lipid metabolism among apparently similar animals maintained under carefully controlled conditions should also be held in mind in interpreting the results of experiments *in vitro* where the use of fewer animals, homogenates of pooled tissues and very precise biochemical tools tend to obscure the animal variation.

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