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# Studies in Lipogenesis *in vivo*

## FATTY ACID AND CHOLESTEROL SYNTHESIS DURING STARVATION AND RE-FEEDING

BY G. R. JANSEN, M. E. ZANETTI AND C. F. HUTCHISON
*Merck Institute for Therapeutic Research, Rahway, N.J. 07065, U.S.A.*

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1. Lipogenesis *in vivo* has been studied in mice given a 250mg. meal of [U-$^{14}$C]-glucose (2·5$\mu$c) or given an intraperitoneal injection of 25$\mu$g. of [U-$^{14}$C]glucose (2·0$\mu$c). 2. The ability to convert a [U-$^{14}$C]glucose meal into fatty acid was not significantly depressed by 6–7hr. of starvation. In contrast, incorporation of $^{14}$C into fatty acid in the liver after the intraperitoneal dose of [$^{14}$C]glucose was depressed by 80% and by more than 90% by 1 and 2hr. of starvation respectively. Carcass fatty acid synthesis from the [U-$^{14}$C]glucose meal was not depressed by 12hr. of starvation, whereas from the tracer dose of [U-$^{14}$C]glucose the depression in incorporation was 80% after 6hr. of starvation. 3. Re-feeding for 3 days, after 3 days' starvation, raised fatty acid synthesis and cholesterol synthesis in the liver fivefold and tenfold respectively above the levels in non-starved control mice. These increases were associated with an increased amount of both fatty acid and cholesterol in the liver. 4. After 18hr. of starvation incorporation of a [U-$^{14}$C]glucose meal into carcass and liver glycogen were both increased threefold.

The ability to convert a 250mg. meal of [U-$^{14}$C]-glucose into fatty acid has been shown to be depressed by approx. 80% in both the liver and extrahepatic tissues after 18hr. of starvation, whereas after 7hr. of food deprivation no decrease was observed (Jansen, Hutchison & Zanetti, 1966*a*; Jansen, Zanetti & Hutchison, 1966*b*). In the present study lipogenesis after such a meal is contrasted with lipogenesis after tracer doses of [U-$^{14}$C]glucose. As shown below, under the latter conditions fatty acid synthesis in the liver is depressed to a considerable extent by as little as 1hr. of food deprivation. In addition, the effect of refeeding on fatty acid and cholesterol formation from [U-$^{14}$C]glucose has been investigated.

## MATERIALS AND METHODS

Young adult male mice of the Merck, Sharp and Dohme colony (ICR strain) weighing approx. 20–35g. each were used in these studies. For each experiment the weight range of animals was less than 4g. The mice were maintained for 1–2 weeks on a purified diet containing 70% of glucose, 20% of casein and 1% of corn oil (diet 2; Jansen *et al.* 1966*a*). The techniques used in dosing and bleeding mice, preparing and analysing tissues for fatty acids, cholesterol and glycogen determination and measuring radioactivity were as described previously (Jansen *et al.* 1966*a*).

The conditions employed for the saponification of both mouse and rat carcasses are given in detail below. Pairs of mice were saponified by refluxing for 6hr. in 160ml. of 3N-KOH in 50% (v/v) ethanol after being kept overnight in half this volume of 6N-KOH. After refluxing, the digests were filtered and made up to 250ml. Rats weighing up to 140g. were saponified individually by refluxing for 6hr. in 300ml. of 3N-KOH in 50% (v/v) ethanol, again after standing overnight in 6N-KOH. These digests were made up to 500ml. For rats weighing 140–180g., 260ml. of ethanolic KOH was used and the reflux time prolonged to 9hr. Complete hydrolysis of body lipid to fatty acid occurs in 6 or 9hr. at ethanolic KOH/body weight ratios (v/w) 2 and 1·5 respectively.

For the isolation of carcass glycogen only, the following saponification procedure was used. At the appropriate times the mice were killed, decapitated and immediately eviscerated (liver, gastrointestinal tract, pancreas, spleen, kidneys, adrenal glands, testes and attached mesentery). The liver was saponified in 6N-KOH and glycogen was isolated as described previously (Jansen *et al.* 1966*a*). The remaining carcasses (muscle, subcutaneous fat, skin, heart, lungs and bone) were each placed in 20ml. of 6N-KOH, refluxed for 2hr. and filtered. Glycogen was isolated from these digests as described for the isolation of liver glycogen (Jansen *et al.* 1966*a*).

The [U-$^{14}$C]glucose was obtained from New England Nuclear Corp. (Boston, Mass., U.S.A.) and had a specific activity of 10–15mc/m-mole.

## RESULTS

*Effect of starvation and re-feeding on lipogenesis from* [$^{14}$C]*glucose.* In the first experiment groups of mice on their normal high-carbohydrate diet (diet 2;

Table 1. *Effect of starvation and re-feeding on cholesterol and fatty acid synthesis in mice*

Groups of eight mice were starved for the periods shown and then given either 250 mg. of [U-$^{14}$C]glucose (2·5 $\mu$c) orally or 25 $\mu$g. of [U-$^{14}$C]glucose (2 $\mu$c) intraperitoneally. Other groups were starved for 18 hr. and then re-fed with diet 2 (see the Materials and Methods section) for the periods shown before being fed or injected with the [U-$^{14}$C]glucose. The diet was removed from the cages at the time of dosing with [U-$^{14}$C]glucose. One hr. after giving the [U-$^{14}$C]glucose the animals were killed and the fatty acid and cholesterol fractions were isolated. Results are expressed as means ± S.E.M.

| | | Radioactivity (counts/min./g. of liver or carcass) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 250 mg. of [U-$^{14}$C]glucose orally | | | | 25 $\mu$g. of [U-$^{14}$C]glucose intraperitoneally | | | | |
| | | Cholesterol | | Fatty acid | | | Cholesterol | | Fatty acid | |
| Time Starved (hr.) | Group | Liver | Carcass | Liver | Carcass | Group | Liver | Carcass | Liver | Carcass |
| 0 | 1 | 58±22 | 50±4 | 20350±2530 | 7730±290 | 6 | 23±4 | 88±12 | 11360±2220 | 6340±1520 |
| 6 | 2 | 26±3 | 60±6 | 14570±1920 | 8910±690 | 7 | 4±1 | 96±6 | 520±50 | 1460±110 |
| 12 | 3 | 32±14 | 54±2 | 11600±1740 | 8060±580 | 8 | 5±1 | 44±8 | 320±31 | 760±66 |
| 18 | 4 | 2±1 | 28±2 | 2220±370 | 3850±350 | 9 | 2±1 | 26±2 | 250±14 | 610±67 |
| 24 | 5 | 4±1 | 24±2 | 4250±680 | 3030±540 | 10 | <1 | 20±2 | 230±15 | 460±33 |
| Time re-fed (hr.) | | | | | | | | | | |
| 0 | 11 | 5±1 | 24±2 | 2520±680 | 2430±520 | 16 | 3±1 | 16±2 | 250±9 | 520±20 |
| 6 | 12 | 13±2 | 26±4 | 6110±1180 | 5250±360 | 17 | 26±10 | 60±16 | 13650±9900 | 7160±1490 |
| 12 | 13 | 45±18 | 42±3 | 12450±1820 | 5640±500 | 18 | 28±4 | 70±2 | 9080±2440 | 8310±930 |
| 18 | 14 | 23±4 | 47±5 | 16590±1670 | 8620±1180 | 19 | 63±19 | 108±6 | 15530±4540 | 5790±1070 |
| 24 | 15 | 56±12 | 65±3 | 22070±3230 | 10220±1180 | 20 | 50±91 | 91±4 | 12940±4480 | 7250±820 |

Jansen *et al.* 1966*a*) were starved for various periods before being either given a meal of 250mg. of [U-$^{14}$C]glucose (2·5 $\mu$c) or injected intraperitoneally with 25 $\mu$g. of [U-$^{14}$C]glucose (2 $\mu$c). Other groups were starved for 18hr. and then re-fed with diet 2 before being given [U-$^{14}$C]glucose either orally or by injection.

As shown in Table 1 the ability to convert the [U-$^{14}$C]glucose meal into fatty acid outside the liver remained undiminished after 12hr. of starvation (group 3 versus group 1) and then declined by 50–70% after 18–24hr. of starvation (groups 4, 5 and 11 versus group 1). In liver, 50 and 80% decreases respectively occurred after 12hr. (group 3) and 18–24hr. of starvation (groups 4, 5 and 11). The 25% decrease at 6hr. was not statistically significant at $P$ 0·05 (analysis of variance; Snedecor, 1946). Recovery of full potential for fatty acid synthesis required 18–24hr. of re-feeding (groups 14 and 15 versus group 1).

Results showing the conversion of the [U-$^{14}$C]-glucose meal into cholesterol are also given in Table 1. Starvation for 6–12hr. caused a 50% decrease in conversion in the liver and 18–24hr. without food essentially eliminated hepatic cholesterol synthesis. Extrahepatic cholesterol synthesis appeared to be more resistant to the effects of starvation, with no fall after 12hr. of starvation. After 24hr. carcass cholesterol synthesis was decreased by 60%. In both sites, restoration of cholesterol synthesis to the level in non-starved mice required 12–24hr. of re-feeding.

When a tracer dose of [U-$^{14}$C]glucose was given intraperitoneally after various periods of starvation and re-feeding, the extent of incorporation of $^{14}$C into fatty acids and cholesterol was considerably different from that seen after the 250mg. meal. As shown in Table 1, incorporation of $^{14}$C into fatty acid was decreased by 95% in the liver and by almost 80% outside the liver by 6hr. of starvation (group 7 versus group 6). Incorporation of the tracer dose into liver cholesterol was virtually eliminated by 6hr. of starvation. Extrahepatic cholestrol synthesis was more resistant to the effects of starvation, with a 75% decrease after 18hr. without food (group 9 versus group 6). The ability to convert the tracer dose of [U-$^{14}$C]glucose into fatty acid and cholesterol, which was lost easily, was also regained rapidly, with complete restoration after re-feeding for 6hr. (group 17 versus group 6).

To explore these differences in response further both methods of administration were used to study the conversion of [U-$^{14}$C]glucose into fatty acid in the liver after periods of starvation of 1 to 7hr. In this experiment, as shown in Table 2, the ability of the liver to convert the [U-$^{14}$C]glucose meal into fatty acid was not depressed by 7hr. of starvation. In contrast, the incorporation of the tracer dose into fatty acid was depressed by 80% and by more than 90% after 1 and 2hr. of starvation respectively.

Table 2. *Effect of short periods of starvation on fatty acid synthesis in the livers of mice*

The experimental details were as for the starved groups of mice described in Table 1. Results are given as means ± S.E.M.

| | Radioactivity (counts/min./g. of liver) | |
|---|---|---|
| Time starved (hr.) | 250mg. of [U-$^{14}$C]glucose orally | 25 $\mu$g. of [U-$^{14}$C]glucose intraperitoneally |
| 0 | 16190±3200 | 14510±3540 |
| 1 | 16790±2970 | 2900±960 |
| 2 | 16490±1650 | 954±158 |
| 3 | 15420±2540 | 1040±410 |
| 5 | 15060±2500 | 416±40 |
| 7 | 15990±3730 | 409±46 |

Table 3. *Effect of short periods of starvation on plasma glucose concentrations in mice*

Groups of eight mice were starved for 1–6hr. during the day. At various times the animals were bled and the plasma glucose concentrations were determined. Control groups were bled throughout the day at times within 30min. of each experimental group. Results are given as means ± S.E.M.

| | Concn. of plasma glucose (mg./100ml.) | |
|---|---|---|
| Time starved (hr.) | Starved mice | Non-starved control mice |
| 1 | 120±6 | 137±11 |
| 2 | 116±4 | 141±8 |
| 3 | 117±4 | 154±15 |
| 4 | 131±5 | 143±11 |
| 6 | 148±6 | 179±16 |

We have previously reported that the concentration of glucose in the plasma of mice given the high-carbohydrate diet used in this study is depressed 50% by 18hr. of starvation (Jansen *et al.* 1966*a*). Changes in the concentration of plasma glucose during daytime starvation periods of up to 6hr. are shown in Table 3. In this experiment control groups were fed *ad libitum* and bled at the appropriate times during the day. The plasma glucose concentration was consistently lower in the starved groups with an average depression of approx. 20%. The variations in plasma glucose concentration shown by both groups could have been due to human activity in the laboratory, although an attempt was made to keep this to a minimum. Alternatively, they were perhaps related to normal diurnal changes independent of feeding. More data are required to

Table 4. *Effect of up to 72 hr. of starvation and re-feeding on lipogenesis in mice*

Groups of eight mice were starved for 0, 24, 48 or 72 hr. and re-fed for 0, 24, 48 or 72 hr. as indicated. The mice were then given 250 mg. of [U-$^{14}$C]glucose (2·5 $\mu$c) orally and killed 60 min. later. Food was removed from the cages at the time of dosing. All the groups were dosed between 9 a.m. and 10 a.m. Control groups fed with the same diet throughout were dosed at the same time as the test groups. Since there were no significant differences among the control groups, the results for these have been presented as averages. The average weight of the mice before starvation was 32·5 g. The results are given as means ± S.E.M.

| | | | | Liver fatty acid | | Liver cholesterol | | Carcass fatty acid | | Carcass cholesterol | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Group | Treatment | Body wt. (g.) | Liver wt. (g.) | (%*) | ($10^{-3}$× counts/min./liver) | (%*) | (counts/min./liver) | (%†) | ($10^{-3}$× counts/min./mouse) | (%†) | (counts/min./mouse) |
| 1 | Starved for 24 hr., not re-fed | 29·7±0·5 | 1·77±0·05 | 9·61±1·50 | 2·92±0·32 | 0·49±0·06 | <1 | 8·01±0·46 | 79±9 | 0·25±0·01 | 690±52 |
| 2 | Starved for 24 hr., re-fed for 24 hr. | 32·2±0·4 | 2·18±0·08 | 4·60±0·53 | 36·1±5·8 | 0·45±0·03 | 37±6 | 10·09±1·96 | 307±11 | 0·22±0·01 | 1140±140 |
| 3 | Starved for 24 hr., re-fed for 48 hr. | 33·7±0·4 | 2·26±0·10 | 4·72±0·47 | 58·9±9·9 | 0·40±0·04 | 86±22 | 11·14±1·57 | 403±20 | 0·22±0·01 | 1710±210 |
| 4 | Starved for 24 hr., re-fed for 72 hr. | 34·5±0·5 | 2·27±0·06 | 4·37±0·42 | 50·8±7·7 | 0·37±0·03 | 100±45 | 13·43±2·69 | 452±57 | 0·21±0·02 | 1310±100 |
| 5 | Starved for 48 hr., not re-fed | 27·3±0·4 | 1·55±0·07 | 8·68±1·44 | 0·97±0·06 | 0·54±0·04 | <1 | 7·92±1·25 | 35±2 | 0·26±0·01 | 285±9 |
| 6 | Starved for 48 hr., re-fed for 24 hr. | 29·2±0·6 | 2·10±0·05 | 4·16±0·51 | 46·5±5·4 | 0·37±0·02 | 73±15 | 6·00±0·79 | 165±8 | 0·25±0·01 | 1060±50 |
| 7 | Starved for 48 hr., re-fed for 48 hr. | 29·9±0·7 | 2·03±0·09 | 4·96±0·49 | 82·1±8·7 | 0·55±0·07 | 106±26 | 8·04±0·75 | 305±24 | 0·25±0·01 | 1720±80 |
| 8 | Starved for 48 hr., re-fed for 72 hr. | 31·6±0·5 | 2·11±0·03 | 5·59±0·34 | 63·8±7·2 | 0·52±0·04 | 42±8 | 9·24±1·12 | 298±13 | 0·23±0·01 | 1370±70 |
| 9 | Starved for 72 hr., not re-fed | 23·3±0·5 | 1·08±0·06 | 4·86±0·82 | 0·31±0·09 | 0·75±0·13 | <1 | 3·61±0·85 | 8·0±2·1 | 0·30±0·02 | 88±28 |
| 10 | Starved for 72 hr., re-fed for 24 hr. | 27·5±0·4 | 2·10±0·06 | 4·10±0·24 | 68·9±6·7 | 0·54±0·06 | 82±15 | 4·07±0·52 | 104±7 | 0·25±0·01 | 957±49 |
| 11 | Starved for 72 hr., re-fed for 48 hr. | 27·1±0·5 | 1·90±0·07 | 6·73±0·81 | 89·7±13·0 | 0·61±0·06 | 288±110 | 3·98±0·50 | 161±23 | 0·26±0·02 | 1350±60 |
| 12 | Starved for 72 hr., re-fed for 72 hr. | 30·2±0·7 | 2·70±0·10 | 10·08±0·51 | 171·4±19·6 | 0·63±0·03 | 536±86 | 6·94±0·58 | 236±28 | 0·24±00·1 | 1460±180 |
| | Controls | 34·8±0·6 | 2·30±0·09 | 4·30±0·44 | 35·4±5·8 | 0·39±0·04 | 45±16 | 11·1±1·4 | 310±22 | 0·21±0·01 | 1380±60 |

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

clarify this point. In any event, the changes occurred to a similar extent in both groups.

*Lipogenesis from [$^{14}$C]glucose during 3 days' starvation and 3 days' re-feeding.* To determine the effect of prolonged periods of starvation and re-feeding on the potential for fatty acid and cholesterol synthesis, groups of mice were starved for 0, 1, 2 or 3 days and then re-fed for 0, 1, 2 or 3 days. The diet before starvation, as well as the diet used in re-feeding, was diet 2 (Jansen *et al.* 1966*a*). The experiment was performed in such a way that the starvation periods all ended and the re-feeding periods all began on a Monday. On an appropriate day, as shown in Table 4, each mouse was given orally 250mg. of [U-$^{14}$C]glucose (2·5$\mu$c) and killed 60min. later. On each day a non-starved control group was also given [U-$^{14}$C]glucose.

As shown in Table 4, the capacity for fatty acid and cholesterol synthesis, which was profoundly reduced after 1 day's starvation, was lowered further as the duration of the starvation period was extended (groups 1, 5 and 9 versus control). Lipogenesis in the liver was decreased by starvation by a larger percentage than was lipogenesis in the carcass.

The severity of starvation periods of 1, 2 and 3 days in mice is indicated by the losses of 10, 17 and 30% of the original body weight respectively. The increase in total liver fatty acid after 1 and 2 days' starvation was presumably due to mobilization of fatty acids from the depots (Robinson, 1964). After 3 days' starvation, when very little mobilizable fat would be left, the liver fat content was not elevated.

The response to re-feeding of fatty acid and cholesterol synthesis in the liver was dramatically different from that in the remainder of the carcass. The more severe the starvation, the longer it took to restore the capacity for lipogenesis outside the liver to normal. Three days' re-feeding, after 3 days' starvation, did not restore extrahepatic fatty acid synthesis to normal (group 12 versus control). Extrahepatic cholesterol synthesis recovered more quickly and appeared normal after 2 days' re-feeding (group 11 versus control). In the carcass the percentage of total fatty acid was completely restored to normal in 1–2 days' re-feeding after 1 day's starvation (groups 3 and 4 versus control). However, after 3 days' starvation the loss in body fat stores was so great that 3 days' re-feeding was not able to replete these depots completely (group 12 versus control).

In the liver both fatty acid and cholesterol synthesis were completely restored in 24hr., even after 3 days' starvation. After 2 additional days' re-feeding, five- and ten-fold elevations were observed in the extent of incorporation of the [U-$^{14}$C]glucose meal into fatty acid and cholesterol respectively (groups 11 and 12 versus control). The twofold increase in the percentage of cholesterol and of total fatty acid in the liver at this time as compared with the non-starved controls was presumably the result of this enhanced lipogenesis.

The amounts of hepatic and extrahepatic cholesterol are listed in Table 5. The constancy of the extrahepatic cholesterol is striking. In the liver, little cholesterol was lost during food deprivation and the increased percentage of cholesterol (Table 4) was caused by the greatly decreased mass of liver tissue. However, the increased synthesis of cholesterol in the liver during re-feeding after 3 days'

Table 5. *Effect of starvation and re-feeding on the amounts of liver and extrahepatic cholesterol*

Experimental details are as given in Table 4. The control values are given as means ± S.E.M.

| Treatment | Cholesterol (mg./liver) | Cholesterol (mg./carcass) |
|---|---|---|
| Starved for 24hr., not re-fed | 8·7 | 70 |
| Starved for 24hr., re-fed for 24hr. | 9·8 | 64 |
| Starved for 24hr., re-fed for 48hr. | 9·1 | 68 |
| Starved for 24hr., re-fed for 72hr. | 8·4 | 67 |
| Starved for 48hr., not re-fed | 8·4 | 67 |
| Starved for 48hr., re-fed for 24hr. | 7·8 | 68 |
| Starved for 48hr., re-fed for 48hr. | 11·2 | 70 |
| Starved for 48hr., re-fed for 72hr. | 11·0 | 69 |
| Starved for 72hr., not re-fed | 8·1 | 67 |
| Starved for 72hr., re-fed for 24hr. | 11·4 | 64 |
| Starved for 72hr., re-fed for 48hr. | 11·6 | 65 |
| Starved for 72hr., re-fed for 72hr. | 17·0 | 65 |
| Controls | 9·0±0·6 | 66±4 |

Table 6. *Effect of re-feeding on glycogen synthesis in the liver and carcass of mice*

Groups of eight mice were starved for 18hr. and then re-fed with diet 2 (see the Materials and Methods section) for the periods shown before being given 250mg. of [U-$^{14}$C]glucose (0·5 $\mu$c) orally. The diet was removed from the cages at the time of dosing. One hr. after dosing the animals were killed and carcass and liver glycogen was isolated. Results are given as means ± S.E.M.

| Treatment | Liver glycogen | | Carcass glycogen | |
|---|---|---|---|---|
| | (mg./liver) | (counts/min./liver) | (mg./mouse) | (counts/min./mouse |
| Fed | 163±14 | 12650±4160 | 48±8 | 3790±1150 |
| Starved for 18hr., not re-fed | 35±3 | 34010±1690 | 14±3 | 14200±4360 |
| Starved for 18hr., re-fed for 1hr. | 67±7 | 39500±2540 | 32±5 | 14410±1810 |
| Starved for 18hr., re-fed for 2hr. | 68±11 | 38010±3330 | 36±8 | 10610±1800 |
| Starved for 18hr., re-fed for 3hr. | 85±12 | 36930±1720 | 50±10 | 13030±1890 |
| Starved for 18hr., re-fed for 5hr. | 125±6 | 21010±2470 | 57±7 | 8180±2080 |
| Starved for 18hr., re-fed for 7hr. | 160±6 | 20530±1850 | 58±6 | 6350±1700 |

starvation was reflected in a doubling in the total hepatic cholesterol content.

*Effect of starvation and re-feeding on glycogen synthesis from* [$^{14}$C]*glucose.* The effects of starvation (18hr.) and subsequent re-feeding (7hr.) on the conversion of a [U-$^{14}$C]glucose meal into carcass and liver glycogen are shown in Table 6. After 18hr. of starvation there was more than a threefold stimulation in the conversion of the [$^{14}$C]glucose meal into both carcass and liver glycogen. After 5hr. of re-feeding these elevated synthesis rates started to return to normal as the glycogen stores in both compartments were repleted.

## DISCUSSION

In isotope-incorporation experiments such as have been described a major complication is the question of the specific activities of the immediate precursors in different nutritional conditions. We have presented arguments that use of the glucose-meal technique should minimize differential dilution of the isotope with unlabelled acetyl-CoA produced endogenously by decreasing lipolysis, fatty acid degradation and glycogenolysis (Jansen *et al.* 1966*a*). Results previously reported show that the specific activity of the circulating glucose for 10–60min. after such a meal is similar in fed and starved animals (Jansen *et al.* 1966*a*). Therefore, although not directly measured in these re-feeding experiments, it seems reasonable to assume that there were no major differences in the specific activity of the plasma glucose after the glucose meal. In the experiment where a tracer dose of [$^{14}$C]glucose was given intraperitoneally, differential isotope dilution is more likely. We have reported the specific activities of the plasma glucose after such a dose given to fed and starved animals (Jansen *et al.* 1966*a*). The results indicate that, presumably because of lowered concentration and decreased utilization, the specific activity of the plasma glucose was two to four times as high in mice that had been starved for 18hr. as in control animals fed *ad libitum*. Therefore, on the basis of these findings alone, the effect of starvation on fatty acid synthesis is probably greater than the present results suggest. However, in no experiments were the tissue concentrations or specific activities of more immediate lipid precursors such as acetyl-CoA measured, and the following interpretation of our findings should be considered with this in mind.

In previous work (Jansen *et al.* 1966*b*) we reported that food deprivation in mice for up to 7hr. did not diminish the ability of the liver to convert a 250mg. meal of [U-$^{14}$C]glucose into fatty acid *in vivo*, though after longer periods of starvation lipogenesis was depressed. In the present work this finding has been confirmed. These results are in sharp contrast with those obtained when a tracer dose of [U-$^{14}$C]glucose was given intraperitoneally. In this case, food deprivation for 1hr. and for 2hr. decreased incorporation of [$^{14}$C]glucose into fatty acid by 80 and 90% respectively. It would appear that incorporation of the tracer dose may reflect the actual fatty acid synthesis rate in the body at a moment in time, whereas incorporation of the meal reflects the capacity or potential for fatty acid synthesis.

It is reasonable to ask what meaning 1 and 2hr. of starvation periods have in mice and whether the decrease in the conversion of the tracer dose over such short periods might not represent a normal diurnal variation. We have not investigated the food-consumption habits of mice in detail but we have observed that, under our conditions, they do in fact eat at intervals throughout the day and the differences in plasma glucose concentration reported in Table 3 are in accord with this. On balance

therefore we feel that the decreased conversion of the tracer dose of [U-$^{14}$C]glucose into fatty acids in the liver after deprivation of food for 1 and 2hr. represents an extremely rapid adaptation to the supply of substrate, rather than diurnal variation. However, more direct evidence on the normal feeding habits of mice along with incorporation studies on control groups dosed at intervals throughout the day are required. A clear-cut distinction between the effects of starvation on glycogen synthesis and on fatty acid synthesis is indicated by the results presented in this paper. After 18hr. of food deprivation, conversion of the [U-$^{14}$C]glucose meal into fatty acid was inhibited by 80% at the time its conversion into glycogen was stimulated threefold. It is noteworthy that in both fed and starved mice the total amount of liver glycogen recovered was about three times that of carcass glycogen and that the total incorporation of [$^{14}$C]glucose into liver glycogen was about three times that into carcass glycogen.

In attempting to explain the increased glycogen synthesis and decreased fatty acid synthesis in the extrahepatic tissues in the course of metabolizing a 250mg. meal after 18hr. of starvation, it appears reasonable to postulate that muscle glycolysis, and probably also glycolysis in adipose tissue, is lower than in mice fed on their normal diet and that this causes an increase in tissue concentrations of glucose 6-phosphate (Randle, 1964; Newsholme & Randle, 1964). This increase could have the dual effect of decreasing glucose uptake by inhibiting hexokinase and of increasing the conversion of the glucose that is taken up into glycogen. However, it is noteworthy that starvation also decreased the conversion of glucose into fatty acid and increased its conversion into glycogen in the liver. In the rat glucokinase, and not hexokinase, is believed to regulate glucose uptake in the liver (DiPietro, Sharma & Weinhouse, 1962) and this enzyme is not inhibited by glucose 6-phosphate (Viñuela, Salas & Sols, 1963). Though comparable data pointing to such a physiological role for glucokinase in mouse liver are not yet available, the enzyme has been reported to be present in mouse liver and its activity does not appear to fall during starvation (Cahill, 1965).

Since the conversion of the [U-$^{14}$C]glucose meal into glycogen was stimulated after 18hr. of starvation, the amounts or activities of the enzymes concerned in glycogen synthesis do not appear to have been diminished at this time. The amounts or activities of certain of the enzymes involved in fatty acid and cholesterol synthesis apparently were lowered, however. The large decrease in the conversion of a tracer amount of [U-$^{14}$C]glucose into fatty acid after as little as 1hr. of starvation may involve regulation at the level of enzyme activity and diminished availability of substrate. After 18hr. of starvation, however, restoration of cholesterol and fatty acid synthesis to normal required 18–24hr. of re-feeding, and after 3 days' starvation the rate of extrahepatic fatty acid synthesis was not restored to normal even after 3 days' re-feeding. It appears likely that this slow recovery after prolonged periods of starvation involves an increase in the amount of enzyme protein present. Potter & Ono (1961) reported that the induction of glucose 6-phosphate-dehydrogenase activity that occurs in rat liver during re-feeding is inhibited by puromycin. Hicks, Allmann & Gibson (1965) also reported that the increases in activity of various rat-liver enzymes during re-feeding were inhibited by actinomycin D or puromycin. However, in neither paper were any data on food consumption or weight gain given, and it is known that these antibiotics are toxic and can inhibit growth (Hechter & Halkerston, 1965). Therefore, although actinomycin D and puromycin may inhibit the recovery of the capacity for fatty acid synthesis during re-feeding, a specific inhibition, completely dissociated from a toxic effect on food consumption, does not as yet appear to have been demonstrated.

The results in Table 6 show that overnight food deprivation in mice caused a considerable loss of carcass as well as liver glycogen. This is not in agreement with the view that the stores of muscle glycogen are extremely resistant to the effects of starvation (Keys, Brozek, Henschel, Mickelsen & Taylor, 1950; Stetten & Stetten, 1954). In our experiments all animals were eviscerated and frozen in a deep-freeze within 10min. of death. The fact that the rate of incorporation of a 250mg. meal of [U-$^{14}$C]glucose into carcass glycogen was greatly increased after starvation and then decreased as the amount of glycogen was restored by re-feeding supports the contention that carcass glycogen was in fact lost after 18hr. of food deprivation.

The large increase in the extent of synthesis of fatty acid observed *in vivo* during re-feeding agrees with earlier work done with liver slices *in vitro* (Tepperman & Tepperman, 1958*b*, 1961). However, Tepperman & Tepperman (1958*a*) reported, in contrast with our results *in vivo*, that the incorporation of [1-$^{14}$C]acetate into liver cholesterol was depressed during re-feeding.

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