Studies on Lipogenesis *in vivo*

LIPOGENESIS DURING EXTENDED PERIODS OF RE-FEEDING AFTER STARVATION

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1. Lipogenesis was studied in mice re-fed for up to 21 days after starvation. At appropriate times [$U\text{-}^{14}\text{C}$]glucose was given by stomach tube and incorporation of ^{14}C into various lipid fractions measured. 2. In mice starved for 48 hr. and then re-fed for 4 days with a diet containing 1% of corn oil, incorporation of ^{14}C from [$U\text{-}^{14}\text{C}$]glucose into liver fatty acids and cholesterol was respectively threefold and eightfold higher than in controls fed *ad libitum*. The percentages by weight of fatty acids and cholesterol in the liver also increased and reached peaks after 7 days. Both the radioactivity and weights of the fractions returned to control values after 10–14 days' re-feeding. These changes could be diminished by re-feeding the mice with a diet containing 20% of corn oil. Incorporation of ^{14}C from [$U\text{-}^{14}\text{C}$]glucose into extrahepatic fatty acids (excluding those of the epididymal fat pads) was not elevated during re-feeding with a diet containing either 1% or 20% of corn oil. However, incorporation of ^{14}C from [$U\text{-}^{14}\text{C}$]glucose into the fatty acids of the epididymal fat pads was increased in mice re-fed with either diet, as compared with non-starved controls. 3. Lipogenesis was also studied in mice alternately fed and starved. Mice given a diet containing 1% of corn oil for 6 hr./day for 4 weeks lost weight initially and never attained the weight or carcass fat content of controls fed *ad libitum*. Incorporation of ^{14}C from dietary [$U\text{-}^{14}\text{C}$]glucose into the fatty acids of the epididymal fat pads was elevated threefold in the mice allowed limited access to food, although the incorporation into the remainder of the extrahepatic fatty acids was not different from that found for controls. Mice given a diet containing 20% of corn oil for 6 hr./day adapted to the limited feeding regimen quicker and in 4 weeks did attain the weight and carcass fat content of controls. Incorporation of ^{14}C from [$U\text{-}^{14}\text{C}$]glucose into the fatty acids of the epididymal fat pads and the remainder of the extrahepatic fatty acids was respectively fivefold and threefold higher than in controls fed *ad libitum*. 4. The elevation in liver lipogenesis during re-feeding was greatest on a diet containing 1% of corn oil, whereas in extrahepatic tissues the increase in lipogenesis was greater when the mice were re-fed or were allowed limited access to a diet containing 20% of corn oil. These results suggest that the causes of the increased rate of incorporation of ^{14}C from [$U\text{-}^{14}\text{C}$]glucose into fatty acids during re-feeding may be different in liver from that in extrahepatic tissues.

Previously, we have reported fivefold and tenfold increases respectively in the incorporation of ^{14}C from [$U\text{-}^{14}\text{C}$]glucose into liver fatty acids and cholesterol in mice re-fed with a high-carbohydrate low-fat diet after a period of starvation (Jansen, Zanetti & Hutchison, 1966c). The increase in incorporation into liver fatty acids is consistent with the reported increase in incorporation of [$1\text{-}^{14}\text{C}$]acetate into fatty acids in liver slices of starved rats re-fed with a high-carbohydrate diet (Tepperman & Tepperman, 1958b, 1961). However, these workers did not find an increase in incorporation of [$1\text{-}^{14}\text{C}$]acetate into liver chole-

sterol in the livers of starved and re-fed rats (Tepperman & Tepperman, 1958a). In the present work we have determined how long it takes these altered parameters of lipid metabolism to return to normal and have studied the role of dietary fat. We have also investigated lipogenesis from [$U\text{-}^{14}\text{C}$]glucose in mice maintained for 4 weeks on a limited feeding schedule.

MATERIALS AND METHODS

The mice used in these experiments were young adult males from the Merck, Sharp and Dohme colony (ICR strain). Their body weights are given in the legends to the

Tables and Figures, and for each experiment were within a 4g. range. The techniques used in dosing and bleeding, preparing and analysing tissues and determining radioactivity were as described previously (Jansen, Hutchison & Zanetti, 1966a), as also were the conditions used for saponification of carcasses (Jansen *et al.* 1966c). The [^{14}C]glucose was obtained from New England Nuclear Corp. (Boston, Mass., U.S.A.) and had a specific radioactivity of 10–15 mc/m-mole. In certain experiments the labelled glucose was added to 250 mg. of unlabelled glucose and given by stomach tube as a 50% (w/v) solution; in other experiments the labelled glucose was added to the diet given to the animals for 24 hr., during which period the food consumption was measured. The mice were housed individually in screen-bottomed cages in an air-conditioned room maintained at approx. 24° and were supplied with food and water *ad libitum*, except as noted.

RESULTS

Effect of duration of starvation and re-feeding periods. Mice with an average weight of 24.0 g. were starved for 48 hr. and then re-fed with a diet containing 1% of corn oil for 21 days. The changes in body weight and the incorporation of ^{14}C from [^{14}C]glucose into carcass fatty acids are shown in Fig. 1. In mice that were starved, it required 10–14 days' re-feeding for the body weight and percentage of extrahepatic fat to reach the corresponding values obtained for the non-starved controls. Incorporation of ^{14}C from [^{14}C]glucose into extrahepatic fatty acids was restored to the value for non-starved controls after 10 days' re-feeding. Although not shown, incorporation into carcass cholesterol was restored to control values by only 2 days' re-feeding. At no time during the 21-day re-feeding period did the incorporation into either extrahepatic fatty acids or cholesterol exceed the values obtained for non-starved controls.

This is in sharp contrast with the results for the liver shown in Fig. 2. Control values for incorporation into both liver fatty acids and cholesterol were reached after 1 day's re-feeding. By 4 days' re-feeding, incorporation of ^{14}C from [^{14}C]glucose into fatty acids and cholesterol was respectively threefold and eightfold higher than the corresponding values obtained for the non-starved controls. The percentages by weight of fatty acids and cholesterol in the liver reflected this increased synthesis and reached peaks after 7 days' re-feeding. The increased levels of incorporation into liver cholesterol and fatty acids fell to the values for non-starved controls after 10 days and the percentages of liver cholesterol and fatty acids returned to normal after 14 days' re-feeding. A similar experiment was carried out in which incorporation of ^{14}C from [^{14}C]glucose into liver fatty acids and cholesterol during re-feeding with the diet containing 1% of corn oil was determined after a 24 hr. rather than a 48 hr. starvation period.

Fig. 1. Changes in body weight and the incorporation of ^{14}C from [^{14}C]glucose into carcass fatty acids of mice starved for 48 hr. and re-fed for up to 21 days. Carcass refers to the body with the liver removed. The diet used contained 1% of corn oil (diet 2; Jansen *et al.* 1966a) and was given for 5 days before the starvation period as well as for 21 days after. On the days indicated in the Figure the starved and re-fed mice, along with non-starved control mice maintained on the same diet, were given 250 mg. of [^{14}C]glucose (2.5 μC) orally and killed 60 min. later (eight mice/group). Food was removed from the cages at the time of dosing. The average weight of the mice before starvation was 24.0 g. The results plotted are the means obtained from determination of individual body weights or saponification of two carcasses/flask. ○, Non-starved; ●, starved for 48 hr. and re-fed.

Alterations were found similar to those occurring after the longer starvation period. All changes were less in degree, however, and were restored to normal by 7 days' re-feeding.

Until this time the [^{14}C]glucose had been administered by stomach tube as a 250 mg. meal in all re-feeding experiments. It appeared desirable to compare the results obtained with this technique with those that would be obtained by adding the [^{14}C]glucose directly to the diet given for a 24 hr. period. A detailed comparison of lipogenesis in mice in which these two methods of [^{14}C]glucose administration were used has been published previously (Jansen, Zanetti & Hutchison, 1967a). Mice were starved for 48 hr. and re-fed for up to 8 days with the [^{14}C]glucose added to the diet containing 1% of corn oil for the last 24 hr. of each feeding period. Non-starved control groups were treated similarly. Because food consumption for

Fig. 2. Incorporation of ^{14}C from $[\text{U-}^{14}\text{C}]\text{glucose}$ into liver fatty acid and cholesterol in mice starved for 48 hr. and re-fed for up to 21 days. For further details see the legend to Fig. 1. The results plotted are the means obtained after analysing the liver of each mouse separately. $\circ$, Non-starved; $\bullet$, starved for 48 hr. and re-fed.

the re-fed mice differed from the controls (20% less on day 1, 15% more on day 4 and the same on day 8), incorporation is expressed as a percentage of the ingested $[\text{U-}^{14}\text{C}]\text{glucose}$ dose. As shown in Fig. 3, the radioactivity of the fatty acids of the epididymal fat pads and the remainder of the carcass reached control values after 4 days' re-feeding. At this time the radioactivity of the liver fatty acid fraction was elevated almost threefold (Fig. 3). As shown in Fig. 4, the increase in incorporation into liver cholesterol and the increase in weight of this fraction were similar in magnitude to that previously found 1 hr. after the single oral dose of $[\text{U-}^{14}\text{C}]\text{glucose}$, suggesting that there was no appreciable difference in the turnover rate of liver cholesterol in the re-fed mice compared with that in non-starved controls. This is in contrast with the increase in percentage of liver cholesterol seen in genetically obese mice given the diet containing 1% of corn oil and which appeared to result from decreased degradation of cholesterol rather than increased synthesis (Jansen, Zanetti & Hutchison, 1967b).

Role of dietary fat in re-feeding response. To evaluate the role of dietary fat or lack thereof in initiating these increases in lipogenesis in the liver, an experiment was carried out in which the pre-starvation diets were formulated to contain either

1% or 20% of corn oil. The mice were starved for 48 hr. and re-fed with diet containing either 1% or 20% of corn oil. All results obtained with pre-starvation diets containing 1% and 20% of corn oil are shown in Tables 1 and 2 respectively. The pre-starvation weights of mice used in this experiment were higher than those used earlier (Fig. 1) and 2 days' starvation did not represent as severe a dietary stress. As a result the percentages of carcass fatty acids and the levels of incorporation of ^{14}C from $[\text{U-}^{14}\text{C}]\text{glucose}$ into carcass fatty acids were in all cases restored to the values for the corresponding non-starved controls by 3 days' re-feeding, although body weights were still slightly depressed. As was reported previously (Jansen *et al.* 1966a), conversion of $[\text{U-}^{14}\text{C}]\text{glucose}$ into fatty acids was considerably depressed by dietary fat.

Feeding with the diet containing 1% of corn oil for 3 days after 7 days on the diet containing 20% of corn oil did not completely raise the level of incorporation of ^{14}C from $[\text{U-}^{14}\text{C}]\text{glucose}$ into extrahepatic fatty acids to that typical for this low-fat diet (Table 2). In mice fed with the diet containing 20% of corn oil before starvation and re-fed with the diet containing 1% of corn oil the level of incorporation of ^{14}C from $[\text{U-}^{14}\text{C}]\text{glucose}$ into extrahepatic fatty acids was more typical of mice fed with the low-fat diet during the entire

Fig. 3. Incorporation of ¹⁴C from dietary [U-¹⁴C]glucose into fatty acid in mice starved for 48 hr. and re-fed for up to 8 days. Carcass refers to the body with the liver and epididymal fat pads removed. The diet used contained 70% of glucose and 1% of corn oil (diet 2; Jansen *et al.* 1966a) and was given for 3 days before starvation as well as for 8 days after. For the last 24 hr. of each feeding period [U-¹⁴C]glucose was added to the diet (0.2 μ C/g. of diet) and food consumption measured for both the starved-re-fed mice and non-starved control groups. There were eight mice in each group and their average weight before starvation was 23.4 g. The incorporation of ¹⁴C from [U-¹⁴C]glucose in various fractions is expressed as a percentage of the ingested dose. The results plotted are the means obtained by analysis of tissues of individual mice or saponification of two carcasses/flask. ○, Non-starved; ●, starved for 48 hr. and re-fed.

experimental period (Table 2). Overall there was very little evidence for increased lipogenesis in this fraction during re-feeding, regardless of the fat content of either the pre- or the post-starvation diet.

The incorporation of ¹⁴C from [U-¹⁴C]glucose into the fatty acids of the epididymal fat pads decreased when mice previously fed with the diet containing 1% of corn oil were transferred to the diet containing 20% of corn oil (Table 1). Very little increase in fatty acid synthesis in the epididymal fat pads was found for mice fed with the diet containing 1% of corn oil for 3 days after being transferred from the diet containing 20% of corn oil without being starved. This observation, along

Fig. 4. Incorporation of ¹⁴C from dietary [U-¹⁴C]glucose into cholesterol in mice starved for 48 hr. and re-fed for up to 8 days. Carcass refers to body with liver and epididymal fat pads removed. For further details see the legend to Fig. 3. ○, Non-starved; ●, starved for 48 hr. and re-fed.

with the small increase in incorporation into carcass fatty acids previously noted, suggests that these mice did not adapt quickly enough to the dietary fat content, and probably as a consequence both body weight and carcass fat content were lowered. However, a difference in the initial palatability of the diet could also have influenced these results. In contrast with the results for carcass fatty acids, incorporation of ¹⁴C from [U-¹⁴C]glucose into the fatty acids of the epididymal fat pads was elevated over the values for non-starved controls regardless of the fat content of either the pre- or the post-starvation diet. The smallest proportional rise over the control value occurred under the conditions that gave the highest control value for lipogenesis, i.e. pre- and post-starvation diets both containing only 1% of corn oil (Table 1). However, the highest absolute level of incorporation into the fatty acids of the epididymal fat pads was observed in these mice. In mice fed with the diet containing 20% of corn oil, incorporation into the fatty acids of the epididymal fat pads was approximately twice as high as in non-starved controls, regardless of the pre-starvation diet.

In confirmation of previous results, incorporation of ¹⁴C from [U-¹⁴C]glucose into liver cholesterol and fatty acids and the percentages by weight of

Table 1. *Lipogenesis during re-feeding in mice fed with the diet containing 1% of corn oil before starvation*

Groups of mice (eight/group) were maintained for 1 week on a diet containing 1% of corn oil (diet 2; Jansen *et al.* 1966a). They were then either starved for 48 hr. or continued on diet 2 for this period. The starved mice were re-fed for 3 days with a diet containing either 1% (diet 2) or 20% (diet 5; Jansen *et al.* 1966a) of corn oil with the non-starved mice treated similarly. At this time each mouse was given 250 mg. of [U-¹⁴C]glucose (2.5 μ c) orally and killed 2 hr. later, food being removed from the cages at the time of dosing. The average weight of the mice before starvation was 29.0 g. Carcass refers to the body with liver and epididymal fat pads removed. The results are given as means $\pm$ S.E.M.

	Non-starved			Starved for 48 hr.		
	Initial day	1% corn oil for 3 days	20% corn oil for 3 days	Not re-fed	1% corn oil for 3 days	20% corn oil for 3 days
Body wt. (g.)	32.4 $\pm$ 0.9	33.8 $\pm$ 1.2	34.0 $\pm$ 1.2	23.0 $\pm$ 0.8	31.2 $\pm$ 1.1	32.2 $\pm$ 1.0
Liver wt. (g.)	2.17 $\pm$ 0.08	2.02 $\pm$ 0.11	2.14 $\pm$ 0.11	1.29 $\pm$ 0.08	2.32 $\pm$ 0.15	2.16 $\pm$ 0.11
Liver fatty acid						
% of liver*	6.03 $\pm$ 0.70	6.15 $\pm$ 0.90	4.89 $\pm$ 0.38	3.23 $\pm$ 0.18	10.19 $\pm$ 0.80	6.92 $\pm$ 0.69
Counts/min./liver ($\times 10^{-3}$)	73.2 $\pm$ 9.4	40.3 $\pm$ 6.0	8.01 $\pm$ 0.91	4.87 $\pm$ 0.53	105.5 $\pm$ 12.5	16.9 $\pm$ 2.4
Liver cholesterol						
% of liver*	0.44 $\pm$ 0.03	0.43 $\pm$ 0.04	0.37 $\pm$ 0.03	0.63 $\pm$ 0.08	0.67 $\pm$ 0.03	0.37 $\pm$ 0.03
Counts/min./liver	243 $\pm$ 105	126 $\pm$ 22	45 $\pm$ 17	4 $\pm$ 1	631 $\pm$ 129	159 $\pm$ 31
Carcass fatty acid						
% of carcass†	12.0 $\pm$ 1.1	11.9 $\pm$ 1.4	12.2 $\pm$ 1.0	4.0 $\pm$ 0.8	11.2 $\pm$ 0.8	12.8 $\pm$ 0.6
Counts/min./mouse ($\times 10^{-3}$)	377 $\pm$ 33	468 $\pm$ 22	292 $\pm$ 29	104 $\pm$ 23	505 $\pm$ 10	322 $\pm$ 12
Carcass cholesterol						
% of carcass†	0.23 $\pm$ 0.01	0.22 $\pm$ 0.01	0.22 $\pm$ 0.01	0.31 $\pm$ 0.01	0.22 $\pm$ 0.01	0.22 $\pm$ 0.01
Counts/min./mouse	2420 $\pm$ 110	2210 $\pm$ 240	2410 $\pm$ 180	950 $\pm$ 140	2510 $\pm$ 50	2370 $\pm$ 170
Epididymal fat pad						
Wt. of fatty acid (mg./pad)	231 $\pm$ 26	293 $\pm$ 40	308 $\pm$ 28	51 $\pm$ 18	216 $\pm$ 28	210 $\pm$ 23
Fatty acid (counts/min./pad) ($\times 10^{-3}$)	7.20 $\pm$ 1.26	11.4 $\pm$ 1.9	5.26 $\pm$ 0.90	0.26 $\pm$ 0.09	15.0 $\pm$ 1.0	10.9 $\pm$ 1.3

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

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

these fractions were considerably elevated in mice re-fed with the diet containing 1% of corn oil compared with non-starved controls. When the diet containing 20% of corn oil was given during the re-feeding period, these changes were decreased but not eliminated. Incorporation of ¹⁴C from [U-¹⁴C]glucose into liver cholesterol after re-feeding with the diet containing 1% of corn oil was twice as high in mice given the diet containing 20% of corn oil before the starvation period (Table 2) as in those previously given the diet containing 1% of corn oil (Table 1). This showed clearly that the composition of the diet given before a starvation period as long as 48 hr. can have a considerable influence on the metabolic changes during re-feeding.

Lipogenesis in mice alternately starved and fed. The preceding experiments were carried out with mice completely unadapted to food deprivation. It was decided to compare lipogenesis in mice trained

to eat their daily food in a short feeding period with those fed *ad libitum*. Although adult rats have been successfully maintained when fed for 2 hr./day it was found in preliminary experiments that a daily feeding period of 6 hr. was more satisfactory for mice.

Groups of mice were given the diet containing 1% or 20% of corn oil either *ad libitum* or for 6 hr. on each day (9 a.m.-3 p.m.) for a 4-week period with [U-¹⁴C]glucose added to the diet for the last 24 hr. (or 6 hr.). Mice given the low-fat diet for 6 hr./day lost weight during the first week, but thereafter maintained a rate of gain similar to that of controls fed *ad libitum* (Fig. 5). After 4 weeks they had not attained the weight of the controls, although it seems likely that they would have done so in another 1-2 weeks. Those mice given the high-fat diet for only 6 hr./day maintained their weight during the first week and after 4 weeks reached the weight of the controls fed *ad libitum*.

Table 2. *Lipogenesis during re-feeding in mice fed with the diet containing 20% of corn oil before starvation*

Groups of mice (eight/group) were maintained for 1 week on a diet containing 20% of corn oil (diet 5; Jansen *et al.* 1966a). They were then either starved for 48 hr. or continued on diet 5 for this period. The starved mice were re-fed for 3 days with a diet containing either 1% (diet 2; Jansen *et al.* 1966a) or 20% (diet 5) of corn oil with the non-starved mice treated similarly. At this time each mouse was given 250 mg. of [U-¹⁴C]glucose (2.5 μ C) orally and killed 2 hr. later, food being removed from the cages at the time of dosing. The average weight of the mice before starvation was 31.1 g. Carcass refers to the body with liver and epididymal fat pads removed. The results are given as means $\pm$ S.E.M.

	Non-starved			Starved for 48 hr.		
	Initial day	1% corn oil for 3 days	20% corn oil for 3 days	Not re-fed	1% corn oil for 3 days	20% corn oil for 3 days
Body wt. (g.)	33.6 $\pm$ 1.2	31.2 $\pm$ 1.0	35.1 $\pm$ 1.1	25.4 $\pm$ 0.6	31.1 $\pm$ 1.3	33.4 $\pm$ 1.5
Liver wt. (g.)	2.18 $\pm$ 0.14	1.72 $\pm$ 0.09	2.04 $\pm$ 0.11	1.38 $\pm$ 0.05	1.98 $\pm$ 0.10	2.22 $\pm$ 0.10
Liver fatty acid						
% of liver*	4.10 $\pm$ 0.18	3.59 $\pm$ 0.22	4.20 $\pm$ 0.23	3.61 $\pm$ 0.34	5.79 $\pm$ 0.58	6.93 $\pm$ 0.75
Counts/min./liver ($\times 10^{-3}$)	10.4 $\pm$ 1.5	26.8 $\pm$ 3.1	9.32 $\pm$ 1.49	1.57 $\pm$ 0.13	106.6 $\pm$ 15.9	21.5 $\pm$ 3.8
Liver cholesterol						
% of liver*	0.30 $\pm$ 0.01	0.30 $\pm$ 0.01	0.35 $\pm$ 0.01	0.36 $\pm$ 0.02	0.44 $\pm$ 0.04	0.41 $\pm$ 0.03
Counts/min./liver	184 $\pm$ 49	269 $\pm$ 58	354 $\pm$ 23	14 $\pm$ 5	1334 $\pm$ 403	204 $\pm$ 41
Carcass fatty acid						
% of carcass†	11.0 $\pm$ 0.7	8.2 $\pm$ 1.6	12.9 $\pm$ 1.8	6.3 $\pm$ 1.4	9.2 $\pm$ 1.5	12.2 $\pm$ 0.9
Counts/min./mouse ($\times 10^{-3}$)	234 $\pm$ 26	305 $\pm$ 8	235 $\pm$ 7	69.0 $\pm$ 7.0	409 $\pm$ 22	234 $\pm$ 27
Carcass cholesterol						
% of carcass†	0.23 $\pm$ 0.01	0.24 $\pm$ 0.01	0.21 $\pm$ 0.01	0.31 $\pm$ 0.01	0.23 $\pm$ 0.01	0.22 $\pm$ 0.02
Counts/min./mouse	2700 $\pm$ 130	2780 $\pm$ 80	2740 $\pm$ 190	1140 $\pm$ 90	3300 $\pm$ 130	2910 $\pm$ 70
Epididymal fat pad						
Wt. of fatty acid (mg./pad)	229 $\pm$ 34	222 $\pm$ 38	350 $\pm$ 38	100 $\pm$ 30	203 $\pm$ 70	277 $\pm$ 59
Fatty acid (counts/min./pad) ($\times 10^{-3}$)	2.22 $\pm$ 0.33	2.95 $\pm$ 0.31	2.50 $\pm$ 0.57	0.13 $\pm$ 0.04	9.40 $\pm$ 1.17	7.23 $\pm$ 1.36

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

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

(Fig. 5). As shown in Table 3 incorporation of ¹⁴C from dietary [U-¹⁴C]glucose into liver fatty acids and the percentage of liver fatty acid by weight were similar for the two feeding schedules irrespective of diet. As has been discussed previously (Jansen *et al.* 1967a), when the [U-¹⁴C]glucose was administered in the diet a considerable portion of the labelled fatty acids synthesized from [U-¹⁴C]glucose in the liver was probably transported to peripheral tissues during the feeding period. Incorporation of ¹⁴C from [U-¹⁴C]glucose into carcass fatty acids was not elevated in the mice with the diet containing 1% of corn oil for only 6 hr./day, but was almost threefold higher in mice fed with the diet containing 20% of corn oil for 6 hr./day than in mice fed with this diet *ad libitum*. Incorporation of ¹⁴C from [U-¹⁴C]glucose into the fatty acids of the epididymal fat pads of mice allowed limited access to the diets containing 1% and 20% of corn oil respectively was threefold and fivefold higher than the corresponding values

obtained for mice given these diets *ad libitum*. With either diet the incorporation of ¹⁴C from [U-¹⁴C]glucose into both liver and carcass cholesterol was considerably depressed in the mice allowed limited access to the food. Since the percentage by weight of these fractions was similar with the two feeding schedules, it would appear that cholesterol turnover was depressed in the mice allowed limited access to their diet.

DISCUSSION

Experiments on lipogenesis during starvation and starvation followed by a short period of re-feeding with a low-fat diet have been published previously (Jansen *et al.* 1966b,c). The present work confirms and extends these studies. The incorporation of ¹⁴C from [U-¹⁴C]glucose into the total extra-hepatic fatty acid fraction (excluding that of the epididymal fat pads) during re-feeding did not rise appreciably above the values for the corresponding

Fig. 5. Changes in body weight of mice fed for 6hr./day or 24hr./day for 4 weeks. For further details see the legend to Table 3. ○, 1% corn oil given 24hr./day; ●, 1% corn oil given 6hr./day; □, 20% corn oil given 24hr./day; ■, 20% corn oil given 6hr./day.

non-starved controls regardless of the fat content of the diet. However, incorporation into the fatty acids of the epididymal fat pads was increased during re-feeding, especially with the diet containing 20% of corn oil. It would appear that the constraint placed on fatty acid synthesis by dietary fat was present to a smaller degree during re-feeding with the high-fat diet after starvation than when this diet was given *ad libitum*.

The changes that occurred in the cholesterol and total esterified fatty acid fractions of liver during re-feeding were considerably more marked than those in extrahepatic tissues. Allman, Hubbard & Gibson (1965) have studied fatty acid synthesis in the liver *in vitro* during the re-feeding of starved rats with a diet devoid of fat. They found an increased incorporation of [^{14}C]acetate into all fatty acids, and a net accumulation of fatty acids and triglycerides. At the same time there was a marked fall in the linoleate content of the liver fatty acid fractions. These changes were similar to those reported to occur during early linoleic acid deficiency in the mouse (Allman & Gibson, 1965). In neither study was cholesterol synthesis investigated. However, Norby (1965) has reported that liver cholesterol also accumulated during essential fatty acid deficiency. We have not investigated directly the essential fatty acid adequacy of our low-fat re-feeding diet. Feigenbaum & Fisher (1963) have reported development of a fatty liver when chickens were re-fed with a fat-free diet after starvation. The fatty liver could be prevented by feeding with a diet containing fat, although differences in

effectiveness were seen among a variety of fat sources studied. More data are required to separate the role of dietary fat content from that of specific fatty acids in these alterations in lipid metabolism.

The increased incorporation of ^{14}C from [$\text{U-}^{14}\text{C}$]glucose into fatty acids that occurred in adipose tissue appears to be in a different category since, rather than being diminished, it occurred to the greatest proportional extent when the re-feeding diet contained 20% of corn oil. Leveille (1967*a,b*) has investigated the effect of dietary fat on lipogenesis in the adipose tissue of meal-fed rats as measured *in vitro*. In contrast with our results *in vivo*, he reported that dietary fat greatly diminished or abolished the elevated lipogenic response in meal-fed rats.

Metabolic responses to alternating periods of feeding and starvation have been widely studied in rats (Hollifield & Parson, 1962*a,b*; Cohn & Joseph, 1960; Stevenson, Feleki, Szlavko & Beaton, 1964; Leveille & Hanson, 1965; Leveille, 1966), but much less extensively in mice (Kekwick & Pawan, 1966). In our experiments mice fed with the diet containing 20% of corn oil for 6hr./day required 4 weeks before they reattained the average body weight of controls fed *ad libitum*. Although an enhanced rate of conversion of dietary [$\text{U-}^{14}\text{C}$]glucose into total body fatty acid was found in these animals, the excess of fat synthesized each day was utilized for energy in the 18hr. daily starvation period, since the carcass fat content and epididymal-fat-pad weights were no greater and probably were slightly less in the mice given limited access to the diet than in the controls. It would appear that the factors responsible for lowering the rate of fatty acid synthesis in mice fed with a high-fat diet were not present to the same extent in the animals given limited access to the diet as in the controls fed *ad libitum*. Mice allowed limited access to the diet containing only 1% of corn oil for 4 weeks weighed less and contained a lower proportion of fat than did controls. Apparently these animals could not greatly elevate the already high rate of lipogenesis that occurs when this low-fat diet is given *ad libitum*.

An important purpose of the present experiments was to examine the role of lipogenesis *in vivo* in the total lipid economy of the mouse under conditions of re-feeding after starvation or daily periods of feeding and starvation. The results suggest that the pre-starvation body weight, composition of the pre-starvation diet, duration of starvation period, duration of re-feeding period and composition of re-feeding diet all are important variables in determining the rates of fatty acid and cholesterol synthesis during starvation-re-feeding conditions. The liver showed enhanced lipogenesis during re-feeding most markedly in mice fed with the diet containing 1% of corn oil. In contrast adipose and

Table 3. *Lipogenesis in mice alternately fed and starved*

Groups of mice (eight/group) were fed with diet containing 1% or 20% of corn oil (diet 2 or diet 5; Jansen *et al.* 1966a) on a 6 hr./day or 24 hr./day schedule for 4 weeks. For the last 6 hr. period (9 a.m.–3 p.m.) or 24 hr. period (9 a.m.–9 a.m.) [^{14}C]glucose was added to the diets ($0.4\mu\text{C/g.}$ of diet) and food consumption measured. In this experiment carcass refers to the body with the liver and epididymal fat pads removed. The results are given as means $\pm$ S.E.M.

Diet	1% corn oil		20% corn oil	
Feeding schedule.....	6 hr./day	24 hr./day	6 hr./day	24 hr./day
Body wt. (g.)	36.4 $\pm$ 1.0	38.1 $\pm$ 0.8	39.9 $\pm$ 0.5	40.2 $\pm$ 0.8
Liver wt. (g.)	2.27 $\pm$ 0.05	2.30 $\pm$ 0.08	2.27 $\pm$ 0.05	2.08 $\pm$ 0.05
Food consumption (g.)	5.5 $\pm$ 0.3	6.6 $\pm$ 0.7	5.5 $\pm$ 0.5	5.4 $\pm$ 0.2
[^{14}C]Glucose dose (counts/min.) ($\times 10^{-6}$)	2.2 $\pm$ 0.1	2.6 $\pm$ 0.3	2.0 $\pm$ 0.2	2.0 $\pm$ 0.1
Liver fatty acid				
% of liver*	4.19 $\pm$ 0.44	4.07 $\pm$ 0.18	3.84 $\pm$ 0.15	4.07 $\pm$ 0.26
Counts/min./liver ($\times 10^{-3}$)	12.6 $\pm$ 1.3	16.4 $\pm$ 2.2	1.94 $\pm$ 0.10	2.60 $\pm$ 0.10
Counts/min./liver (% of dose)	0.57 $\pm$ 0.06	0.62 $\pm$ 0.08	0.10 $\pm$ 0.01	0.13 $\pm$ 0.01
Liver cholesterol				
% of liver*	0.36 $\pm$ 0.03	0.29 $\pm$ 0.01	0.23 $\pm$ 0.01	0.29 $\pm$ 0.01
Counts/min./liver	14 $\pm$ 9	93 $\pm$ 20	47 $\pm$ 18	143 $\pm$ 8
Counts/min./liver (% of dose)	0.0006 $\pm$ 0.0004	0.0035 $\pm$ 0.0007	0.0022 $\pm$ 0.0004	0.0072 $\pm$ 0.0004
Carcass fatty acid				
% of carcass†	5.70 $\pm$ 0.97	8.86 $\pm$ 0.80	12.0 $\pm$ 0.5	13.1 $\pm$ 1.1
Counts/min./mouse ($\times 10^{-3}$)	203 $\pm$ 8	231 $\pm$ 21	151 $\pm$ 1	59 $\pm$ 2
Counts/min./mouse (% of dose)	9.3 $\pm$ 0.4	8.8 $\pm$ 0.8	7.4 $\pm$ 0.1	3.0 $\pm$ 0.1
Carcass cholesterol				
% of carcass†	0.21 $\pm$ 0.01	0.22 $\pm$ 0.01	0.21 $\pm$ 0.01	0.21 $\pm$ 0.01
Counts/min./mouse	420 $\pm$ 30	1560 $\pm$ 140	540 $\pm$ 30	1780 $\pm$ 100
Counts/min./mouse (% of dose)	0.019 $\pm$ 0.001	0.060 $\pm$ 0.005	0.027 $\pm$ 0.002	0.090 $\pm$ 0.005
Epididymal fat pad				
Total wt. (mg./pad)	234 $\pm$ 55	300 $\pm$ 19	497 $\pm$ 29	588 $\pm$ 75
Wt. of fat (mg./pad)	193 $\pm$ 53	273 $\pm$ 18	395 $\pm$ 25	519 $\pm$ 73
Wt. of fatty acid (mg./pad)	180 $\pm$ 47	262 $\pm$ 16	372 $\pm$ 22	476 $\pm$ 60
Total counts/min./pad	9020 $\pm$ 700	3730 $\pm$ 580	4170 $\pm$ 690	1760 $\pm$ 180
% of pad ^{14}C as fat	94 $\pm$ 1	95 $\pm$ 1	51 $\pm$ 3	63 $\pm$ 4
% of fat ^{14}C as fatty acid	87 $\pm$ 1	78 $\pm$ 1	46 $\pm$ 3	17 $\pm$ 1
Fatty acid ^{14}C /pad (% of dose)	0.34 $\pm$ 0.03	0.11 $\pm$ 0.02	0.056 $\pm$ 0.001	0.010 $\pm$ 0.001

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

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

other extrahepatic tissues demonstrated increased fatty acid synthesis during re-feeding or limited feeding to the greatest extent when the mice were fed with the diet containing 20% of corn oil. These findings suggest that the mechanisms involved in elevating lipogenesis in the liver under re-feeding or limited feeding conditions may not be the same as the mechanisms in extrahepatic tissues.

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