CXLIX. THE EFFECT OF VARIOUS FATS IN THE PRODUCTION OF DIETARY FATTY LIVERS.

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(*Received April 30th, 1936.*)

THE work described in this paper is the result of a study of the degree to which certain fats of widely varying chemical composition and physical properties accumulate in the liver when fed to animals under certain dietary conditions. It was undertaken in order to obtain information concerning the nature of the fatty acids which appeared in the liver under these different conditions and because knowledge of this kind should provide evidence as to the part played by the chemical nature of the food fat in fatty liver production. Further, it seemed possible that additional evidence of the part played by the liver in fat metabolism might be so obtained. Such a study has not so far been reported, for in investigations of the fatty liver problem, Best and his colleagues have used beef fat and "Crisco" as their dietary fats for the routine production of fatty livers, whilst for the same purpose the former fat only has been used in this laboratory. In the first experiments carried out in 1933 we employed the mixed grain diet with 40% fat which has been much used by the Toronto workers since their original discovery [Best *et al.*, 1932]. Groups of animals were fed for three weeks on this diet with butter, beef dripping, palm oil, coconut oil, olive oil and cod-liver oil as the dietary fats. The average percentages of fat (fatty acids and unsaponifiable matter) present in the livers were 3·25, 5·70, 6·99, 6·33, 6·02 and 3·42 respectively. This and other failures to produce fatty livers by the use of the grain diet with 40% fat caused us to leave the problem at that time and led to the investigations which resulted in the finding that the amount of fat occurring in the liver was governed by the amount of protein in the diet, irrespective of any action of choline [Channon and Wilkinson, 1935; Beeston *et al.*, 1935]. The results of the repetition of the work, in which use was made of this finding, are recorded here.

EXPERIMENTAL.

Six groups, each of ten rats, were fed on a basal diet of alcohol- and ether-extracted caseinogen 5, marmite 5, glucose 45, salt mixture 5 parts, with 1 drop of cod-liver oil per rat every 3 days, together with 40 parts of one of the following fats: butter fat, beef fat, palm oil, coconut oil, olive oil and cod-liver oil. The diets were administered for 14 days, and the animals were then guillotined and the livers removed. The total ether-soluble material was extracted from the

Table I. *Weight records of the animals.*

Group no.	1	2	3	4	5	6
Fat in diet	Butter fat	Beef fat	Palm oil	Coconut oil	Olive oil	Cod-liver oil
Av. initial body wt. (g.)	185	186	195	186	180	187
Av. final body wt. (g.)	187	186	179	177	177	166
Av. gain or loss (g.)	+2	±0	−16	−9	−3	−21
Food intake (g. per rat per day)	10·0	9·5	8·1	9·5	9·2	6·9

pooled livers and its contents of lecithin, cholesterol, cholesteryl esters and glyceride estimated as described by Channon and Wilkinson [1934]. The data relevant to the animal side of the experiment are recorded in Table I.

Results.

1. *Fatty liver production.* Table II records the percentages of each constituent present in the fresh liver and also the absolute weight of each constituent in the liver of the 100 g. rat. These are arranged in order of decreasing fat content of the livers. This table shows that all the fats have caused fatty livers, although the

Table II. *The liver lipoids.*

(*a*) Percentage of each constituent (g./100 g. fresh liver).
(*b*) Weight of each constituent present in the liver of the 100 g. rat (g.).
$\left(\frac{a \times \text{liver as percentage of body weight}}{100}\right)$.

Group no.	1		2		3		4		5		6	
Dietary fat	Butter fat		Beef fat		Palm oil		Coconut oil		Olive oil		Cod-liver oil	
	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)
Lecithin	2·59	0·12	2·57	0·12	2·25	0·10	2·92	0·11	3·02	0·10	3·60	0·13
Cholesterol	0·14	0·01	0·20	0·01	0·25	0·01	0·19	0·01	0·23	0·01	0·28	0·01
Cholesteryl oleate	0·64	0·03	0·56	0·03	0·31	0·01	0·31	0·01	0·85	0·03	0·26	0·01
Glyceride	27·30	1·30	23·70	1·06	23·54	1·05	17·12	0·67	11·47	0·39	3·04	0·11
Total % of liver	30·67	—	27·03	—	26·35	—	20·54	—	15·57	—	7·18	—
Wt. per liver of 100 g. rat (g.)	—	1·46	—	1·22	—	1·17	—	0·80	—	0·53	—	0·26
I.V. of food fat	33		40		49		10		80		145	

intensity of the accumulation in the liver varies. Save for cod-liver oil, in the case of which the livers are only slightly fatty (7·18 %), the remaining groups contain total lipoids varying from 15·57 %, in the case of olive oil, to 30·67 % for butter fat; the very high values for butter fat, beef dripping and palm oil, 30·67, 27·03 and 26·35 % respectively, are noteworthy in view of the short period of experiment, 14 days only. The results again demonstrate clearly the effectiveness of the 5 % protein diet in fatty liver production with a variety of oils and it seems likely that such variations in the nature and amount of the fatty acids present in the livers may provide a means for further elucidation of the fatty liver problem. The percentages of the individual liver lipoids call for little comment. Those of lecithin fall with increasing fat content and the absolute weight of this substance in the liver of the 100 g. rat varies over a roughly similar range. The free cholesterol content varies little from the normal value, 8 mg. in the liver of the 100 g. rat. Both the percentages and absolute weights of the cholesteryl esters show a definite increase, the normal figure being of the order of 0·01 %.

As was to be anticipated, the differences in the degree of lipoid accumulation in the livers are entirely due to the differing glyceride contents, which vary from 3·04 % in the case of cod-liver oil to 27·30 % in that of butter. Thus the livers of the animals receiving cod-liver oil contained 0·11 g., three times the normal amount, whilst those of animals receiving butter gave the highest figure and contained 1·303 g., about forty times the normal. Best [1934] remarks that he had found beef fat more effective in producing fatty livers than butter, and the present result is contrary to this finding. Best however used butter, whilst in our experiments filtered butter fat was used and it is possible that the explanation of the different finding lies here. Whilst any caseinogen present in the butter would tend to exercise a lipotropic effect, its amount would be too small

significantly to affect the result. In other studies our colleague, Dr Loach, has demonstrated the presence in milk of choline compounds other than phosphatides. It is possible that these may accumulate in the butter and exert a lipotropic effect, yet be removed in the preparation of butter fat by filtration.

The figures in Table II show that in general the intensity of the fat infiltration of the liver rises inversely to the iodine values of the dietary fat. Thus cod-liver oil, the most unsaturated fat fed in this experiment, I.V. 145, has caused only a very small increase in the glyceride content of the liver, whilst butter fat, I.V. 33, beef fat, I.V. 40 and palm oil, I.V. 49 have resulted in very large increases in the glyceride content of the livers, such increases being however of about the same order. Olive oil, with an intermediate I.V., gives a glyceride content between these two extreme values. Coconut oil is the one exception, for with the lowest I.V., 10, it should have caused the greatest accumulation of liver fat, if the degree of unsaturation were the only controlling factor. It is possible however that the short-chain fatty acids, which constitute a very considerable portion of the fatty acids of this oil, are more readily metabolised and do not therefore play so prominent a part in fatty liver production.

In Table III are recorded the average percentage compositions of groups of the fatty acids of the various food fats used in these experiments. The degree of fat infiltration in the liver runs parallel with the percentages of C_{14}–C_{18} saturated

Table III. *Average percentage composition of fatty acids of the various food fats.*

	Saturated fatty acids		Unsaturated fatty acids			Intake of saturated
	C_{12} or lower	C_{14}–C_{18}	Lower than C_{18}	C_{18}	Higher than C_{18}	C_{14}–C_{18} acids g./rat/day
Butter fat	12	52	—	36	—	2·08
Beef fat	—	52	—	48	—	1·98
Palm oil	—	48	—	52	—	1·55
Coconut oil	63	29	—	8	—	1·11
Olive oil	—	12	—	87	—	0·44
Cod-liver oil	—	15	—	40	44	0·41

acids present in the different fats, except in the cases of olive oil and cod-liver oil. The last column of Table III shows the actual daily intake by each animal of saturated C_{14}–C_{18} acids calculated from the food intake. These figures fall into the same order as do the percentages of liver fat, although from the fact that the intakes by the olive oil and cod-liver oil groups are similar, 0·44 and 0·41 g. per day respectively, it might have been anticipated that these two fats would have caused similar degrees of fat accumulation. The actual amounts of glyceride are however 11·47 % for olive oil and 3·04 % for cod-liver oil. Whilst a certain deduction cannot be made from these results, the amount of C_{14}–C_{18} saturated acids ingested appears to govern in a general manner the accumulation of fat in the liver. Worthy of notice in this connection are the observations that the inclusion of a relatively saturated fat in the diet of depancreatised dogs, receiving controlled insulin administration, considerably shortened the period before the appearance of signs of liver failure [Allen *et al.*, 1924; Hershey and Soskin, 1931]. One further point needs emphasis regarding the liver fat percentage of the cod-liver oil group; in all the other groups the daily food intake varied from 8·1 to 10·0 g. per animal, while in this group it was only 6·9 g. Further, there are many reports of the toxicity of cod-liver oil which has been demonstrated in a variety of ways. These two factors make it necessary to regard the result with this oil with caution.

2. *The fatty acids present in the livers.* Table IV records the I.V. and mol. wt. of the total liver acids obtained by hydrolysis of part of the total ethereal extract and removal of the unsaponifiable matter. The phosphatide and glyceride

Table IV. *Analysis of the total liver fatty acids.*

Group no. ...	1	2	3	4	5	6
Fat in diet ...	Butter fat	Beef fat	Palm oil	Coconut oil	Olive oil	Cod-liver oil
Total fatty acids in liver (g./100 g. liver)	28·23	24·73	24·19	18·57	13·46	5·55
I.V.	83·5	85·9	87·9	67·9	91·5	158·2
Mol. wt.	270	275	274	257	280	287
Fatty acids of fat fed. I.V.	35	42	51	12	84	150
Mol. wt.	245	276	266	210	280	290

Table V. *Analysis of the liver glyceride fatty acids.*

Group no. ...	1	2	3	4	5	6
Fat in diet ...	Butter fat	Beef fat	Palm oil	Coconut oil	Olive oil	Cod-liver oil
Glyceride fatty acids (g./100 g. liver)	26·25	22·72	22·16	16·77	12·88	3·31
I.V.	77	74	87	60	83	173
Mol. wt.	271	270	281	261	284	291
	Twitchell separation.					
Liquid fatty acids %	62·2	66·3	68·1	57·7	78·1	76·1
I.V.	116	106	121	101	109	176
Mol. wt.	296	300	298	266	289	313
Solid fatty acids %	37·8	33·7	31·9	42·3	21·9	23·9
I.V.	—	14	5	7	10	49
Mol. wt.	—	267	269	257	267	310

Table VI. *Analysis of the liver phosphatide fatty acids.*

Group no. ...	1	2	3	4	5	6
Fat in diet ...	Butter fat	Beef fat	Palm oil	Coconut oil	Olive oil	Cod-liver oil
Phosphatide fatty acids (g./100 g. liver)	1·65	1·59	1·42	1·52	2·11	2·63
I.V.	138	134	149	132	148	143
Mol. wt.	295	296	288	276	291	337
	Twitchell separation.					
Liquid fatty acids %	58·3	60·5	59·6	57·3	57·8	57·6
I.V.	185	176	205	189	179	192
Mol. wt.	368	369	350	334	380	350
Solid fatty acids %	41·7	39·5	40·4	42·7	42·2	42·4
I.V.	15	16	21	20	28	15
Mol. wt.	299	289	273	264	319	343

fractions of the total ethereal extracts of the livers were then obtained by means of acetone precipitation. The fatty acids were prepared from each fraction in the usual way and the liquid and solid fatty acids separated by a modified Twitchell process [Hilditch and Priestman, 1931]. The results of these analyses are set out in Tables V and VI.

Discussion.

(*a*) *Total fatty acids.* From Table IV it is seen that where a greatly increased deposition of fat in the liver has occurred, *i.e.* in all but the cod-liver oil group, the I.V. of the total fatty acids have decreased quite considerably from the normal (about 115), although the I.V. and mol. wt. of the dietary fats are well reflected in the corresponding values of the liver fatty acids. This is seen particularly well in groups 4, 5 and 6, receiving coconut, olive and cod-liver oils respectively.

(*b*) *Glyceride fatty acids.* With the exception of the cod-liver oil group, I.V. of the glyceride fatty acids given in Table V are considerably lower than that of the glyceride normally present in the liver (I.V. about 90). These liver glyceride acids reflect in general the nature of the acids fed. Thus those of group 4 have the lowest I.V., 60, and the lowest mol. wt., 261 (coconut oil fatty acids have I.V. 8; mol. wt. 257). With these may be contrasted those from groups 5 and 6. The liver glyceride acids of group 5 have I.V. 83 and mol. wt. 284 (olive oil fatty acids have I.V. 84; mol. wt. 280). Those of group 6 are I.V. 173 and mol. wt. 291 (cod-liver oil fatty acids have I.V. 150; mol. wt. 290). Consideration of the results with these three fats, which provide the best contrast because of their chemical nature, indicates that the composition of the glyceride fatty acids accumulating in the liver is markedly influenced by the dietary fat. Similarly the highest percentages of unsaturated acids are found in the olive and cod-liver oil groups, 78·1 and 76·1 respectively, while the lowest occurs with coconut oil, 57·7. The mol. wts. of these three groups of acids, 289, 313 and 266, also reflect those of the acids of the fats fed. It is noteworthy that apart from the cod-liver oil group the I.V. of the unsaturated acids vary only from 106 to 121, in spite of the varying characteristics of the fats administered. Attention is particularly directed to the I.V. of the acids from the coconut oil group, 101, which differs little from that of the olive oil group, 109, in spite of the fact that coconut oil contains but a very small proportion of unsaturated acids (about 8 % of oleic acid, I.V. 90), whilst olive oil (I.V. 83) contains some 87 % of C_{18} unsaturated acids. The mol. wt. of the coconut oil group acids, 266, together with the I.V., 101, indicates the presence of considerable amounts of unsaturated acid of less than eighteen carbon atoms and may be evidence of the liver having desaturated the saturated acids of this oil. It is of interest also that there has not been a greater deposition of saturated acids in groups 1, 2 and 3, for it has already been pointed out that the intake of higher saturated acids seems to govern the intensity of the fat accumulation.

(*c*) *The phosphatide fatty acids.* The I.V. of the phosphatide fatty acids vary only from 132 to 149 and support the finding of Sinclair [1931] that the I.V. of the fatty acids of the food fat and that of the liver phosphatide fatty acids do not run parallel. The I.V. of the liver phosphatide fatty acids from group 4 is also similar to results obtained by Sinclair [1932] when coconut oil was fed. There is however a very considerable difference between the result obtained in group 6 (cod-liver oil) and those obtained by Sinclair [1931; 1932]. That author recorded considerable increases in the I.V. of the liver phosphatide fatty acids up to 170–180, when feeding cod-liver oil in small amounts and up to 21·3 % of the diet. His values were much higher than those obtained after coconut and olive oil feeding. In this experiment 40 % of cod-liver oil in the diet has been fed, yet the I.V. of the phosphatide fatty acids has increased only to 143, which is not as great as that due to feeding olive oil and only slightly higher than that due to the inclusion of coconut oil in the diet. Further the I.V. of the acids of the glyceride fraction, 173, is considerably higher than that of the phosphatide acids. No

explanation for this divergence can be given. Although the I.V. of the phosphatide fatty acids does not bear any relationship to the I.V. of the food fatty acids, the mol. wts. of these fatty acids do appear to reflect to some extent those of the food fatty acids, save in the case of butter fat, this indicating that the composition of the liver phosphatides may vary to some extent with the dietary fat. Consideration also shows that little difference occurs in the percentages of the liquid and solid fatty acids present in the different groups, the range for the unsaturated acids being 57·3 to 60·5. There is also little variation in the I.V. of the liquid fatty acids, which are considerably higher than those of the glyceride fatty acids, save in the case of group 6 which are only slightly raised. The mol. wts. of the liquid fatty acids are extremely high and fall within a very narrow range. It is possible that these values are inaccurate, since a considerable period of time unavoidably elapsed before the completion of the analyses and chemical change may have occurred, even though the I.V. have remained at a high level. The mol. wts. of the solid fatty acids, although higher than those of the glyceride fatty acids, again reflect to some extent the mol. wts. of the food fatty acids.

These results thus appear to show that the fat occurring in the fatty liver when different fats are fed, if not derived exclusively from the dietary fat, is considerably modified by its nature, which also governs the degree of accumulation occurring. Further discussion of these results, from which many points emerge, will be deferred until work at present in progress has been completed. After removal of the livers and gastro-intestinal tracts of all the animals, the carcasses were pooled and the total fatty acids of each group were worked up. These are being analysed by the ester distillation method after being submitted to the Twitchell process. Discussion of the liver results will be further facilitated when the composition of the depot fats is thus made available. Further, the results at present reported made it appear worth while to put large groups of animals on to the diet used in this paper, with coconut and olive oils, in order to obtain sufficient liver fatty acids for ester distillation, since light on the liver desaturation process, in which choline may be involved, as well as more accurate information concerning the nature of the fatty acids accumulating in the fatty liver under different dietary conditions, will be so obtained.

Summary.

1. Groups of rats have been fed on the diet previously found effective in producing fatty livers and containing 5% caseinogen with 40% fat. Various fats were used and the effectiveness of this diet is shown by the fact that the livers of all the groups were very fatty.

2. The total lipoids present in the livers corresponding to the various fats at the end of 14 days were: butter fat 30·67, beef fat 27·05, palm oil 26·35, coconut oil 20·54, olive oil 15·57, cod-liver oil 7·18% of the fresh liver weight.

3. Factors influencing the variations in the intensity of fat deposition in the livers with the different fats are discussed.

4. The total lipoids were fractionated into phosphatide and glyceride fractions. The fatty acids from all the fractions were analysed by the Twitchell procedure and the I.V. and mol. wt. determined. The results indicated that the nature of the fat in the livers was markedly influenced, both in the glyceride and phosphatide fractions by that of the dietary fat from which much of it was derived.

The expenses of this research were defrayed by a grant from the Medical Research Council, to whom we wish to express our thanks.

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