XLIV. CYSTINE AND THE DIETARY PRODUCTION OF FATTY LIVERS.

BY ALAN WILMOT BEESTON AND HAROLD JOHN CHANNON.

From the Department of Biochemistry, the University of Liverpool.

(Received December 30th, 1935.)

IN a previous paper it was shown that the amount of fat appearing in the livers of rats receiving a diet of low choline content (choline intake 1.5 mg. per rat per day) and containing 40% of fat was controlled by the amount of caseinogen present in the diet. In a later paper the findings concerning this lipotropic action of caseinogen were extended to embrace the glyceride fraction of the "cholesterol" fatty liver [Channon and Wilkinson, 1935; Beeston *et al.* [1935], whilst more recently results were reported which suggested that the caseinogen used in our diets possessed a lipotropic action equivalent to 8 mg. of choline per g. [Beeston and Channon, 1935].

In the first-mentioned paper it was suggested that further information concerning this control of liver fat by dietary protein fractions might be obtained by supplementing diets low in caseinogen with individual amino-acids, and a systematic study of this problem is in progress. The present paper records results already obtained with cystine. Contrary to the original expectation, it has been found that supplementing a diet low in choline and containing 5% caseinogen and 40% fat with small amounts of cystine causes a profound increase in the amount of liver fat.

EXPERIMENTAL.

The control groups of animals, 8-10 in each group, received a diet consisting of caseinogen 5, beef dripping 40, marmite 5, glucose 44, salt mixture 5 and cod-liver oil 1 part. Other groups received this diet supplemented with amounts of cystine varying from 0.05 to 1.0% of the dry weight of the diet. The cystine was thoroughly incorporated with the protein before admixture with the rest of the diet and the amount ingested calculated from the food intake which was measured daily. The periods of the experiments varied from 14 to 21 days. At the end of each experiment the animals were killed by guillotine and determinations were made of the amount of unsaponifiable matter and fatty acids present in the livers by the methods previously used [Channon and Wilkinson, 1935], and in the tables which follow the "fat" content of the liver represents the sum of the fatty acids and the unsaponifiable matter.

In all the experiments save No. 1, in which the livers were pooled, determinations of the fat contents of the individual livers were made; for brevity however the average results obtained for the different groups in the various experiments will form the basis of discussion and the individual figures for one experiment only will be discussed in detail.

In Table I there are recorded the essential practical data concerning all the experiments. The figures show that the food intake of the animals in the different experiments was adequate and the amounts eaten by the control and experimental groups were quite comparable; they show further that any changes in body weight during the experiments were too insignificant to play any part in the interpretation of the results. Because the variations in the final weights

Table I. *Experimental data and analyses of livers of rats receiving basal diet with and without added cystine. (Mean values.)*

Exp.	No. of animals	Cystine content of diet %	Food intake g./rat/day	Av. final body wt. g.	Av. change in body wt. $\pm$ % initial body wt.	Period of exp. days	Av. wt. of fat in liver g.	Av. wt. of liver g.
1. Control	9	—	10.0	197	- 2.5	20	0.87	7.07
Control + cystine	10	1	8.0	204	- 0.1	—	2.13	10.20
2. Control	9	—	8.3	168	+ 0.2	21	2.59	8.43
Control + cystine	10	0.2	8.7	183	+ 17.3	—	5.04	12.73
Control + cystine	10	0.6	7.8	170	+ 4.0	—	3.79	10.28
3. Control	9	—	6.9	145	- 4.9	14	1.16	6.98
Control + cystine	8	0.2	6.9	146	- 4.0	—	2.24	9.20
4. Control	10	—	7.3	141	- 6.5	18	1.70	7.16
Control	8	—	5.7	139	- 8.1	—	1.71	7.27
Control + cystine	10	0.05	6.5	143	- 4.4	—	2.04	7.66
Control + cystine	8	0.1	7.5	155	- 1.7	—	3.09	9.27
Control + cystine	10	0.2	6.7	149	- 3.2	—	2.10	8.15
Control + cystine	10	0.4	6.7	153	- 2.9	—	3.04	9.42
Control + cystine	10	0.6	7.3	156	$\pm$ 0.0	—	4.04	10.76

attained by the animals of the different groups prevent ready comparison of the individual figures recorded in Table I, the more essential figures are presented in Table II, calculated from the final body weights on the basis of the 100 g. rat, together with the daily cystine intake per animal. The figures in Table II show

Table II. *Fat in liver of animals receiving basal diet with and without added cystine. (The figures refer to the 100 g. rat.)*

Exp.	No. of animals	Liver as % of body wt.	Fat in liver % fresh wt.	Wt. of fat in liver g.
1. Control	9	3.60	23.74	0.745
Control + cystine (80 mg.)	10	5.26	39.65	1.921
2. Control	9	5.12	26.34	1.365
Control + cystine (17.4 mg.)	10	7.10	37.38	2.845
Control + cystine (46.8 mg.)	10	6.07	36.34	2.249
3. Control	9	4.83	14.54	0.799
Control + cystine (13.8 mg.)	8	6.16	24.71	1.342
4. Control	10	5.20	21.67	1.214
Control	8	5.16	21.18	1.263
Control + cystine (3.25 mg.)	10	5.38	24.93	1.644
Control + cystine (7.50 mg.)	8	6.41	34.50	2.158
Control + cystine (13.40 mg.)	10	5.56	24.40	1.409
Control + cystine (26.80 mg.)	10	6.10	27.86	1.981
Control + cystine (43.80 mg.)	10	6.59	35.42	2.593
Mean control	Total 45	4.78	21.50	1.077
Mean control + cystine	Total 86	6.06	31.78	2.02

the profound effect of cystine in increasing the fat content of the livers of animals receiving this particular diet. Thus in Exp. 1 the weight of fat in the liver is some $2\frac{1}{2}$ times as great as in those of the control animals, a result which is compounded from two factors, namely the increase in the percentage of fat in the liver from 23.74 to 39.65 % of the fresh liver weight, together with the increase in the size of the liver from 3.60 to 5.26 % of the body weight.

The results for Exp. 2 in which the cystine intakes of the two cystine groups were reduced to 17.4 and 46.8 mg. respectively are scarcely less striking. Here

again the amount of liver fat is more than doubled in the one case and some 60% greater in the other, whilst the percentages of fat in the liver are 37.38 and 36.34% of the wet weight, compared with the 26.34% of the control animals. There are also similar corresponding increases in the liver size.

Exp. 3 was then carried out on groups of animals introduced from an outside source and these received 13.8 mg. of cystine daily. These animals, which were utilised as soon as they were imported into the laboratory, were nervous and unaccustomed to handling, a fact which may perhaps account for the low fat percentage (14.54%) given by the control group. The same results appear however, for the liver fat is increased to almost double the control value, with increases in the percentage of fat in the liver and the liver size of the same order as in the previous experiments.

In an endeavour to determine whether there was any relationship between the amount of cystine ingested and the amount of liver fat which resulted, Exp. 4 was carried out. Because of large individual variations it was deemed wise to run two control groups of animals in this experiment, whilst five groups received added cystine. From the figures in Table II it does not appear that there is a quantitative relationship between the amount of cystine ingested and the weight of fat in the liver, for there is no regular gradation with the increasing cystine intake, whilst one group, *i.e.* those receiving 13.4 mg., has given a figure for liver fat which is no more than 16% greater than the controls. It appears possible that this lack of gradation with increasing dosage may be due to the relatively small groups of animals used, because of the large individual variation which occurs, particularly among cystine-fed animals. This point will be discussed in the next section. The results of Exp. 4 however bear out those of the first three experiments.

It seemed worth while to draw up mean values for all the control animals and all those which received cystine, irrespective of the amounts of cystine received and the duration of the experiments. These figures appear at the bottom of Table II, where the mean value for the weight of liver fat for 45 control animals is 1.077 g., whereas that for 86 animals which received cystine is 2.02 g. This 87% increase is due to two factors, the increase in liver size from 4.78 to 6.06% of the body weight and the increase of the liver fat percentage from 21.50 to 31.78% of the fresh weight. It appears then from these results that the effect of the inclusion of small amounts of cystine in a diet which contains 40% of fat and is low in choline is to double the amount of the already high deposition of fat resulting from the control diet. It is to be remembered that the liver of the normal 100 g. rat weighs about 3.5 g. and contains about 0.12 g. of total fatty acids.

In order to make clear to other workers the great variations which may occur, the individual values obtained for the animals in a typical experiment, Exp. 2, are recorded in Table III, in which the figures again refer to the 100 g. rat.

In the control group the liver varies from 3.98 to 6.69% of the body weight, whilst in the two cystine groups the variations are from 3.53 to 11.88% and 4.91 to 7.96% respectively. The overlap shown in these figures appears also in the percentage of fat in the liver. Thus in the control animals the variations are from 14.33 to 40.35%, whilst in the cystine groups they are from 15.18 to 48.96% and from 29.58 to 43.14%. Hence a similar overlap occurs in the actual weight of fat in the liver. In the control group this varies from 0.657 to 2.271 g., whilst in the cystine groups it is from 0.536 to 5.817 g. and 1.371 to 3.434 g. respectively. Superficially it might appear that these individual variations are such as to render dubious the finding that the addition of cystine

Table III. *Individual values for liver fat of animals receiving basal diet with and without added cystine (Exp. 2).*

Control				Control + 0.2% cystine				Control + 0.6% cystine			
Animal no.	Liver wt. g.	Liver fat % fresh wt.	Fat in liver g.	Animal no.	Liver wt. g.	Liver fat % fresh wt.	Fat in liver g.	Animal no.	Liver wt. g.	Liver fat % fresh wt.	Fat in liver g.
1	5.09	30.35	1.545	10	5.59	31.52	1.762	20	7.24	35.85	2.595
2	5.98	37.96	2.271	11	5.71	37.83	2.160	21	4.91	29.58	1.452
3	4.47	24.23	1.082	12	8.34	42.41	3.535	22	7.96	43.14	3.434
4	3.98	16.50	0.657	13	6.78	40.13	2.720	23	6.37	39.64	2.524
5	6.69	40.35	2.179	14	6.63	39.00	2.586	24	6.67	41.38	2.760
6	5.55	14.33	0.795	15	9.13	48.53	4.431	25	5.58	32.74	1.827
7	4.94	18.82	0.930	16	5.51	27.46	1.513	26	4.60	29.78	1.371
8	5.53	19.14	1.058	17	3.53	15.18	0.536	27	5.28	38.58	2.036
9	4.97	35.34	1.757	18	7.92	42.85	3.394				
				19	11.88	48.96	5.817				
Mean	5.12	26.34	1.365		7.10	37.38	2.845		6.07	36.34	2.249

has had an effect, but statistical analysis shows that the results recorded in Table III are significant. Further, the similar results of Exps. 1, 3 and 4 seem to provide ample confirmation of the finding. Necessity for brevity precludes discussion in detail of the distribution of the figures about the so-called mean value and this distribution must be seen by inspection of Table III.

Typical difficulties of individual variations encountered may be illustrated by reference to these animals. The liver of rat No. 17 (0.2% cystine) was but 3.53% of the body weight (which is the usual figure for the normal animal), and contained 15.18% fat; there was thus only 0.536 g. present in this liver. Yet the liver of animal No. 16, which had the next smallest liver of this group, constituted 5.51% of the body weight, contained 27.46% fat and there were present in it 1.513 g. of fat, a quantity nearly three times as great. Further, the liver of rat No. 19 contained 5.817 g. fat, which is eleven times as much fat as that of No. 17. The considerable variations in the amount of fat in the livers of rats receiving choline-free diets has been discussed by Best and Channon [1935], but has no explanation at present. It does not appear to be due to varied food intake, nor can these individual variations in this type of experiment be overcome by use of litter-mates [Channon and Smith, 1936]. The addition of cystine both in the experiment under discussion and in the others described in this paper appears to make for even greater individual variations.

It is of some importance to draw attention to the magnitude of the changes of the livers of some of the cystine-fed animals. The most striking example is that of rat No. 19, the liver of which was 11.88% of the body weight and which contained 48.96% of its wet weight as fat, or 5.817 g. The presence of 5.817 g. of fat in the liver of a 100 g. animal is very striking, for it has to be remembered that the normal animal contains some 0.12 g. of total fatty acid in its liver. Further, about 90 mg. of this fatty acid are present as phosphatide, and therefore the amount of the glyceride present in the liver of this particular rat is something like 200 times greater than the normal. Further, this weight, 5.817 g., is approximately $1\frac{1}{2}$ times the fresh weight of the liver of the normal animal! Although we did not estimate the amount of body fat in these animals, it is usual to find a figure of something like 12% after experiments in which rats have received the control diet employed. This means then that the liver of this particular animal contained an amount of fat equal to one-half of the total fat in the body of the normal control animal.

It seems to us that this finding of a means of further enhancing the amount of liver fat should make easier the problem of investigating the source of this fat, for if any serious proportion of the liver fat in these livers comes from the fat depots, the quantity present is so great as to make its disappearance from the depots easily demonstrable. The difficulty hitherto has been that the amount of fat appearing in the liver has been quantitatively too small to make investigation of this problem in regard to the depots possible and to this problem we are now giving attention.

At the present stage it would be unwise to attempt to interpret the mechanism of the cystine action until further work has been carried out, for so far as we are aware, the only recorded finding of a similar nature was that of Curtis and Newburgh [1927], who recorded a single instance of fatty infiltration and severe necrosis in a rat receiving a diet containing 15 % caseinogen with 4 % cystine. In this connection it may be well to state that, although we have not yet carried out experiments on the action of choline in preventing the cystine effect, we have observed that, if the protein content of the diet is increased to 30 % with the fat remaining constant at 40 %, fat infiltration does not occur, which shows that an increased caseinogen intake can prevent not only the fat infiltration due to the high fat diet but also the superimposed effect of cystine.

We may add that one experiment has been carried out with each of the amino-acids lysine, glutamic acid, aspartic acid, serine, glycine and phenylalanine. Whilst deductions cannot be made with certainty from single experiments in this type of work, it may be mentioned that no effect either in increasing or decreasing the amount of liver fat has been as yet observed. These studies are being extended to the other amino-acids and related compounds.

SUMMARY.

1. Groups of rats have been fed on a diet containing caseinogen 5, fat 40, glucose 44, marmite 5 and salts 5, with and without the addition of cystine, such that each rat ingested from 3.25 to 80 mg. of cystine daily over periods of 14 to 21 days.

2. If all the results be pooled and expressed on the basis of the 100 g. rat, the average amount of fat in the livers of 45 control animals was 1.077 g., whereas that of the cystine-fed animals was 2.02 g., the amount of liver fat being doubled by the addition of cystine. This increase is caused by some 50 % increase in the percentage of fat in the liver, together with a 25 % increase in the liver weight.

3. Some abnormally high values were obtained in animals which received cystine, the highest being an animal in which the liver constituted 11.88 % of the body weight and contained 48.96 % of its fresh weight as fat, which corresponds to the presence in the liver of a 100 g. rat of 5.817 g. of fat.

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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