21. The Effect of Variations of Diet Fat on Dietary Fatty Livers in Rats

By Harold John Channon, Sidney William Firth Hanson and
Petros Antonios Loizides, *From the Department of Biochemistry,
University of Liverpool*

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Hershey & Soskin [1931] reported that fat infiltration into the liver of depancreatized dogs was accelerated when a relatively saturated fat, such as beef suet, was added to a diet of lean meat and sucrose; in contrast, the addition of the highly unsaturated fat cod-liver oil had no such effect, for a dog receiving it showed no symptoms of liver dysfunction and at post-mortem examination the fat content of its liver was found to be normal. Further study of the effect of the nature of food fat on the amount of fat deposited in the liver was made by Channon & Wilkinson [1936] in experiments on dietary fatty liver production in rats. In this work six different fats—butter fat, beef dripping, palm oil, coconut oil, olive oil and cod-liver oil—chosen because of the wide differences in their chemical composition, were used. The results showed that with the single exception of coconut oil (i.v. 10) the amounts of fat deposited in the liver varied inversely with the iodine value, butter (i.v. 33) causing 6 times the degree of fat infiltration which resulted from cod-liver oil (i.v. 145). When the results were considered in relation to the saturated acid content of the fats, it seemed that without exception the fat deposition depended directly on the intake of C_{14} – C_{18} saturated acids. Since choline prevents this fat deposition, its action might be due to its being concerned in a process of desaturation of fatty acids occurring either in the liver or elsewhere; in its absence, the inability of the tissues to dispose of the saturated acids in the normal way might lead to their accumulation in the liver. This possible mode of action of choline would explain the contrasting effects of saturated and unsaturated acids in their effects on fat deposition. On the other hand, the effect of saturated acids might depend not on their chemical nature but on their physical properties, and in particular perhaps their melting points, for the ability of the mammalian liver to take up excessive quantities of unsaturated acids which are liquid might well be limited on physico-chemical grounds. It seemed of value, therefore, to attempt to determine whether saturated acids exerted their effect because of their chemical nature or because of their physical properties, and work with this purpose in view is described in the present paper. The method adopted was to study the effects of progressive hydrogenation of a particular oil, and the effects of fats containing solid unsaturated acids, on fat deposition in the liver.

In the experiments of Channon & Wilkinson [1936] the very varied chemical composition of the different fats used complicated to some extent the interpretation of the results; the extremes in composition of the fats were provided on the one hand by coconut oil, which contains 63 % of saturated acids of 12 C atoms or less, 29 % of higher saturated acids and 8 % only of oleic acid, and, on the other hand, by cod-liver oil which contains no lower fatty acids, only 15 % of higher saturated acids and 84 % of highly unsaturated acids with 18 or more carbon atoms. In order to avoid complications of this character, one fat only, and that containing mostly acids of one chain length, was used for all the groups in each experiment. In Exp. 1 linseed oil was chosen since it consists almost entirely (93 %) of C_{18} acids and is highly unsaturated (i.v. 175); the oil was progressively hydrogenated to particular iodine values and groups of animals received these different hydrogenated oils. It was hoped thus to learn the effect of progressive saturation of one

particular oil on fat deposition. Linseed oil possesses the further advantage that on hydrogenation solid unsaturated or *iso*-oleic acids [Cocchinaras, 1932] are produced, and its use therefore enabled the effects of the presence of solid unsaturated acids to be studied at the same time. In the remaining experiments olive oil (87 % C_{18} unsaturated acids and a negligible percentage C_{18} saturated acids) was used, and the effect of solid unsaturated acids was further studied by adding elaidin in varying proportions, elaidic acid, M.P. 45, being a solid isomer of oleic acid.

EXPERIMENTAL

Animal feeding. Groups, each of 10 animals (usually 5 ♂♂ and 5 ♀♀), were fed on diets containing fat 35 or 40, caseinogen or albumin 8, glucose 51 or 46, and salt mixture 5 parts; vitamins A and D were supplied either as 1 part cod-liver oil or as concentrates, and each rat received 10 μ g. aneurin daily. The cages were kept on filter paper pads to enable faeces and rejected food to be collected.

Exp. 1. Five groups of animals received, for 14 days, diets which included 40 parts of either linseed oil (i.v. 175) or the same oil after hydrogenation to i.v. 142, 123, 90 and 55; caseinogen was the protein and to avoid the use of cod-liver oil, a vitamin A concentrate and calciferol were dissolved in the food fats. Animals were offered 10 g. of diet but food intakes were not measured.

Exp. 2. Four groups received, for 19 days, diets containing either beef dripping 40 (group 6), olive oil 40 (group 7), olive oil 30 + elaidin 10 (group 8), or olive oil 10 + elaidin 30 parts (group 9); albumin was the protein.

Exp. 3. Feeding period 20 days; the fats included in the different diets were: olive oil 40 (group 10), olive oil 30 + elaidin 10 (group 11), olive oil 30 + mixture 'H' 10 (group 12), olive oil 20 + elaidin 20 (group 13), olive oil 20 + mixture 'H' 20 (group 14) and beef dripping 35 parts (group 15). Mixture 'H' was composed of tristearin 75 and triolein 25 % (see p. 218).

Preparation of the fatty acids. At the end of the experiments the rats were guillotined and the fatty acids, freed from unsaponifiable matter, were prepared in the usual way from the livers.

For reasons which appear later, the fatty acids were also prepared from the carcasses. After removal of the gastro-intestinal tract and the tail the combined carcasses of each group were heated for 2 hr. with 2 l. 20 % NaOH; fat which rose to the surface was removed with benzene; after further heating of the aqueous residue, the mixture was acidified and the liberated fatty acids were extracted with benzene. After evaporation to dryness of all the benzene extracts the material was resaponified and the fatty acids prepared after removing unsaponifiable matter. It was not necessary in all cases to prepare and analyse the fatty acids from the whole carcass. In Exp. 2 the omental and perirenal fats were taken as representative of the depot fat; comparison of the analytical results for carcass fatty acids in other similarly fed groups showed that the procedure was justified.

The faeces were collected daily and stored under alcohol and extracted three times with hot alcohol containing 10 % conc. HCl; the residue was ground and extracted with ether after hydrolysis with aqueous acid. The fatty acids were then prepared after resaponification of the material obtained by evaporation of all the extracts.

The determination of the amounts of saturated acids, solid unsaturated acids and liquid unsaturated acids was carried out by use of the lead soap method of Twitchell as modified by Hilditch & Priestman [1931]. In this method the lead soaps insoluble in alcohol contain the higher saturated acids and those unsaturated acids which are solid; determination of the i.v. of the acids prepared from the insoluble lead soaps thus enables the amount of solid unsaturated acid to be calculated. Since the estimation of the amount of solid unsaturated acid was made in this way, it was clearly of the greatest importance

to prevent as far as possible any contamination of the insoluble lead soaps by the soluble lead soaps which contain the unsaturated acids. In our hands, the recrystallization of the insoluble lead soaps from alcohol was not entirely successful and it was found advantageous to free the solid acids from the insoluble lead soaps after the initial crystallization and then to convert them again into lead soaps; these soaps were then crystallized from alcohol as before. Figures to illustrate this are recorded in Table 1 in which olive oil, which contains no solid unsaturated acids, was used.

Table 1

	Solid acids % total fatty acid	Mean	i.v. of solid acids	Mean	% of solid unsaturated acid, calculated as % elaidic acid in the solid fractions
1. Direct recrystallization of the lead soaps [Hilditch & Priestman, 1931]	(a) 11.2 (b) 11.8	11.5	9 11	10	11
2. Pb soaps crystallized and decom- posed; then reformed and crystal- lized	(a) 11.5 (b) 12.1	11.8	0.1 0.5	0.3	0.3

Table 1 shows that while both the original method and the modification now introduced yield the same percentage of solid acids in the oil, the acids prepared by the former method had i.v. 10, whereas those resulting from the latter had i.v. 0.3. Calculated as elaidic acid, the results obtained by the Hilditch & Priestman method would thus imply the presence of 11 % elaidic acid, whereas olive oil contains no solid unsaturated acid. Since the modified procedure reduced this figure to 0.3 %, it was adopted. It must be realized that this method for determining the amount of solid unsaturated acids tends to be inaccurate when the amount of solid unsaturated acid is small. Except in Exp. 1 it was applied to all the fatty acid preparations made from the food fats, livers, carcasses and faeces, so that the effects of the different amounts of saturated and solid unsaturated acids in the food fats might be correlated with the findings on the tissues and with the degree of absorption.

RESULTS AND DISCUSSION

(a) *Food-fat and liver-fat deposition*

The results will be first considered from the point of view of the effect of the food fat on the liver-fat deposition. The simplest form of presentation is that adopted in Tables 2 and 3, in which the percentages of saturated and solid unsaturated acids in the dietary fats are compared with the weights of fat deposited in the 100 g. rat liver.

This method has been adopted justifiably since the variations in the daily fat intake of the different groups in each experiment, which was measured by the intake-excretion method, were found to be insignificant.

Table 2. *The effect of feeding hydrogenated linseed oil on liver-fat deposition (Exp. 1)*

Group	Dietary fat	Composition of dietary fat			Liver fat	
		Saturated acids %	iso-Oleic acid %	Total solid acids %	g./100 g. fresh tissue	g./100 g. rat
1	Hydrogenated linseed oil: i.v. 55	41.1	11.9	53.0	13.0	0.62
2	i.v. 90	14.9	5.6	20.5	11.1	0.44
3	i.v. 123	11.1	4.7	15.8	13.2	0.48
4	i.v. 142	9.4	5.1	14.5	7.4	0.28
5	Linseed oil: i.v. 175	8.1	(0.8)	8.9	7.2	0.26

The results of this experiment are less satisfactory than might be desired for two reasons. First, the degrees of hydrogenation, arbitrarily carried out until the i.v. of the

hydrogenated fats differed from one another by about 30 units, have not resulted in a gradation of the amounts either of the saturated or of the solid unsaturated acids. Thus the dietary fat of group 1 has a total solid acid content of 53 %, and contains 11.9 % of solid unsaturated acid. The total solid acids in the 'hydrogenated' groups vary, however, only from 14.5 to 20.5 %, and the solid unsaturated acids only from 4.7 to 5.6 %. As a result, pronounced differences in the amount of fat deposition of the latter groups cannot be expected. Secondly, the unpalatability of these linseed oil diets resulted in low food intakes and made it desirable to limit the experiment to a period of 14 days only, when the animals in all the groups had lost on the average about 10 % of their initial body weight. As a result, the disadvantages arising from the lack of pronounced variation in the composition of the dietary fat of groups 2-5 were accentuated, and there was, therefore, relatively limited fat deposition in the livers. Nevertheless, certain deductions are possible. The results show for instance that the progressive hydrogenation of a particular oil to different degrees has resulted in increasing liver-fat contents, just as in the previous work, the amounts of fat deposited were inversely related to the I.V. of the different fats used. The greatest weight of fat in the 100 g. rat liver (0.62 g., group 1) is found in the group receiving the highest percentage both of saturated and solid unsaturated acids.

The results as a whole suggest, however, that the 11.9 % of solid unsaturated acid present in the food fat of group 1 exerts little, if any, effect, for the amount of fat deposited in groups 2-5 seems to be unrelated to the intake of *iso*-oleic acid. While recognizing the difficulty of drawing final conclusions from results which, for the reasons given above, are less clear than had been hoped for, we formed the preliminary opinion that fat deposition was mainly dependent on the intake of saturated acids, and that solid unsaturated acids played but a minor part.

In order to obtain clearer information, the use of linseed oil was discontinued, and a series of experiments, of which two only need be described, was carried out using olive oil with the addition of varying amounts of elaidin. The elaidin, prepared by the British Drug Houses Ltd., was found to contain approximately 50 % elaidic acid. In Exp. 2 it was decided to focus attention on the effect of varying widely the intake of elaidic acid and to ignore for the moment the relatively small changes in the percentage of dietary saturated acids resulting from the presence of 25 % of saturated acids in the added elaidin. Thus the percentages of elaidic acid present in the dietary fatty acids of groups 7, 8 and 9 were respectively 0, 12.5 and 37.5; in contrast, the appropriate addition of elaidin had a relatively insignificant effect on the percentage of saturated acids, increasing it from 12.0 in group 7 to 15.3 and 21.8 in groups 8 and 9 respectively. In order to obtain a further contrasting figure, group 6 received beef dripping. Like olive oil this fat contains mainly C_{18} acids; it has, however, 56 % of saturated acids compared with the 12 % of olive oil, while neither of these fats contains any solid unsaturated acid. The results of Exp. 2 as well as those of Exp. 3, which will be described later, are given in Table 3.

The results obtained from groups 7-9 (Table 3) show clearly that elaidic acid has exerted no effect in increasing fat deposition. Thus the percentage of solid (saturated) acids in the dietary fat of group 7 which received olive oil was 12.0 and the resulting liver fat was 0.80 g. Increasing the percentage of solid acids to 27.8 in group 8 and to 59.3 in group 9 by the replacement of 10 and 30 parts of olive oil by elaidin has not resulted in an increase in the amounts of liver fat, which are 0.68 and 0.60 g. respectively. So far from causing an increase in fat deposition, these large additions of elaidic acid have tended, if anything, to decrease the amount of liver fat. This tendency seems more significant if it be remembered that with these increases in elaidic acid, the saturated acids themselves were also increased from 12.0 % in group 7 to 21.8 % in group 9; while these latter increases are not great, they might have been expected to raise the liver fat slightly. No conclusion on this point, however, can be reached, for, as may be seen in Table 4, the rats of groups 8 and 9 ate less fat than those of group 7 and this might, in part, account for

Table 3. *Comparison of the effects on liver-fat deposition of saturated with those of 'solid' and 'liquid' unsaturated acids*

Group	Dietary fat	Composition of dietary fat			Liver fat	
		Saturated acids %	Elaidic acid %	Total solid acids %	g./100 g. fresh tissue	g./100 g. rat
		Exp. 2				
6	Beef dripping 40	56.0	0	56.0	23.9	1.21
7	Olive oil 40	12.0	0	12.0	18.8	0.80
8	Olive oil 30 + elaidin 10	15.3	12.5	27.8	15.9	0.68
9	Olive oil 10 + elaidin 30	21.8	37.5	59.3	14.5	0.60
Exp. 3						
10	Olive oil 40	12.0	0	12.0	16.2	0.82
11	Olive oil 30 + elaidin 10	15.3	12.5	27.8	15.4	0.74
12	Olive oil 30 + mixture 'H' 10	27.8	0	27.8	18.5	1.03
13	Olive oil 20 + elaidin 20	18.5	25.0	43.5	14.5	0.65
14	Olive oil 20 + mixture 'H' 20	43.5	0	43.5	21.7	1.29
15	Beef dripping 35	56.0	0	56.0	21.9	1.54

Table 4. *Fat intake and fatty acid composition of food, liver, carcass and faeces (Exp. 2)*

Group	Fat/100 g. rat/day			Net intake g.	Fatty acid composition							
	Food	Faeces	% absorption		Food fat		Liver fat		Carcass fat		Faeces fat	
					% un-saturated	% elaidic	% un-saturated	% elaidic	% un-saturated	% elaidic	% un-saturated	% elaidic
6	1.63	0.31	83	1.32	44.0	0	69.1	0	66.6	0	30.2	0
7	1.78	0.08	95	1.70	88.0	0	76.4	0	72.3	0	46.8	0
8	1.59	0.14	90	1.45	84.8	12.5	75.9	3.8	71.1	3.7	45.3	8.9
9	1.54	0.23	86	1.31	78.3	37.5	74.5	15.4	71.9	12.6	44.0	21.4

the lower amounts of liver fat in these groups. Apart from showing the lack of effect of elaidic acid, this experiment again shows strikingly the effects of increasing the saturated acid intake. Thus the 100 g. rat liver of group 6 which received beef dripping, containing 56 % of higher saturated acids, has 1.21 g. fat compared with the 0.80 g. in group 7 which received 12 % saturated acids in the dietary olive oil. This result appears even more definite if the liver fat figures are considered in relation to the fat intake and excretion values (Table 4). Groups 6 and 9 had the same daily fat intake, 1.3 g./100 g. rat, containing almost identical percentages of solid acids; the amounts of liver fat are very different, 1.21 and 0.60 g. respectively, and this difference must be due to the replacement of some of the saturated acids of group 6 by elaidic acid. The contrasting effects of saturated and unsaturated acids are evident too from the liver fat results of groups 6 and 7; group 7, with a daily fat intake of 1.7 g. olive oil, had liver fat 0.80 g./100 g. rat, while group 6, consuming a smaller amount of beef dripping (1.3 g.), had liver fat 1.21 g.

As already mentioned, the fatty acids of the elaidin used in Exp. 2 comprised 50 % elaidic, 25 % stearic and 25 % oleic acids, and as a result, the substitution of part of the olive oil in groups 8 and 9 by elaidin raised the percentage of saturated acids in the diets of these two groups as compared with the control group 7. In order to define yet more closely the effect of elaidic acid, the conditions in Exp. 3 were so arranged that the dietary fats were identical in their content of solid acids and differed only in the percentage of elaidic acid. This was effected by the use of mixture 'H', which was made up of 75 % tristearin and 25 % triolein. In the dietary fat of group 11, 10 parts of the olive oil of the control group 10 were replaced by 10 parts elaidin, and in that of group 12 by 10 parts of mixture 'H'. As a result, the dietary fats of groups 11 and 12 differed only in that the elaidic acid of the one was replaced by stearic acid in the other; similarly for groups 13 and 14 in which 20 % of elaidin and mixture 'H' were used. A further group, 15, received

35 % beef dripping; since this fat contains 56 % of saturated acids, the use of a lower percentage of dietary fat in this group provided an opportunity for studying the effect of further increasing the proportion of saturated acids.

The results in Table 3 fully confirm the results of Exp. 2, for the amount of liver fat deposited is entirely unrelated to the intake of total solid (saturated and unsaturated) acids. The daily intake of all six groups was so nearly the same as fully to justify comparison of the liver-fat values directly with the percentage figures of the fatty acid make-up of the diet fat. A comparison of groups 10 and 11 shows that more than doubling the total solid acid intake by the addition of elaidin has had no effect; likewise the liver fat of group 12, which received a mixture identical in solid acid content with that of group 11, is nearly 50 % greater than that of the latter group; similarly the liver fat of group 14 is double that of group 13, in spite of the two groups having the same proportion of solid acids in their diets. In contrast to the failure of elaidic acid to increase the fat deposition, the gradation in the amounts of liver fat in groups 15, 14, 12 and 13 from 1.54 to 0.65 g. by successive large steps runs parallel with the intake of dietary saturated acids; the results in groups 10 and 11 (0.82 and 0.74 g.), which received proportions of saturated acids differing not greatly from that of group 13, are probably not significantly different from the 0.65 g. of the latter group. It is interesting to note that the amounts of liver fat in groups 11 and 13 which received elaidin are actually slightly lower than that of the control group 10; these differences are probably insignificant, but are in the same direction as the corresponding finding in Exp. 2.

(b) *Food fat and liver, carcass and faecal fats*

Table 4 includes the percentages of saturated and elaidic acids in food, liver, carcass and faecal fats in Exp. 2, the results of which are typical. The outstanding features are: (1) the presence of elaidic acid in both the livers and fat depots of those rats to which it has been fed; (2) the increase in its percentage in the tissues as its percentage in the diet was increased; (3) the uniformity of the proportions of liver, carcass and faecal unsaturated acids in groups 7, 8 and 9 although 0, 12.5 and 37.5 % respectively of the total fatty acids of the diet were supplied as elaidic acid. These three observations leave no doubt that elaidic acid is metabolized normally by the animal body, as was found by Barbour [1933] and by Sinclair [1935]. In view of the uniformity of the unsaturated fatty acid content of the liver fat in groups 7, 8 and 9, it is interesting to note that in Exp. 1, the i.v. of the total liver fatty acids, which were 73 and 97 for groups 1 and 2, though depressed by the feeding of relatively saturated fats, were not appreciably raised above 125–130 when the diet fat was more unsaturated (groups 3–5).

The analysis of the faecal fat was not an integral part of this investigation, but it was carried through to determine whether the faecal fatty acids were modified in make-up by the different diets. The faecal fat of group 6, which received a more saturated fat, contains a higher percentage of saturated acids (69.8) than that of groups 7, 8 and 9, and there is a further reflexion of the dietary fat in the elaidic acid content of the faecal fats of groups 8 and 9. It is, of course, not possible to decide whether this represents unabsorbed material or a modification of the composition of the excreted fat in response to the presence in the blood of an increased amount of elaidic acid. The variations in the amounts of faecal fat in groups 7, 8 and 9 lie in the direction which might be expected from diminished absorption with increasing solid acid content of the food, but the similarity in the percentages of unsaturated acids in the three groups lends support to the view that the faecal fat may be largely excretory in origin.

SUMMARY

1. In continuation of the work of Channon & Wilkinson [1936] the influence of dietary fat on the extent of fat infiltration of the liver of the rat has been further investigated, with a view to assessing whether choline was concerned with the desaturation of fatty acids in the liver.

2. Comparison has been made of the effects of liquid unsaturated acids, solid unsaturated acids and solid saturated acids on liver-fat deposition.

3. It has been shown that on diets high in fat and low in choline, the extent of fatty infiltration is related to the proportion of C_{14} - C_{18} saturated fatty acids. Solid unsaturated acids exert no effect.

4. Elaidic acid is treated normally in the body, appearing in liver, carcass and faecal fats in proportions dependent on its amount in the diet.

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