XX. THE EFFECT OF LIVER FEEDING ON THE "FAT" CONTENT OF THE LIVER.

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THE investigations recorded in this paper were carried out as a result of the findings of Blatherwick *et al.* [1931; 1932; 1933, 1, 2] on the effect on the "fat" content of the liver of the feeding to rats of diets consisting largely of fresh liver, dried liver or certain liver extracts. These authors observed that feeding raw or dried liver resulted in the production of fatty livers; that the addition of lecithin to the diets had no effect in preventing the fatty liver; and further that it appeared possible that there was present in the liver a water-soluble substance, which was responsible for the deposition of liver "fat", or alternatively that aqueous extraction of liver caused some change in the constituents of the liver residue, as a result of which the amply demonstrated property of cholesterol in causing "fat" deposition in the liver was opposed [Okey, 1933, 1, 2; Chanutin and Ludewig, 1933; Best *et al.*, 1934; Channon and Wilkinson, 1934].

Consideration of the relative amounts of fat and cholesterol in liver led us to think that the apparent failure of lecithin to influence the fat deposition was largely concerned with the high proportion of fat and cholesterol present in such diets. The provision of 2.3 % choline in a synthetic diet containing 2 % of cholesterol and 20 % of fat will not entirely prevent cholesteryl ester deposition [Best *et al.*, 1934]. Hence it might be anticipated that the addition of further lecithin to a liver diet, which would already contain some 13 % phosphatide as well as 1 % cholesterol and 30 % fat, might not cause any further decrease in "fat" deposition, since the maximum choline effect was already being exercised by the lecithin contained in the liver.

In order to investigate this possibility, the work described in this paper on the feeding of liver to rats was carried out. The effect of the withdrawal from the dietary liver of the constituent lecithin, cholesterol and glyceride, and the replacement of these substances by an equivalent amount of cholesterol or beef dripping, either singly or together, has been studied. In other experiments more cholesterol than was originally present in the liver has been added. By these means a series of results of a strictly comparable nature has been obtained and from them the effect of the individual constituents of dietary liver in fatty liver production may be assessed.

In the work of Blatherwick *et al.* [1933, 2] the results of the different experiments are not strictly comparable, because each diet did not contain the equivalent of the constituents of the original liver and in some cases the extracts employed were not from the main bulk supply. At the same time opportunity was taken to investigate the possibility of the presence in liver of a water-soluble factor responsible for fatty liver production.

EXPERIMENTAL.

Composition of the diets.

The composition of the diets is set out in Table I.

Table I. *Composition of the diets.*

Diet No.	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Raw liver	—	—	—	—	—	—	—	—	100	100	100
Dried liver	75	75	—	—	—	—	—	100	—	—	—
Dried liver after alcoholic extraction	—	—	—	51·8	51·8	—	—	—	—	—	—
Dried liver after aqueous extraction	—	—	52·8	—	—	—	—	—	—	—	—
Dried liver after alcoholic and aqueous extraction	—	—	—	—	—	29·6	29·6	—	—	—	—
Residue after evaporating aqueous extract of dried liver	—	—	—	—	—	—	22·2	—	—	—	—
Marmite	5	5	5	5	5	5	5	—	—	—	—
Salt mixture	2	2	2	2	2	2	2	—	—	—	—
Glucose	17	15	39·2	17	17	37·2	15·0	—	—	—	—
Cod-liver oil	1	1	1	1	1	1	1	—	—	—	—
Choline chloride	—	2	—	—	—	—	—	—	—	0·2	0·4
Beef dripping	—	—	—	23·2	22·45	22·45	22·45	—	—	—	—
Cholesterol (as present in liver)	—	—	—	—	0·75	0·75	0·75	—	—	—	—
Cholesterol	—	—	—	—	—	2·0	2·0	—	—	—	—

The liver and preparations made from it were obtained as follows.

Dried liver. This was a powdered preparation which had been specially prepared through the kindness of Dr F. H. Carr of the British Drug Houses, Ltd. by drying liver *in vacuo* below 60°. Analysis showed that this preparation contained 30·91% of ether-alcohol-soluble material and 29·5% of water-soluble material; the ether-alcohol-soluble material was completely analysed, and the original dried liver was found to contain 13·4% "lecithin", 0·32% cholesterol, 1·2% cholesteryl ester (calculated as oleate), 11·39% glyceride (triolein) and 4·59% of other material.

Dried liver after alcoholic extraction. Dried liver (3 kg.) was extracted exhaustively in a Soxhlet apparatus with alcohol, followed by ether, each for one working day. The final residue was dried by electric fan at room temperature and finally for a short time in an electric oven at 105°. This was used for diets IV and V.

Dried liver after aqueous extraction. Dried liver (4·5 kg.) was extracted several times with boiling water. The combined filtrate (16·5 l.) was evaporated *in vacuo* to a syrup which was used in diet VII. The liver residue was dried at 105° and was used for diet III.

Dried liver after alcoholic followed by aqueous extraction. The dried liver exhaustively extracted with alcohol as above was then extracted with boiling water as described and the residue after drying was used in diets VI and VII.

In all the diets III–VII the amount of the liver residue or extract used is that corresponding to the 75 parts of dried liver used in Exps. I and II.

In diet II the only change from diet I was the addition of two parts of choline chloride at the expense of two parts of glucose.

In diet III the material removed by aqueous extraction was replaced by glucose.

In diet IV the material removed by alcohol-ether extraction was saponified and its percentage of total fatty acids and cholesterol determined; the alcohol-ether extract was then replaced in the diet by an equivalent weight of beef dripping.

In diet V the same procedure was adopted, save that the total cholesterol present in the alcoholic extract was replaced as free cholesterol.

In diet VI the residue from the dried liver which had been extracted both with water and alcohol was fed, with the addition of the corresponding amount of beef dripping and cholesterol as in diet IV whilst in addition 2% of cholesterol was added, the aqueous extract being replaced by glucose.

In diet VII the same procedure was adopted with the addition of the aqueous extract at the expense of the additional glucose added in diet VI.

In diet VIII the animals received dried liver alone for a longer period of time.

In diet IX the animals received raw minced liver only, which was not that from which the dried liver used in all the other experiments had been prepared.

In diets X and XI the same raw liver with the addition of 0.2% and 0.4% of choline chloride respectively was used.

All the groups of rats received these diets over a period of 21 days, except groups IX, X, XI and group VIII, which were fed for 28 and 52 days respectively. After these periods of feeding the animals of groups I–VIII were guillotined, whilst those of the three groups receiving raw liver (IX, X and XI) were killed by a blow on the head. The pooled samples of the livers obtained were analysed as described by Channon and Wilkinson [1934]. The data relating to the animal side of the experiment, the food, choline, fatty acid and cholesterol intakes are recorded in Table II. The percentages of the different individual

Table II. *Weight records of animals and food intakes.*

Group No.	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
No. of animals	6	6	6	6	6	6	6	4	8	8	8
Days on exp.	21	21	21	21	21	21	21	52	28	28	28
Average gain or loss (% of body wt.)	+1.3	+4.3	-1.7	+13.1	+13.8	+5.7	-2.0	+14.1	+23.8	+18.6	+22.2
Average food intake (g./rat/day):											
(a) Dry wt.	12.3	12.3	12.3	12.3	12.3	12.3	11.0	—	—	—	—
(b) Wet wt.	—	—	—	—	—	—	—	—	29.5	27.8	29.8
Average fatty acid intake (g./rat/day)	2.0	2.0	2.0	2.7	2.6	2.6	2.4	—	1.3	1.2	1.3
Average cholesterol intake (mg./rat/day)	92	92	92	—	92	338	338	110	85	80	86
Choline intake (mg./rat/day):											
Added to diet	—	214	—	—	—	—	—	—	—	56	119
Present as phosphatide	185	185	185	—	—	—	—	—	181	170	183
Total	185	399	185	—	—	—	—	—	181	226	302

Table III. *Percentage composition of the liver lipoids (g./100 g. liver).*

Group No.	Lecithin %	Free cholesterol %	Cholesteryl ester %	Glyceride %	Total %
I	4.34	0.310	1.94	3.32	9.91
II	4.56	0.302	1.82	0.90	7.58
III	4.40	0.302	2.02	1.53	8.25
IV	4.56	0.257	0.45	1.67	6.94
V	3.90	0.309	3.62	3.19	11.02
VI	4.84	0.356	5.45	3.88	14.53
VII	4.58	0.354	5.71	2.61	13.25
VIII	4.27	0.363	4.16	3.23	12.02
IX	3.26	0.294	1.85	1.84	7.24
X	3.32	0.325	1.39	1.76	6.79
XI	3.34	0.247	1.32	1.60	6.51

lipoid constituents present in the livers were calculated in the manner of Best *et al.* [1934] and the results are recorded in Table III.

DISCUSSION.

Before discussing the results, it is to be pointed out that the amount of glyceride and cholesteryl ester in the liver will depend to a considerable extent on the choline content of the diet [Best *et al.* 1934]. A further additional factor which may be involved is the protein content of the diet, for it has been found,

at least with caseinogen, that the amount of protein in the diet exercises a profound effect in controlling liver glyceride, irrespective of any effect of choline [Beeston *et al.*, 1935]. Whether this effect of the protein fraction applies to proteins other than caseinogen has yet to be determined, but it seems possible that such is the case.

(a) *The effect of dried liver.* The results for groups I and VII, in which the diet consisted mainly of dried liver, confirm the findings of Blatherwick *et al.* that a dried liver diet causes fatty livers. This fatty liver is characterised by an increase in the amount of cholesteryl esters which rise to the extent of about 1.8% after 21 days' feeding (Group I) and to 4.1% after 52 days' feeding. Such a progressive accumulation in the amount of cholesteryl esters on diets containing cholesterol has been observed before [Chanutin and Ludewig, 1933]. With this increase in cholesteryl esters there is also a rise of some 2% in the glyceride and in experiments which we have previously reported it is usual to find, even when the diet contains choline or a lipotropically active protein, the presence of rather more glyceride than normal in the liver, and the present results are similar in substance. The degree of "fat" infiltration after 3 weeks is however relatively small, for the normal figures for rat liver may be regarded as lecithin 3.8%, free cholesterol 0.3%, cholesteryl ester 0.05% and glyceride 1.0–1.5%, giving a total of 5.1–5.6% as against 9.91% of group I. This is to be connected with the high lecithin content of the liver diet and possibly with its high protein content also.

(b) *The effect of choline.* The addition of choline to the dried liver diet, so that each animal received an extra 214 mg. choline daily in group II, has had no effect on the amount of cholesteryl esters, but has lowered the glyceride content to 0.9%. This decrease in glyceride from 3.2 to 0.9% may be significant, although the significance of such a fall in the pooled livers of groups of six animals must be accepted with reserve.

(c) *The effect of alcoholic extraction of the liver.* In groups IV and V the liver used was the residue remaining after the dried liver had been extracted with alcohol. In group IV there was added beef dripping equivalent in weight to the total material removed by alcohol. This diet was thus lacking both in the phosphatide and cholesterol of diet I. The result shows quite clearly that it is the cholesterol in the liver which is mainly responsible for the fatty liver produced, for the cholesteryl ester has decreased to 0.45% as compared with 1.94% in group I. It might have been anticipated that it would have decreased to the normal value, 0.05%, and it is possible that its failure to do this depends on the difficulty of completely extracting dried liver with alcohol in bulk in the laboratory. Coincidentally with this decrease in cholesteryl ester, the glyceride falls to 1.67%, a more or less normal figure. In group V the diet was the same as in group IV, save that the amount of cholesterol in the original liver was replaced and comparison with group IV shows the result of that replacement. The cholesteryl ester fraction has increased from 0.45% to 3.62%, with a corresponding increase in the glyceride to 3.19%, showing quite clearly the effect of cholesterol in the diet in causing fatty liver production. This result seems to bring out another point, because the cholesteryl ester content, 3.62%, is considerably greater than that of the original dried liver of group I, 1.94%, and this implies that the absence of lecithin in group V is the explanation of the increase over the control figures.

(d) *The effect of the aqueous extraction of the liver.* In group III the residue after aqueous extraction was used, with the replacement of the aqueous extract as glucose. Analysis of the aqueous extract showed that the liver residue con-

tained virtually all the original cholesterol, phosphatide and glyceride. The results show that the amount of cholesteryl ester resulting is unchanged from that of the control group. This result is in contrast with that of Blatherwick *et al.*, who found that feeding water-extracted liver residue as 75% of the diet did not result in fatty livers. It was on this evidence that they suggested the possibility that there was a water-soluble factor necessary for fatty liver production, for they pointed out that the original cholesterol of the liver was still present in the liver residue. Our results however do not provide evidence for this suggestion, because the amount of cholesteryl ester is unchanged in group III. It is true that the glyceride content of group III, 1.53%, is some 1.8% lower than that of the control group, but it is probable that this fall is not of significance. Six animals only were used in each group and it is our experience that it is necessary to be guarded in interpreting changes in the glyceride of this magnitude derived from the pooled tissues of such small groups.

In group VI the residue remaining after alcohol and water extraction was used as a source of liver, but the total alcoholic extract was replaced as beef dripping and its cholesterol also replaced with a further 2% cholesterol. This diet differed therefore from the original dried liver of group I in lacking the phosphatide and water-soluble material but containing 2% of added cholesterol. The figures in Table III show that there is increased deposition of cholesteryl ester consequent upon the inclusion of an extra 2% of cholesterol in the diet.

Diet VII was identical with diet VI, save that the water extract was replaced, and the results are very similar to those of diet VI.

Comparisons then both of the results of group III with those of group I, and of those of group VII with those of group VI, do not seem to provide evidence for the presence of a water-soluble factor involved in cholesteryl ester deposition or for the alternative explanation mentioned in the introduction, because in all of these cases the amount of cholesteryl ester appearing in the livers is related to the cholesterol content of the diets and is uninfluenced by the presence or absence of the dietary liver aqueous extract.

One further point for notice concerning all the groups I-VIII is that the figures for lecithin in these groups are above the normal figure, which we regard as 3.7-3.8%. This may be connected with one of two factors. It has been shown that the presence of phosphatide in diets results in the deposition of phosphatide in the liver [Rewald, 1928] and secondly that diets of high caseinogen content cause increased values for liver phosphatide [Beeston *et al.*, 1935]. Either or both these explanations may be applicable in the case of these liver diets, although the former cannot apply to groups IV and V, in which the lecithin had been removed by alcoholic extraction.

(e) *The effect of raw liver.* In groups IX, X and XI the animals received raw liver as an exclusive diet for 28 days. The results are generally similar to those of group I which received dried liver, except that the amount of glyceride is lower than in group I; this is to be correlated with the fact that whereas on both the dried and the raw liver diets, groups I and IX, the cholesterol intakes were virtually identical, 92 and 85 mg. per rat per day respectively, the fat intake was considerably lower for the raw liver group, IX, namely 1.3 g. per rat per day, as compared with 2 g. per day of the dried liver group I.

The effect of adding choline to the raw liver diets was studied in groups X and XI, in which 0.2 and 0.4% of choline chloride were added. The increased choline intakes of groups X and XI compared with group IX were 56 and 119 mg. per rat per day respectively and this has caused no significant change in the amount of fat in the livers.

SUMMARY.

1. In confirmation of the findings of Blatherwick *et al.* it is found that the feeding of rats on diets consisting mainly of liver, either raw or dried, causes the production of fatty livers.

2. These fatty livers are characterised by the presence after 3 weeks of some 2 % of cholesteryl ester with a small increase in the percentage of glyceride.

3. The addition of choline to the diet had no significant effect in decreasing the amount of fat infiltration. In this connection it is to be remembered however that the liver diets contain much lecithin, the choline of which may be exercising a maximum effect already.

4. Attempts were made to confirm the suggestion of Blatherwick *et al.* that there was a water-soluble factor present in liver, necessary for fatty liver production. The results obtained however do not support the suggestion.

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