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Studies on Lipogenesis *in vivo*

COMPARISON OF CHOLESTEROL AND FATTY ACID SYNTHESIS IN RATS AND MICE

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1. The importance of fatty acid synthesis as a pathway for the disposal of ingested glucose has been evaluated in rats and mice given a purified diet high in glucose and low in fat. [U-^{14}C]Glucose was either added to the diet and fed for 24hr. or given by stomach tube as a 250mg. (mice) or 1000mg. (rats) meal. The two methods of isotope administration gave similar results. 2. Under the conditions employed fatty acid synthesis appeared to be a more important pathway for glucose disposal in mice than in rats. In mice 15·3% of ingested [U-^{14}C]glucose was converted into fatty acid and in rats the corresponding value was 8·6%. In contrast, the conversion of [U-^{14}C]glucose into cholesterol, as a percentage of dose, was twice as high in rats as in mice. 3. The effect of 20% of corn oil in the diet on the conversion of dietary [U-^{14}C]glucose into fat was also investigated. Mice given diets containing 1% or 20% of corn oil converted 14·6% or 7·0% respectively of dietary [U-^{14}C]glucose into fatty acid over a 24hr. period. There was no effect of fat on the incorporation of the isotope into cholesterol. 4. In mice given diets containing 1% or 20% of corn oil approx. 10% and 2% respectively of newly synthesized fatty acids were found in the liver. Hepatic fatty acid synthesis appears to be more sensitive to dietary fat than is extrahepatic synthesis.

In previous papers we described experiments in which lipogenesis was studied in mice by measuring the incorporation of a 250mg. meal of [U-^{14}C]-glucose, given by stomach tube, into fatty acids and cholesterol (Jansen, Hutchison & Zanetti, 1966*a*; Jansen, Zanetti & Hutchison, 1966*b*). We referred to this procedure as the glucose-meal technique. During these experiments results were obtained that suggested the possibility that rats and mice differ in the importance of lipogenesis as a pathway for the disposal of ingested glucose. Arguments have been presented that the use of the glucose-meal technique should minimize the problem of differential isotope dilution by decreasing the production of unlabelled acetyl-CoA from fatty acid or glycogen breakdown (Jansen *et al.* 1966*a*). Nevertheless it would be desirable to compare this technique with the procedure of giving the [^{14}C]-glucose in the diet for 24hr. In the present work we have compared lipogenesis from [U-^{14}C]glucose in mice with that occurring in rats by using both these methods of isotope administration. Also evaluated in mice was the effect of a moderate amount of dietary fat on lipogenesis.

METHODS

The techniques used in dosing, bleeding, preparing and analysing tissues, and analysing for radioactivity were those described previously (Jansen *et al.* 1966*a*), as were the conditions used for saponification of the carcasses (Jansen, Zanetti & Hutchison, 1966*c*). In the experiments described below, carcass refers to the body from which the liver and epididymal fat pads have been removed. The rats were of the Charles River CD strain and the mice were from the Merck, Sharp and Dohme Colony (ICR strain). Both species had been maintained for 1 week on a purified diet containing 1% of corn oil (diet 2; Jansen *et al.* 1966*a*) and only males were used. Details as to the number of animals in each group and their ages and weights are given in the legends to each of the Tables. The [U-^{14}C]glucose was either given as a 50% (w/v) solution by stomach tube or was fed in the diet for 24hr. The [U-^{14}C]glucose (10–15mc/m-mole) was obtained from New England Nuclear Corp. (Boston, Mass., U.S.A.). The animals were housed in individual screen-bottomed cages in an air-conditioned room maintained at approx. 24° and were supplied with food and water *ad libitum.*

RESULTS

The time-course of labelling in rats after the administration of a solution of [U-^{14}C]glucose by

Table 1. *Time-course of labelling in rats after* [U-^{14}C]*glucose meal*

The rats, six/group, weighing 270g. to 290g., were each given 1500mg. of [U-^{14}C]glucose (3μc) by stomach tube. The animals were then bled and killed at 30, 60 or 180min. after dosing. For 1 week before the experiment the rats were maintained on a low-fat high-glucose diet (diet 2; Jansen *et al.* 1966*a*). Food was removed from the cages at the time of dosing. The results are given as means ±S.E.M.

Time after [U-^{14}C]glucose (min.)	Plasma glucose (mg./100ml.)	Epididymal fat pads: Wt. of pad (mg.)	Epididymal fat pads: Counts/min./pad	Liver fatty acids Counts/min./g. of liver
0	114±13	—	—	—
30	180± 2	506±59	1150± 90	1403±160
60	172± 2	493±55	2350± 290	2520±300
180	139± 5	518±34	11090±2270	7160±810

stomach tube is shown in Table 1. The observed peak in the concentration of glucose in the plasma was reached in 30min. and was still slightly elevated after 3hr. Uptake of the label, by the epididymal fat pad, and its incorporation into fatty acid in the liver both continued to increase over the entire period studied and the total uptake by the epididymal fat pads after 3hr. was 5 times as great as the uptake after 1hr.

The incorporation of [U-^{14}C]glucose into fatty acid and cholesterol was measured in rats and mice after the administration of [^{14}C]glucose, either as a solution by stomach tube, or after feeding the [^{14}C]glucose in the diet for 24hr. The results are summarized in Table 2. For rats, only the incorporation rates for 3hr., after the single dose of [U-^{14}C]glucose, are given, but the values at 6hr. were also determined and were shown to be similar. The time-intervals were compared, and 2hr. in mice and 3hr. in rats were chosen since essentially maximal incorporation into fat has been obtained by these times. For both rats and mice, except for fatty acid synthesis in the liver, the similarity in conversion of the [^{14}C]glucose into fatty acid and cholesterol, as a percentage of the dose, by using these two methods of isotope administration was striking. Although the amount of glucose given by stomach tube was only 6–7% of that consumed over a 24hr. period it would appear that the metabolic fate of the small dose was reasonably representative of the fate of dietary carbohydrate consumed over the 24hr. period. In mice, the incorporation of ^{14}C into liver fatty acid was twice as high (as a percentage of dose), after the single dose of [^{14}C]glucose, as after giving the [^{14}C]glucose in the diet for 24hr. This occurred presumably because of the rapid turnover of liver fatty acids, which have a half-life of about 16hr. after a glucose meal (Jansen *et al.* 1966*a*). This difference was also seen in rats, although to a smaller degree.

Conversion of [U-^{14}C]glucose into fatty acid in both liver and extrahepatic tissues was almost twice as high, as a percentage of administered isotope, in mice as in rats when both species had been maintained for 1 week on the same high-glucose low-fat diet. The percentage of carcass fat (as fatty acid) was also twice as high in mice as in rats and this difference was reflected in the weight of the epididymal fat pads. In mice this fat depot represented almost 3 times the percentage of body weight as it did in rats. Conversion of [U-^{14}C]-glucose into fatty acid was several times as high, per g. of white adipose tissue (epididymal fat), as per g. of liver. If the results were expressed per mg. of N the comparison would be more striking because of the much lower nitrogen content of epididymal fat.

The rat converted approximately twice as much of the ingested [U-^{14}C]glucose into cholesterol in both the liver and extrahepatic tissues as did the mouse. However, the total conversion of [U-^{14}C]-glucose into cholesterol was only 2% and 1% respectively of the total conversion into fatty acid in rats and mice respectively.

These results confirmed that a small dose of [^{14}C]glucose given by stomach tube can give a reasonably good indication of the fate of dietary glucose. It was important to determine if this is is also true under conditions of lowered lipogenesis, such as result from giving a moderate amount of fat in the diet. Therefore purified diets containing 1% or 20% of corn oil were given to mice for 1 week; [U-^{14}C]glucose was added to the diet for the last 24hr. of this period. Data on weight gain and food consumption and on the uptake of [^{14}C]glucose by the epididymal fat pads are shown in Table 3. The uptake of [^{14}C]glucose by epididymal fat pads under these conditions was similar to that previously found after the [^{14}C]glucose meal (Jansen *et al.* 1966*a*). Results showing the incorporation of radioactivity into cholesterol and fatty acid in both liver and the remainder of the extrahepatic tissues are listed in Table 4. Again the similarity between these results and those obtained previously

Table 2. *Conversion of* [U-^{14}C]*glucose into fatty acid and cholesterol in rats and mice*

The rats, six/group, weighing 116–128g. each and approx. 5 weeks of age, were fed for 1 week on a low-fat high-glucose diet (diet 2; Jansen *et al.* 1966*a*). The average weight gain was 40·1g. One group of the rats was given 1000mg. of [U-^{14}C]glucose (10μc) by stomach tube and killed 3hr. later. The food was removed from the cages at time of dosing. [U-^{14}C]Glucose was added to the diet (0·4μc/g.), which was then given *ad libitum* to the remaining rats for the last 24hr. of the 7-day feeding period. The mice, eight/group, weighed 21–24g. each and also were approx. 5 weeks old. They were given diet 2 (Jansen *et al.* 1966*a*) for 1 week during which time the average weight gain was 5·5g. The mice were either given 250mg. of [U-^{14}C]glucose (2·5μc) by stomach tube and were killed 2hr. later or were given [U-^{14}C]glucose in the diet for 24hr. as just described. Where appropriate the results are given as means ±S.E.M.

	Rats		Mice	
Measurement	[U-^{14}C]Glucose by stomach tube (after 3hr.)	[U-^{14}C]Glucose in diet (24hr.)	[U-^{14}C]Glucose by stomach tube (after 2hr.)	[U-^{14}C]Glucose in diet (24hr.)
[U-^{14}C]Glucose dose (counts/min.) ($\times 10^{-6}$)	11·2	8·70±0·44	2·86	2·84±0·14
Epididymal fat pads				
Wt. of 2 pads (mg.)	790±70	720±50	375±27	324±41
Counts/min./2 pads ($\times 10^{-3}$)	42·4±4·0	35·6±3·2	16·4±1·5	17·0±2·6
Counts/min./g. of pad wt. ($\times 10^{-3}$)	53·7	49·4	43·7	52·5
Counts/min. in 2 pads (% of dose)	0·38	0·40	0·58	0·60
% of pad ^{14}C as fat	95·6±0·8	100·4±0·6	93·9±0·8	94·2±0·7
% of fat ^{14}C as fatty acid	82·9±2·6	86·2±0·4	81·9±0·6	84·2±1·3
Liver fatty acid				
% of liver*	3·84±0·73	2·55±0·04	4·49±0·32	4·29±0·31
Counts/min./g. of liver ($\times 10^{-3}$)	12·5±2·7	6·02±0·32	24·3±3·5	11·0±1·1
Counts/min./liver ($\times 10^{-3}$)	97·0±17·3	48·4±2·6	50·6±5·6	22·6±2·6
Counts/min. in liver (% of dose)	0·86	0·56	1·77	0·80
Liver cholesterol				
% of liver*	0·23±0·01	0·21±0·01	0·35±0·03	0·36±0·02
Counts/min./g. of liver	160±31	144±16	76±13	67±15
Counts/min./liver	1290±270	1150±120	166±33	140±15
Counts/min. in liver (% of dose)	0·011	0·013	0·006	0·005
Carcass fatty acid				
% of carcass†	4·34±0·24	3·67±0·19	11·33±0·58	9·09±0·94
Counts/min./g. of carcass	5·25	4·40	14·5	15·1
Counts/min./mouse or rat ($\times 10^{-3}$)	799±54	684±56	374±11	390±38
Counts/min. in carcass (% of dose)	7·1	7·9	13·1	13·7
Carcass cholesterol				
% of carcass†	0·20±0·01	0·20±0·01	0·24±0·02	0·25±0·02
Counts/min./g. of carcass	102	92	94	91
Counts/min./mouse or rat ($\times 10^{-3}$)	15·5±0·4	14·3±0·4	2·44±0·22	2·35±0·18
Counts/min. in carcass (% of dose)	0·14	0·16	0·08	0·08

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

† Wt. (g.) of fatty acid or cholesterol (after saponification)/100g. (body wt. − liver wt. − wt. of 2 fat pads).

(Jansen *et al.* 1966*a*) is apparent. Fatty acid synthesis in the liver is seen to be more sensitive to inhibition by dietary fat than synthesis outside the liver. Incorporation of ^{14}C into cholesterol was not significantly affected by the dietary corn oil in either the liver or extrahepatic tissues.

DISCUSSION

Results comparing the overall conversion of ingested [U-^{14}C]glucose into fatty acid in rats and mice have been summarized in Table 5. It would appear that, under the conditions employed, fatty acid synthesis was a more important pathway for the disposal of ingested glucose in mice than in rats. These results are possibly applicable only to the specific strains of animals and the diet used. Nevertheless it would appear to be desirable to compare the results obtained with those previously published.

Masoro, Chaikoff & Dauben (1949) found that mice, given a high-carbohydrate diet labelled with

Table 3. *Effect of amount of dietary fat on incorporation of* [U-^{14}C]*glucose into epididymal fat in mice*

The mice, eight/group, were fed for 1 week on either diet 2 or diet 5 (Jansen *et al.* 1966*a*). For the last 24hr. of the feeding period [U-^{14}C]glucose was added to the diet (0·4μg./g. of diet) and food consumption measured. Where appropriate results are given as means ±S.E.M.

	1% of corn oil	20% of corn oil
Initial body wt. (g.)	25·6±0·3	25·7±0·3
Final body wt. (g.)	30·8±0·5	32·0±0·4
Food consumption (g./day)	6·8±0·4	5·9±0·3
[U-^{14}C]Glucose consumption (counts/min./day) ($\times 10^{-6}$)	3·04±0·18	2·66±0·14
Epididymal fat pads		
Wt. of 2 pads (mg.)	527±33	684±30
Counts/min./2 pads ($\times 10^{-3}$)	22·4±4·7	13·2±2·2
Counts/min. in 2 pads (% of dose)	0·72	0·48
% of pad ^{14}C as fat	94·8±1·2	81·5±3·0
% of fat ^{14}C as fatty acid	82·4±1·3	43·3±2·6

Table 4. *Effect of amount of dietary fat on incorporation of* [U-^{14}C]*glucose in liver and carcass of mice*

For experimental details see Table 3. Where appropriate results are given as means ±S.E.M.

Dietary fat	1% of corn oil	20% of corn oil
Liver fatty acid		
% of liver*	5·05±0·35	5·40±0·22
Counts/min./g. of liver ($\times 10^{-3}$)	11·5±1·8	2·68±0·30
Counts/min. in liver (% of dose)	0·78	0·21
Liver cholesterol		
% of liver*	0·41±0·05	0·31±0·01
Counts/min./g. of liver	76±17	74±16
Counts/min. in liver (% of dose)	0·005	0·006
Carcass fatty acid		
% of carcass†	10·4	13·9
Counts/min./mouse ($\times 10^{-3}$)	400±86	168±32
Counts/min. in carcass (% of dose)	13·1	6·3
Carcass cholesterol		
% of carcass†	0·22±0·02	0·20±0·01
Counts/min./mouse ($\times 10^{-3}$)	2·50±0·40	2·16±0·30
Counts/min. in carcass (% of dose)	0·08	0·08

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

† Wt. (g.) of fatty acid or cholesterol (after saponification)/100g. (body wt. − liver wt. − wt. of 2 epididymal fat pads).

[U-^{14}C]glucose, incorporated 11% of the ingested ^{14}C into fatty acid during a 24hr. period of feeding *ad libitum*. Patkin & Masoro (1964) reported that 4·7% of absorbed [U-^{14}C]glucose was converted into fatty acid in rats 6hr. after a 1g. meal of labelled glucose. Masoro, Asuncion, Brown & Rapport (1957) had studied conversion of [U-^{14}C]-glucose into fatty acid in rats; the labelled glucose was added to the diet, which was given for 24hr., and 2·8% of the ingested [^{14}C]glucose was recovered in total body fatty acid.

Lequin & Steyn-Parvé (1962) administered [U-^{14}C]glucose, by stomach tube, to rats and found that only 0·5–2·3% of the absorbed [U-^{14}C]glucose was converted into fatty acid. DeFreitas & Depocas (1965) continuously infused [U-^{14}C]glucose into rats and found that 4% of the [U-^{14}C]glucose removed from the plasma was converted into fatty acid. In their experiments plasma glucose was maintained at approx. 250mg./100ml. by infusion of unlabelled glucose for 1hr. before the [U-^{14}C]-glucose was added.

Table 5. *Conversion of* [U-^{14}C]*glucose into total body fatty acid*

Except where noted all data are taken from Tables 2–4 of this paper.

	Conversion into fatty acid (% of dose)			
	Mice (1%-corn oil diet)		Rats (1%-corn oil diet)	
	250 mg. meal	24 hr. feeding	1000 mg. meal	24 hr. feeding
Fatty acid compartment				
Liver fat	1·8	0·8	0·9	0·6
Epididymal fat	0·6	0·5	0·4	0·3
Remainder of extrahepatic fat	13·1	13·7	7·1	7·9
Total body fatty acid	15·5	15·0	8·4	8·8

	Conversion into fatty acid (% of dose)			
	Mice (1%-corn oil diet)		Mice (20%-corn oil diet)	
	250 mg. meal	24 hr. feeding	250 mg. meal	24 hr. feeding
Fatty acid compartment				
Liver fat	1·4*	0·8	0·2*	0·2
Epididymal fat	0·4*	0·6	0·2*	0·2
Remainder of extrahepatic fat	13·2*	13·1	8·0*	6·3
Total body fatty acid	15·0	14·5	8·4	6·7

* Data derived from Table 5 of Jansen *et al.* (1966*a*).

Because of the decarboxylation of pyruvate to form acetyl-CoA, if 30% of the ingested glucose were directed toward fatty acid synthesis, as estimated by Stetten & Boxer (1944) from deuterium studies in rats, 20% of the glucose carbon would be incorporated into fatty acid. The maximum incorporation of [U-^{14}C]glucose into fatty acids that we have observed is 75 and 45% of this for mice and rats respectively. It is noteworthy that Leveille (1966) has reported that mouse adipose tissue incorporated significantly more [1-^{14}C]acetate into fatty acids *in vitro* than did comparable tissue from rats.

It is evident that, with either method of isotope administration, the presence of 20% of corn oil in the diet decreased overall fatty acid synthesis by about 50% and synthesis in the liver by 85%. We do not know at this time whether corn oil is typical of dietary fat in general. Reiser, Williams, Sorrels & Murty (1963) investigated the effects of a number of single triglycerides and natural fats on the incorporation of [1-^{14}C]acetate into fatty acid and cholesterol; they found that chain length and saturation of the dietary fatty acids both affected conversion of [1-^{14}C]acetate into fatty acid.

Our results suggest that, in a mixed diet in which over 40% of the calories are supplied by fat, new fatty acid synthesis is of little quantitative importance. Also, under these conditions the conclusion of Favarger & Gerlach (1958), that the conversion of glucose into fatty acid in the liver is of little importance, appears to be valid.

Another important consideration is the role of white adipose tissue in lipogenesis. At present it is widely held that much of the fatty acid-synthetic ability in the body is located in this tissue (Cahill, 1964; Rodahl & Issekutz, 1964). The experimentation of Favarger and co-workers has given support to this concept (Favarger, 1964). However, Patkin & Masoro (1964), from work *in vivo* with rats, have suggested that white adipose tissue is not an important site of conversion of glucose into fatty acid. Our experiments show that, depending on the fat content of the diet, 90–98% of the newly synthesized fatty acid is made outside the liver. However, our results also show that epididymal adipose tissue is less active synthetically than the average of the rest of the extrahepatic tissues. Epididymal adipose tissue of mice, given a high-carbohydrate diet, contained 14% of the total fatty acid but only 4% of the labelled fatty acid. Results presented previously have shown that the turnover of epididymal fat is also considerably less than the remainder of extrahepatic tissues (Jansen *et al.* 1966*a*).

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