113. FAT-DEFICIENCY DISEASE OF RATS. THE INFLUENCE OF TUMOUR GROWTH ON THE STORAGE OF FAT AND OF POLYUNSATURATED ACIDS IN THE FAT-STARVED RAT

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SINCE the growth of tumours in rats, fed on a normal diet rich in fat, seems to be associated with a considerable fall in the amount of highly unsaturated acids present in the subcutaneous fat [Smedley-MacLean & Nunn, 1941] there was a possibility that if the level of this acid could be sufficiently reduced, growth of the tumour might be retarded or even prevented. In previous experiments it had been found that growth of the rat on a fat-free diet ceased in 5–6 months. The level of the essential unsaturated acids would presumably be considerably reduced at an earlier date and we decided to keep rats for 14 weeks on a fat-free diet and then implant tumours. The rats chosen were 30–50 g. in weight. We again enlisted the cooperation of Dr Haddow, who implanted Walker tumour tissue both in these animals and in a set of controls which had received in addition to the fat-free diet a daily supply of linseed oil. The tumours were implanted on the 97th day of fat-starvation and the same fat-free diet was continued with and without the addition of linseed oil until the 113th day when the animals were killed and the analyses made as described in our previous communication. The results are set out in Tables 1 and 2.

Changes in the relative proportions of lipid matter in the tissues of fat-starved rats in which tumours had been implanted

The most striking changes in the relation of lipid matter to fat-free dry weight were those found in the skin and carcass of rats in which tumours had been implanted and had grown to large size in 14 days. These are set out in Table 1. A particularly interesting comparison was afforded by the rats in groups XI and XII which had received exactly the same diet throughout the experiment; it consisted of the basic fat-free ration supplemented by a daily dose of about 100 mg. linseed oil. After a fortnight, in 2 of the rats (group XII) the tumours had developed very slightly and seemed to be regressing. Group XI contained 5 similar rats in which large tumours had developed. The proportion of dry tumour tissue calculated on the dry weight of the total amount of tissues examined was 10 times as much per rat in the 5 rats of group XI as in the 2 rats of group XII where the tumours had failed to reach any considerable size. The fall in the proportion of lipid to dry weight in the tumour-bearing animals was very striking (group XI, skin 44.6%, carcass 23.4%) when contrasted with the high values found in group XII (skin, 90.4%, carcass, 34.5%).

This ratio (lipid : fat-free dry weight) was also very low in the tumour-bearing rats on fat-free diet when compared with that found in normal fat-starved

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Table 1. *Showing the percentage proportions of (1) lipid matter and (2) highly unsaturated acid (calculated as arachidonic acid) to fat-free dry weight in the tissues of fat-starved rats and in those of rats receiving supplements of linoleic acid, after Walker tumours had been implanted*

	% Lipoid			% Arachidonic		
	Skin	Carcass	Liver	Skin	Carcass	Liver
A. Rats fed only on fat-free diet for 113 days. <i>Tumours</i> implanted on 97th day, <i>grew well</i>						
Group IX, 4 rats	42.2	26.7	16.3	0.04	0.30	0.9
Group X, 3 rats	28.6	20.3	14.4	0.05	0.33	1.3
B. Rats fed on fat-free diet with 0.1 g. linseed oil daily for 113 days. <i>Tumours</i> implanted on 98th day						
Group XI, 5 rats. <i>Tumours grew well</i>	44.6	23.4	17.2	0.97	1.56	4.2
Group XII, 2 rats. <i>Tumours remained as small nodules and did not grow further</i>	90.4	34.5	24.7	2.33	1.09	4.1
C. Rats fed on normal diet until tumours implanted, then transferred to fat-starved diet for 14 days						
Group XIII, 3 rats. <i>Tumours grew well</i>	37.6	19.6	22.9	0.77	2.25	5.8
Group XIV, 3 rats. Linseed oil added to fat-free diet. <i>Tumours grew well</i>	31.4	21.7	25.5	0.62	1.72	2.3
D. Normal values found in rats without tumours (for comparison)						
Rats fed on full normal diet						
Mean value	67.5	35.7	16.8	1.33	1.77	3.2
Rats fed on fat-free diet for 172-330 days						
Mean value	82.1	37.1	18.2	0.03	0.21	0.4

rats. Thus in the rats in which no tumours had been implanted the values were for the skin 65.8-98.3%, for the carcass 30.8-46.4%. When the tumours were present, the values had fallen to 28.6-42.2% in the skin and 20.3-26.7% in the carcass. The difference in these fat-starved rats is very much more striking than in previous experiments on rats which had received a normal diet, rich in both fat and unsaturated acid, during the period of tumour growth [Smedley-MacLean & Nunn, 1941].

In the liver no such sharply marked division was apparent. The figures for the tumour-bearing animals did not differ very greatly from those for the rats in which no tumours had been implanted. In the two similar groups which had received the same diet of fat-free ration with the addition of linseed oil, the proportion of liver lipid in the resistant group was considerably higher than that of the group in which large tumours had developed, but the variations in the whole series were irregular and gave no clear indication of any special lowering of the amount of lipid in the livers of the tumour-bearing animals.

The estimations of the total lipid material as a basis of comparison are less satisfactory in the liver than in the skin and carcass, since the liver lipid contains a greater proportion of ether-soluble substances other than fatty acid.

This diminution of the proportion of lipid material (mainly fatty acid) to fat-free dry weight, so strongly marked in the skin and carcass of the rats in which actively growing tumours were present, again suggests that there is some

Table 2. Showing actual weights recorded of (1) wet weight of tissue, (2) fat-free dry weight, (3) total lipid matter and (4) ether-insoluble bromide in rats after implantation of Walker tumours

Group	No. of rats		Skin						Carcass						Liver						Tumour					
			Wet			Wt. ether-insoluble			Wet			Wt. ether-insoluble			Wet			Wt. ether-insoluble			Wet			Wt. ether-insoluble		
			wt.	Dry wt.	lipoid	wt.	g.	mg.	wt.	g.	mg.	wt.	g.	mg.	wt.	g.	mg.	wt.	g.	mg.	wt.	g.	mg.	wt.	g.	mg.
IX	4	Rats received only fat-free diet; tumours implanted on 97th day and rats killed on 113th day	99.3	31.5	13.3	8.8	353.7	97.5	26.1	228	42.2	9.5	1.55	63.3	50.0	6.6	1.20	9.7								
X	3		56.9	20.3	5.8	7.3	265.5	63.9	13.0	163	29.7	6.2	0.89	63.4	56.5	7.5	1.15	6.0								
XI	5	Rats received in addition to fat-free diet 0.1 g. linseed oil daily throughout period of experiment; tumours implanted on 97th day. Rats killed on 113th day	111.2	33.6	15.0	252	388.7	106.9	25.0	1283	53.9	10.9	1.88	349.2	65.4	8.8	1.55	131.7								
XII	2		55.7	15.6	14.1	280	205.1	53.8	18.6	457	18.2	4.3	1.06	134.6	2.8	0.34	0.10	2.9								
XIII	3	Rats of about 100-150 g. wt. which had been fed on normal diet transferred to fat-free diet for 14 days after tumours implanted	48.3	14.7	5.5	86.5	182.0	45.9	9.0	794	24.7	5.2	1.19	231.6	—	6.6	1.77	103.2								
XIV	3	Rats similar to group XIII transferred to fat-free diet + daily dose linseed oil for 14 days after tumour implanted	66.2	19.7	6.2	93.5	244.8	65.9	14.3	870	29.0	6.4	1.63	111.8	—	7.0	1.72	120.2								

connexion between the rapid growth of new tissue and the diminution of sub-cutaneous and carcass fat. A similar phenomenon had already been noted when curative doses of the essential unsaturated acids were given to fat-starved rats in which growth had previously ceased, and may possibly indicate that fatty acids have some direct part to play in the formation of new tissue, when the essential unsaturated acids are supplied. In the fat-starved rats, depletion of fat might be brought about by taking away fat from the fat stores or by inhibiting the synthesis of fat from the freely supplied carbohydrate. There is, however, no indication that there is any interference with the process of conversion of carbohydrate into fat in the fat-starved rat.

The wasting diseases in human subjects in which the fat stores become much depleted are usually associated with fever and increased metabolism or with a diminished food intake caused by starvation whether brought about by lack of food, loss of appetite or mal-absorption. It is possible, however, that utilization of fat in tumour growth might also prove a factor in bringing about the characteristic cachexia of the cancer patient. Here too depletion of the fat stores might be increased because fatty acid was being directly used to provide the growing tumour with some factor necessary for the formation of new cells.

Determinations of the respiratory quotient in slices of the liver and tumour of rats were made by Dickens & Šimer [1930] who found them to be well below unity. The mean value for liver was 0.79, for the Jensen rat sarcoma 0.81–0.84 and slices of tumours of chick, mouse, rat and man gave values from 0.81 to 0.85. The simplest explanations of the low R.Q. is that these tissues are directly burning fat and though evidence from R.Q. determinations is difficult to interpret, they at least indicate that fatty acids are directly oxidized in the tumour cell.

*Changes in the proportion of highly unsaturated acid to dry weight
during tumour growth*

Unfortunately no control rats were available which had been starved for 113 days, the experimental period during which the rats in groups IX and X were kept on the fat-free diet. After 113 days the proportions of arachidonic acid in the skin and carcass of the tumour-bearing rats were comparable with the values found in normal rats fat-starved for the much longer period of 172–330 days. Since increase in weight continues in the fat-starved rat for 150–160 days, it is probable that there was an increased diminution in the proportion of highly unsaturated acid during the period of tumour growth, but satisfactory controls are not available to establish this. In the livers of the tumour-bearing animals the proportion of the unsaturated acid was much higher than in the animals fat-starved for the longer period.

In the two comparable groups, XI and XII, both of which comprised rats fed for 113 days on the basic fat-free diet supplemented by linseed oil, tumours grew well in the 5 animals of group XI whereas in the two animals of group XII the tumours remained as small nodules and failed to develop further. The proportions of highly unsaturated acid to dry weight in the livers of the two groups were practically identical. In the skins of the animals where the tumours had grown freely the proportion of highly unsaturated acid to dry weight of the tissue was strikingly diminished. This is in agreement with the results obtained by Smedley-MacLean & Nunn [1941] who found that when rats fed on a normal diet in which large tumours had grown were compared with normal control animals on the same diet, the proportion of highly unsaturated acid to fat-free dry weight in the skin was always diminished in the tumour-bearing animals. In the rats examined, the skin contained one-third to one-half as much fat as the

rest of the carcass (exclusive of liver and alimentary canal) so that it contained an important part of the storage fat of the whole animal; the results so far obtained suggest that when tumour growth or the restored growth of the fat-starved rat occurs it is especially in the skin that a fall in the arachidonic acid content takes place.

In the two groups XIII and XIV, tumours were implanted in rats previously fed on a normal diet rich in fat; the animals were then transferred to the fat-free diet and those in group XIII received only this until they were killed a fortnight later. The animals in group XIV received in addition a supplement of linseed oil. The values for the proportion of highly unsaturated acid to fat-free dry weight were again very low even in those which had received the linseed oil, when compared with the values found in similar rats on normal diet.

These experiments furnished no evidence therefore that a reduction of the level of the highly unsaturated acid retarded tumour growth. The tumours were implanted on the 97th day of fat starvation and the animals were killed on the 113th day. Rats, however, on this fat-free diet do not attain their maximum weight until the 150–160th day and from the 97th day to the time of maximum growth make an average increase of 26 g. per rat. Since fat-starved rats have continued to grow for 60 days and have made increases in weight up to 26 g. after the 97th day, the day on which the tumours were implanted, it is perhaps not surprising that tumour growth was not stayed. These experiments only show therefore that after 97 days of fat-starvation the diminution of the body stores of highly unsaturated acid did not prevent a further gain in weight when the rat was allowed to continue on the same diet nor did it prevent development of tumour growth after implantation of the Walker tumour. Implanted tumours grew as well in these fat-starved rats as in those which had received in addition a daily dose of 0.1 g. linseed oil. It seems possible that if the tumour were implanted after the 160th day when the level of the essential acid had fallen so low that the animal could no longer increase in weight, failure of tumour growth might then also occur; experiments are now being carried out to investigate this. So far the results suggest that as long as enough essential fatty acid is present to allow the normal fat-starved rat to increase in weight, enough is present to supply the needs of the growing tumour.

The growth of the Walker tumour in the rat seems to have some correlation with a rapid diminution of fat, especially in the subcutaneous tissue, accompanied by a marked lowering in the proportion of highly unsaturated acid to fat-free dry weight in the same tissue. The tumours in the rats which had received the essential unsaturated acids were about the same weight as those in the fat-starved rats, but they contained 10 times as much of the essential acids. Above a certain minimum therefore the presence of the acid can have no effect on the growth of the tumour.

Synthesis of clupanodonic acid from linoleic acid by the rat

Rats in the 4 groups IX, X, XI and XII received the fat-free diet during the 113 days of the experiment; those in the groups XI and XII received also 100 mg. linseed oil daily during the whole of this period and were therefore supplied with linoleic and linolenic acids. A comparison of the percentages of bromine in the ether-insoluble bromides prepared from the liver fatty acids in these four groups showed that when linoleic and linolenic acids had been supplied, the bromide stored was the decabromide of clupanodonic acid. This is shown in Table 3.

Table 3

Group	Ether-insoluble bromide from liver fatty acids mg. per rat	Br %
IX (fat-free diet)	15.8	65.1
X (fat-free diet)	21.1	65.0
XI (fat-free diet with linseed oil)	69.8	70.5
XII (fat-free diet with linseed oil)	67.3	71.3
Theory for:		
Linoleic acid tetrabromide	—	53.3
Linolenic acid hexabromide	—	63.3
Arachidonic acid octabromide	—	67.8
Dihydroarachidonic acid hexabromide*	—	61.1
Clupanodonic acid decabromide	—	70.8

In the case of the rats fed only on the fat-free diet and which have received neither linoleic nor linolenic acids, it seems probable that the bromide stored is a mixture of arachidonic acid and its dihydro derivative, which latter was previously isolated from the liver fatty acids of the fat-starved rat as its hexabromide [Smedley-Maclean & Nunn, 1940]. In the case of the rats which had received linoleic and linolenic acids, it seems to be clupanodonic acid $C_{22}H_{34}O_2$ which has been stored and which must have been synthesized from the less highly unsaturated C_{18} acids supplied.

SUMMARY

1. Walker tumours were implanted in (a) seven rats which had been maintained for 97 days on a fat-free diet, and in (b) seven rats which had received for the same period daily doses of linseed oil in addition to the fat-free diet. Large tumours developed in all the fat-starved rats and in five of those which had received the linseed oil. In two rats receiving linseed oil, small nodules formed but failed to develop further.

2. The five 'linseed oil rats' in which tumours had developed showed a large diminution in the proportion of total lipid material (chiefly fatty acids) in both skin and carcass when compared with the two rats in which the tumours had remained as small nodules. The proportion of lipid material was also much lower in the tumour-bearing fat-starved rats than in fat-starved rats in which no tumours had been implanted.

3. A marked lowering of highly unsaturated acid in the subcutaneous tissue but not in the carcass fat accompanied the development of the tumours. There was no significant change in the proportion of this acid in the liver. The fat-starved rats had not reached the stage of complete cessation of growth, so that any highly unsaturated acid available for normal growth would have been available for utilization in tumour development.

4. The ether-insoluble bromides from the fatty acids of the livers of the rats which had received linseed oil contained 70% Br, corresponding with the decabromide of clupanodonic acid $C_{22}H_{34}O_2Br$. The corresponding bromides from the livers of the fat-starved rats contained only 65% Br which would be in agreement with a mixture of arachidonic octabromide and dihydroarachidonic hexabromide. This suggests the synthesis by the rat of clupanodonic from linoleic or linolenic acid.

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