112. FAT-DEFICIENCY DISEASE OF RATS. THE STORAGE OF FAT IN THE FAT-STARVED RAT

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BURR & BURR [1930], to whom we owe the first description of the fat-deficiency disease of rats, originally attributed the curative effect of the essential unsaturated fatty acids to their power of promoting fat synthesis. According to these observers: 'These small emaciated animals on the fat-free diet consume as much food daily as their larger fat-containing controls yet this food is burned and none is used in growth and fat synthesis. On the addition of the unsaturated fatty acid growth is resumed and a normal amount of subcutaneous and visceral fat is found in the cured animal at autopsy. Therefore fat synthesis is dependent upon the presence in the tissues or blood stream of a certain minimum quantity of the essential fatty acids. What percentage of the growth observed is due to storage of fat and what percentage is due to increase in other compounds (protein and carbohydrate) is not yet known.'

Subsequent investigations of the respiratory quotient, at various stages of the fat-deficiency disease, carried out by Burr and his colleagues indicated that the formation of fat from carbohydrate must be taking place [Wesson & Burr, 1931; Burr & Beber, 1932]. Further confirmation of the synthesis of fatty acid from carbohydrate was supplied by an investigation of the rate of synthesis of phospholipin in the organs of the fat-starved rat. The rate of turnover of phosphorus in the liver, kidney and muscle of the fat-starved rat was as high as in those in which the fat-free diet had been supplemented with methyl linoleate; indeed in muscle tissue, the rate of phospholipin synthesis was significantly higher in those animals which had been fat-starved [Hevesy & Smedley-MacLean, 1940].

Burr & Burr recorded that the fat stores were depleted in this disease but they do not appear to have made any actual estimations of the amount of fat present. Smedley-MacLean & Nunn [1940] found an unexpectedly high percentage of fat in the skin and carcass of rats which had been fat-starved for 197 days and which had then received nine doses of methyl arachidonate; each dose was one drop of the ester weighing approximately 13 mg. given at intervals of 4 days, thus constituting an average daily dose of ' $\frac{1}{4}$ drop'. The percentage of fat was actually higher in these animals than in those which had received four times as much arachidonate and in which regular growth had been resumed. At that time no estimations of the amounts of fat stored by the rat fed for a long time on the fat-free diet were available. Accepting the view that depletion of the fat stores was characteristic of the fat-starved rat, Smedley-MacLean & Nunn [1940] concluded that in their experiments the filling of the fat stores of the rats had been caused by the administration of the minute doses of methyl

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arachidonate. The diminished percentage of body fat and the increased body weight found in the rats receiving the larger doses of arachidonate were attributed to the effect of these larger doses [Hume *et al.* 1940; Smedley-MacLean & Nunn, 1940].

The present investigation was therefore undertaken to determine the condition of the fat stores in rats kept for so long on the fat-free diet that most of the highly unsaturated acids which were originally present would have disappeared.

As in previous investigations, the rats were weaned when 3 weeks old and at once placed on the fat-free diet: the technique followed was fully described in a previous communication [Hume *et al.* 1938].

Since the rats were supplied with a diet from which all ether-soluble substances had been removed the food administered could not have contained any considerable amount of choline. We did not however find that the addition of choline to the diet caused any significant improvement either in growth rate or in the general condition of the animals. The absence of choline had not therefore in these experiments acted as a limiting factor. This is in agreement with the experience of Jacobi *et al.* [1941] who found that the choline content of rats on choline-free diets increased as the body-weights of the rats increased, indicating the synthesis of choline. These authors found that in one case 76 mg. choline were synthesized by one rat in 8 weeks.

Estimation of lipoid matter. The procedure followed was that described by Smedley-MacLean & Nunn [1941] except that the head, tail and paws of the rat were minced with the rest of the carcass. The tissues examined formed approximately 85% of the total weight of the rat. When the weight of total lipoid matter (i.e. the amount of ether-soluble material extracted from the tissue by alcohol and ether) was compared with the weight of fatty acids derived from it, the fatty acid fraction comprised in the skin 95%, in the carcass about 91% of the total lipoid matter. As we were here concerned only with large differences in the amounts of fat stored, the weights of total lipoid material seemed adequate as the basis of comparison. The residual tissue after ether extraction was air-dried for 24 hr. at the laboratory temperature and its weight taken as the 'dry weight'.

Estimation of polyunsaturated acids. The procedure described by Smedley-MacLean & Nunn [1941] was followed, except that the bromination was always

Table 1. *Showing changes in weight (g.) of rats on fat-free diet with and without supplements of linseed oil and methyl arachidonate*

Group	I	II	III	IV	V	VI	VII	VIII
No. of rats in group	1	2	2	1	3	3	3	1
No. of days on fat-free diet	172	280	280	330	197	197	197	330
Subsequent treatment	—	—	—	—	' $\frac{1}{2}$ drop' A for 35 days, then 37 days fat-free diet	' $\frac{1}{2}$ drop' A for 35 days	' $\frac{1}{2}$ drop' A for 68 days	Received for 240 days varying doses of arachidonate, then 0.1 g. linseed oil daily till killed
Weights of rats on 197th day		174 172	176 181	138	173 181 144	172 164 135	165 173 175	214
Change in weight from 197th day to death		-17 + 5	- 8 -16	0	+3 +7 +5	-11 - 1 + 1	-10 + 4 +11	+30

carried out in ether solution. Previous bromine estimations on similar material have shown that the insoluble bromide precipitate consisted almost entirely of the bromides of the C_{20} and C_{22} acids; the quantities of the bromides obtained were in many cases so small that estimations of bromine were not carried out on the insoluble bromide precipitates.

The proportion of lipid material in the tissues of the fat-starved rat

The figures given in Table 2 show that the ratio of total lipid matter to the weight of dry matter left after the ether extraction is quite as high in the carcass and skin of the fat-starved rat as in the corresponding tissue from rats fed on a

Table 2. *Showing the proportions of lipid matter and of arachidonic acid in the tissues of fat-starved rats and in those of rats receiving supplements of esters of unsaturated acids*

Group	No. of rats	No. of days fat-starved	Rats receiving only fat-free diet			% wt. arachidonic, dry wt.		
			% wt. lipid, dry wt.					
			Skin	Carcass	Liver	Skin	Carcass	Liver
I	1	172	65.5	>32.2*	22.9	0.04	0.19	0.30
II	2	280	80.9	40.1	17.4	0.00	0.14	0.57
III	2	280	78.1	46.5	19.9	0.04	0.35	0.30
IV	1	330	93.1	30.9	20.9	0.06	0.09	0.31
Rats receiving $\frac{1}{4}$ drop' doses of methyl arachidonate after being kept for 197 days on fat-free diet only								
V	3	Dosed for 35 days, then given fat-free diet only for 37 days	86.9	29.1	16.5	0.04	0.16	0.80
VI	3	Dosed for 35 days, then killed	65.5	32.8	18.8	0.00	0.28	1.28
VII	3	Dosed for 68 days, then killed	75.3	27.2	19.4	0.23	0.31	1.28
Rat receiving, from time of weaning, with the fat-free diet a daily dose of 7-50 mg. methyl arachidonate or of linseed oil (0.1 g.) for 330 days, serving as control								
VIII	1	—	75.0	41.9	14.4	0.65	0.74	1.01

* About 1/5 of the total volume of the ethereal solution of fat was lost.

normal diet rich in fat—indeed the highest values recorded were in the fat-starved rats. The number of rats killed after a prolonged period of fat starvation without receiving curative doses was not large but it is interesting to see that in the skin of these animals, the proportion of lipid matter to fat-free dry-weight rose as the period of fat-starvation continued.

	% lipid to dry wt. of		
	Skin	Carcass	Liver
1 rat fat-starved 172 days	65.5	>32.2*	22.9
2 rats fat-starved 280 days	80.9	40.1	17.4
2 rats fat-starved 280 days	78.1	46.5	19.9
1 rat fat-starved 330 days	93.1	30.9	20.9

* About 20 % of the fat was lost.

It will be seen from the figures given in Table 2 that the proportion of lipid is somewhat lower in the rats which had received the $\frac{1}{4}$ drop' doses of arachidonate up to the day on which they were killed. The one rat which had been fed with unsaturated acids in addition to the fat-free diet (group VIII) showed a high proportion of lipid, similar to that recorded for rats on normal diet.

Table 3. *Showing actual weights recorded of (1) wet weight of tissue, (2) dry weight (fat-free), (3) total lipid matter and (4) ether-insoluble bromide in fat-starved rats and in those receiving minimal doses of methyl arachidonate*

Group	No. of rats	No. of days on completely fat-free diet	Subsequent treatment	Skin				Carcass				Liver			
				Wet wt. g.	Dry wt. g.	Wt. lipid g.	Wt. ether-insoluble bromide mg.	Wet wt. g.	Dry wt. g.	Wt. lipid g.	Wt. ether-insoluble bromide mg.	Wet wt. g.	Dry wt. g.	Wt. lipid g.	Wt. ether-insoluble bromide mg.
I	1	172	Killed at once	27.1	8.7	5.7	2.6	104.1	27.9	>9.0	41.8	12.0	2.84	0.65	6.6
II	2	280	"	54.5	17.2	13.9	0	215.6	58.1	23.3	63	18.0	3.84	0.67	17
III	2	280	"	57.2	16.9	13.2	5	218.8	54.2	25.2	145?	18.4	3.67	0.73	8
IV	1	330	"	20.3	5.8	5.4	4.9	91.5	23.6	7.3	16.5	7.3	1.53	0.32	3.7
Fat-starved rats receiving minimal doses of arachidonate															
V	3	197	35 days '1/4 drop' doses arachidonate, then 37 days on fat-free diet	81.8	24.1	20.9	8	317.4	85.8	25.0	104	27.4	6.0	0.99	37
VI	3	197	35 days '1/4 drop' doses arachidonate, then killed	75.6	23.2	15.2	0	332.8	88.6	<20.1	194	25.0	5.3	1.00	52
VII	3	197	68 days '1/4 drop' doses arachidonate, then killed	84.7	25.5	19.2	43	348.9	94.2	25.6	227	27.8	6.1	1.18	60
VIII	1	—	Fat-starved rat receiving 7-50 mg. methyl arachidonate or 0.1 g. linseed oil daily with fat-free diet, for 330 days	38.7	12.0	<9.0	60	173.1	43.2	18.1	247	11.4	2.5	0.36	19

The ' $\frac{1}{4}$ drop' doses of arachidonate were insufficient to produce significant increase in weight, though in the majority of the animals the loss in weight was arrested (Table 1).

The proportion of arachidonic acid in the tissues of the fat-starved rat

One of the most striking features of the estimation of the polyunsaturated acids in the fat-starved rat is that while the amount of this acid fell to a very low level during the first 6 months of fat-starvation it remained almost constant in those rats which had had varying periods of fat-starvation ranging from 172 to 330 days (Table 2, groups I-IV). This indicated that during a period of more than 6 months, the variations were almost within the limits of biological variation. Since none of this acid was being supplied, and it apparently cannot be manufactured unless some polyunsaturated acid such as linoleic is given, its rate of utilization in the second 6 months of fat-starvation must have been exceedingly small. During this period, the rat was still able to carry out the functions necessary for life but the growth of the animal ceased, the proportion of fat in the skin and carcass increased and the skin became dry and scaly, the *stratum corneum* being no longer thrown off but remaining as a hard thick layer.

Our interpretation of these results is that the small amount of arachidonic acid, so long and tightly retained in the tissues, was still sufficient to allow those functions which are essential for life to be maintained. In the process of development of new cells both to replace the worn-out cells of the body and to form the new tissues necessary for growth, the polyunsaturated acids must be utilized and some part of them is probably destroyed in the process of utilization. Smedley-MacLean & Nunn [1940] have shown that when the curative doses of the essential acids given to the fat-starved rats were sufficient to cause resumption of growth, a considerable proportion of the arachidonic acid which had been given could not be detected in the skin or carcass. Available evidence seems therefore to point to the view that a free supply of polyunsaturated acid is an essential condition for the formation of new tissue.

The rat, deprived of its essential unsaturated acid, continued to live for 6 months after growth had ceased before being killed. This behaviour is in striking contrast to that of the rat suffering from a deficiency of vitamin A or B₁ where fatal effects rapidly supervene after cessation of growth. It seems probable that a distinction must be made between those factors, a free supply of which is essential for the maintenance of the normal life of the cell and those which must be furnished when new tissue is to be formed.

It may perhaps be noted that where food for the embryo is to be provided, these acids are present in considerable quantity. It is well known that seed fats, where fat is to be utilized for the growth of the embryo, are characterized by a high proportion of linoleic acid [Hilditch, 1940]. The yolk of the egg is also rich in these acids; Nishimoto [1934], in an investigation on the phospholipins of egg-yolk, claims to have isolated a number of kephalins, rich in arachidonic acid, a di-arachidonyl kephalin being described. It is interesting that here, where material is prepared especially for the maintenance of embryo growth, it is the kephalins which are especially rich in arachidonic acid.

Feeding the fat-starved rats with very small doses of arachidonate insufficient to promote growth, improved the general condition of the rats and in the majority of cases stopped the loss of weight; under these conditions only very small changes in the proportion of this acid present in the skin and carcass were

observed; indeed any change was within the limits of variation except in the skin of the rats which had received the minimal ' $\frac{1}{4}$ drop' doses for as long as 68 days where the value retored was significantly higher. On the other hand, all rats which had received these minimal doses showed a notable increase in the proportion of polyunsaturated acid present in the liver. Whereas in the completely fat-starved rats, the proportion of arachidonic acid to the fat-free dry liver tissue lay between 0.30 and 0.57%, in those rats which had received 35 and 68 ' $\frac{1}{4}$ drop' doses the value rose to 1.27%. Even in a group which, after 197 days of fat-starvation, had received 35 daily doses of ' $\frac{1}{4}$ drop' arachidonate and then resumed for 5 weeks the fat-free diet, the liver value was still 0.80%. It seems from these results that the liver has some important part to play in utilizing the arachidonic acid, possibly in some process which furnishes a factor essential for the growth of new cells.

SUMMARY

1. Rats were kept on a fat-free diet for periods varying from 172 to 330 days and the proportions of (a) total lipid and (b) arachidonic acid to fat-free dry weight determined in skin, liver and carcass. In the subcutaneous tissue the proportion of total lipid increased with the duration of fat-starvation.

2. The proportion of lipid to fat-free dry weight showed no diminution when compared with the corresponding values in a rat which had received linseed oil in addition to the fat-free diet throughout the whole period. There was therefore no evidence of any diminished fat-storage in the fat-starved rat.

3. From the 6th to 11th months of fat-starvation, there was very little change in the ratio of arachidonic acid to fat-free dry weight in skin, liver and carcass.

4. The proportion of arachidonic acid to fat-free dry weight was very low in the subcutaneous tissue of the fat-starved rat. The amount of this acid in the subcutaneous tissue was not increased significantly when minimal doses of ' $\frac{1}{4}$ drop' methyl arachidonate were given to rats for 5 weeks after 197 days of fat-starvation: after 10 weeks' dosing, a definite increase in the arachidonic content was detected. The change was less apparent in the carcass.

5. The level of arachidonic acid in the liver rose immediately and markedly on giving minimal doses of the curative acid even for a short period.

6. One rat received a daily dose of 7-50 mg. methyl arachidonate or of 0.1 g. linseed oil with the fat-free diet for a period of 330 days from weaning. Here the percentage of highly unsaturated acid in the skin was especially high.

7. The conclusion was drawn that a plentiful supply of highly unsaturated acid was necessary for the formation of new tissue but not for the maintenance of the normal metabolism of the cell.

8. The addition of choline to the diet produced no significant effect.

REFERENCES

- Burr & Burr (1930). *J. biol. Chem.* **86**, 587.
— & Beber (1932). *J. biol. Chem.* **97**, xxxvi.
Hevesy & Smedley-MacLean (1940). *Biochem. J.* **34**, 1940.
Hilditch (1940). The chemical constitution of natural fats. Chapman and Hall.
Hume, Nunn, Smedley-MacLean & Smith (1938). *Biochem. J.* **32**, 2162.
— — — (1940). *Biochem. J.* **34**, 879.
Jacobi, Baumann & Meek (1941). *J. biol. Chem.* **138**, 571.
Nishimoto (1934). *Proc. imp. Acad. Japan*, **10**, 578.
Smedley-MacLean & Nunn (1940). *Biochem. J.* **34**, 884.
— — — (1941). *Biochem. J.* **35**, 983.
Wesson & Burr (1931). *J. biol. Chem.* **91**, 525.