XIX. THE DIETARY PREVENTION OF FATTY LIVERS. TRIETHYL- β -HYDROXYETHYL-AMMONIUM HYDROXIDE.

BY HAROLD JOHN CHANNON AND JAMES ANDREW
BUCHAN SMITH.

From the Department of Biochemistry, The University of Liverpool.

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THE discovery of the action of choline in preventing fat accumulation in the livers of rats [Best and Huntsman, 1932] has been considerably extended, and it has become apparent that in a variety of experimental conditions this substance exercises both preventive and curative actions on fat deposition in the liver [Best and Huntsman, 1935]. The fact that choline is the base present in lecithin makes it possible that its action may be exercised through synthesis of that substance, in accordance with the original suggestion of Leathes that fatty acids might be transported from the liver to the tissues after incorporation in the phosphatide molecule. Light should be thrown on this question if a base could be found which possessed an action on liver fat similar to that of choline and which could be shown to be present in the liver and other tissues in very small amount by isolation of a suitably characteristic derivative. Such a base might be found to have been incorporated in a new phosphatide molecule in which it had replaced the choline of lecithin. Its absence from the phosphatide would not exclude the possibility that choline exercised its action by enabling lecithin synthesis to occur, but would perhaps make that mode of action less likely. Its presence however would provide a means for investigation of the whole problem from many new aspects.

We have therefore begun a study of the action of substances chemically similar to choline. Whilst considerable difficulties have been encountered because of the toxicity of these compounds, we are able to report in the present paper that triethyl- β -hydroxyethylammonium hydroxide, which differs from choline only in the replacement of the three methyl groups of that substance by ethyl groups, possesses an action on liver fat similar to that of choline. It will be recalled that in their original work Best and Huntsman [1932] reported that betaine was effective, whilst aminoethyl alcohol was without action. The former finding might indicate an action independent of lecithin, whilst the latter seems to preclude kephalin from being involved.

EXPERIMENTAL.

In each experiment three groups of rats were used and all received the basal fatty liver-producing diet, which consisted of beef dripping 40, glucose 45, extracted caseinogen 5 and salt mixture 5, while marmite, 5 parts, was used as a source of B vitamins and vitamins A and D were supplied as cod-liver oil (1 drop per animal every 3 days). One group served as control, whilst in addition to the basal diet the second received triethyl- β -hydroxyethylammonium hydroxide and the third choline, in order to obtain some quantitative comparison of the action with that of choline. These groups are designated "control",

“derivative” and “choline” in the table and discussion which follow. The choline content of the basal diet was about 1.7 mg./100 g. The bases were used as their chlorides and were administered by the methods set out in the table. The animals were kept in groups of two to four per cage and their average daily food intake was determined.

Preparation of triethyl- β -hydroxyethylammonium hydroxide.

Molecular proportions of triethylamine and ethylenechlorohydrin were heated in a sealed tube at 95° for 4 hours. To a solution of the resulting product in absolute alcohol were added acetone and ether. The exceedingly deliquescent fine crystalline material was redissolved in alcohol and similarly reprecipitated four times. (Found: Cl 19.8, N 7.5; $C_8H_{20}ONCl$ requires Cl 19.55, N 7.7 %.) The chloroaurate melted at 225° (C 19.7, H 4.8, N 3.12, Au 40.5 %; $C_8H_{21}ONAuCl_4$ requires C 19.7, H 4.3, N 2.88, Au 40.6 %).

The results of Exps. 1 and 2, in which the livers of all the groups were treated as pooled samples, show that the derivative has prevented the accumulation of liver fat, Exp. 2 suggesting that its action is at least equivalent to that of choline in the amounts given. In both experiments, however, the food consumption of the derivative group was substantially less than that of the control animals, whilst the weight losses were considerably greater. Although weight losses of this magnitude do not affect the percentage of fat in the liver [Best *et al.* 1935], at the same time the much lower food intake in Exp. 2 and the greater toxicity of the compound under examination made further experiments desirable in order to attempt to overcome the possible effects of these factors on the results. In all the remaining experiments the individual livers were investigated. Exps. 3 and 4 were then carried out by administration of a solution of the substance orally by drops and by stomach tube respectively, on the assumption that the exceedingly bitter taste of the derivative was rendering the diet unpalatable. The control and choline groups in these two experiments received solutions of sodium chloride and of choline chloride respectively, administered by the same methods in order to avoid any interfering effect of the administration techniques.

The effect of the compound was shown by the result of Exp. 3, in which the food intake of the derivative group compared with that of the control group was better than in Exp. 2, with closely similar body weight changes, although the effect seemed less than that of choline at an approximately similar daily dosage in contrast to Exp. 2. This may be connected with the fact that the method of administration caused so intense a salivary flow that much of the derivative may have been lost in this way. A further fact was that whereas the individual values for the does ranged from 5.7 to 8.8 % (mean 7.4 %), for the derivative group, compared with the control range of 8.7–24.0 % (mean 14.75 %)—a satisfactory result—for the bucks of the derivative group the range 6.6–11.8 % showed a considerable overlap with the control bucks, 8.4–31.0 %. Evidence regarding this overlap was obtained in further experiments.

The result of Exp. 4 was again satisfactory, for the livers of the derivative bucks contained 9.2 % “fat”, against the control value of 33.1 % and the choline group value of 8.5 %, although the food intake was somewhat less than that of the controls. Thus 27.8 mg. derivative seemed just as effective as 26.9 mg. choline. In this Exp. there was no overlap in the extreme values (controls 19.6–42.4 %; derivative group 4.8–12.0 %).

The results of Exp. 4 led us in Exp. 5 to attempt to observe the effects further by incorporation of the substance in the diet as in Exps. 1 and 2, in the hope that

Table I. *The effect of triethyl- β -hydroxyethylammonium hydroxide in preventing fat deposition in the liver.*

No. of exp.	Duration of exp. (days)	Method of administration of choline or derivative	Choline or derivative ingested/animal/day (mg.)	Group	No. and sex of animals	Wt. gain or loss (%)	Diet eaten per animal per day (g.)	Liver wt. % body wt. (g.)	Fatty acids and unsap. matter in liver (%)	Extreme limits of figures averaged in preceding column	Wt. of fatty acids and unsap. matter in liver of 100 g. rat (g.)
1	17	Incorporated as an aqueous solution in diet	*	Control	6 M & F	- 8.4	9.6	4.1	24.1	Pooled sample	0.99
				Choline	6 "	- 7.7	9.3	3.1	5.2		0.16
				Derivative	6 "	- 10.6	7.3	3.3	7.1		0.23
2	20	As in 1	—	Control 1	3 F	- 7.6	10.0	4.1	20.7	Samples pooled	0.69
			—	" 2	3 F	- 3.7	10.0	4.1	30.5	according to body weight changes	1.25
			—	" 3	3 F	+ 4.3	10.0	4.1	23.7		0.87
			—	" 4	3 F	+ 4.3	10.0	4.1	20.0		0.66
			79.8	Choline 1	5 M	+ 3.0	9.2	2.8	8.9		0.25
				" 2	5 M	- 2.2	9.2	2.8	8.1		0.23
			47.6	Derivative	10 M & F	- 13.6	5.3	3.1	7.0		0.22
3	18	Aqueous solution orally from bu-rette	—	Control 1†	4 F	- 4.2	7.4	4.4	14.75		0.65
			—	" 2	5 M	- 3.0	8.9	3.6	16.75		0.60
			61.6	Choline 1	5 F	+ 1.2	7.9	3.8	5.2		0.20
				" 2	5 M	+ 1.0	9.5	2.8	5.3		0.15
			67.4	Derivative 1	5 F	- 7.7	5.9	3.8	7.4		0.28
				" 2	5 M	- 6.1	8.1	3.7	9.6		0.36
4	18	As aqueous solution by stomach tube	—	Control 1	6 F	- 4.7	8.8	4.1	19.1		0.78
			—	" 2	6 M	- 8.8	8.8	5.8	33.1		1.92
			26.9	Choline 1	6 F	+ 1.6	9.1	3.1	5.9		0.18
				" 2	6 M	- 2.9	9.1	3.3	8.5		0.28
			27.8	Derivative 1	1 F†	- 9.4	7.0	3.7	5.1		0.19
				" 2	6 M	- 13.4	7.0	4.0	9.2		0.37
5	19	As in 1	—	Control 1	5 F	- 8.7	8.4	4.7	26.1		1.23
			—	" 2	5 M	- 6.9	8.4	4.6	30.3		1.39
			20.8	Choline 1	5 F	+ 3.3	9.5	3.2	5.8		0.19
				" 2	5 M	+ 0.5	9.5	3.3	7.5		0.25
			27.8	Derivative 1	5 F	- 1.9	8.8	3.4	8.8		0.30
				" 2	5 M	- 7.5	8.8	3.9	18.7		0.73
6	13	As in 1	—	Control 1	8 F	- 0.2	8.0	4.7	19.6		0.92
			—	" 2	8 M	- 5.8	8.0	6.4	31.4		2.01
			8.8	Choline 1	8 F	+ 2.9	8.4	3.8	8.3		0.32
				" 2	8 M	- 2.4	8.4	4.2	8.1		0.34
			18.3	Derivative 1	8 F	- 5.5	7.0	3.7	8.0		0.30
				" 2	8 M	- 3.8	7.0	4.8	18.7		0.90

* Actual amounts of choline and derivative not measured. The diets contained however 10 mg. of each per g. diet.

† Five animals died.

the low amount of the substance used would render the diet more palatable. In this experiment equimolar concentrations of choline and the derivative were used, although the better food consumption of the choline group rendered the actual intakes of choline and the derivative slightly different. The food intake and body weight changes were strictly comparable in the control and derivative groups and the results of more certain value. These were similar to those of Exp. 3, for whereas the results with the does were entirely satisfactory, the bucks again showed apparently less reaction to the derivative (σ control 17.4–39.2 %, mean 30.3 %; derivative 12.7–29.4 %, mean 18.7 %; choline 6.3–9.8 %, mean 7.5 %).

Exp. 6 was carried out by the same method, but at still lower concentrations of choline and the derivative. Here again the results for the does were completely positive (control 12.8–36.7 %, mean 19.6 %; derivative 5.6–10.3 %, mean 8.0 %), whilst those of the bucks were very similar to those of Exp. 5 (control 15.2–39.9 %, mean 31.4 %; derivative 8.9–27.8 %, mean 18.7 %).

The results of Exps. 1–6 showed therefore that the compound adequately prevented the deposition of fat in the livers of the does and that in this action it was almost as potent as choline. Its effect seemed far less pronounced however with the bucks, as was shown in all four Exps. (3–6) in which individual values were investigated. No obvious explanation appeared for this. The wide range of variation of the liver fat in the control groups of animals, which always occurs, makes any result of this kind liable to suspicion and accordingly a more elaborate experiment was carried out, in which individual food intakes were measured and every effort made to substantiate the findings by adopting as rigorous a method as possible.

In this experiment 72 animals were used and divided into three groups as before, each rat being kept in a separate cage. Of the 24 rats in each group, 12 were does and 12 bucks and of these 6 does and 6 bucks were chosen so that they were from the same litters as the corresponding animals in the other two groups. Thus for each rat in the derivative group there were corresponding litter-mates in the other two groups. The remaining 12 animals in each of the 3 groups (6 bucks and 6 does) were taken from stock and thus served for comparison with the 12 litter-mates also in each group. Further, in order to control as far as possible any effect of varying food intake, the 24 animals of the derivative group, in which in Exps. 1–6 the food intake had tended to be lower, were placed on their diet a day before the choline and control groups. Then throughout the experiment the animals of the latter two groups were fed as nearly as possible with the same amount of food as the derivative animals had consumed during the previous day. In this way at the close of the experiment, which lasted 13 days, the food intake of rat No. 1, say, in the control group was almost the same as that of each of the rats No. 1 in the other two groups and so on.

The results of the experiment are represented diagrammatically in Fig. 1, in which the values for the control animals were plotted in ascending order of magnitude, the lowest being that of rat No. 10, and the highest that of No. 6. The figures for the corresponding animals in the other two groups were then placed in position and the curves obtained as shown. There also appears on the diagram the food intake for the rats of the control group which, as already stated, was almost identical with that of the corresponding members of the other two groups because of the method of feeding adopted.

Certain conclusions may readily be drawn from the figure. In the first place, although the food intake varies from 6.3 to 9.8 g. and the fat content of the liver in the case of the controls from 12.7 to 35.8 %, yet there is no relationship

whatever between them and this is equally true for the other two groups. The individual variation cannot therefore be explained by differences in food intake. In the second place the large individual variation is in no way dependent on whether the rats were litter-mates or simply selected in the usual way from stock. This is clear when it is considered that Nos. 1-12 in any group on the diagram were litter-mates of the corresponding numbers in the other two groups, the variation being as great amongst Nos. 1-12 as amongst Nos. 13-24.

Fig. 1. Percentage of fat in liver of rats with their food intake. The average amount of choline and of derivative eaten per animal per day was 18.2 and 22.4 mg. respectively.

Furthermore from the lines showing the mean values in the three cases it is obvious that, on the average and with the amounts of each substance ingested, the derivative has had an action equal to about 2/3 that of choline, the actual figures being 24.8 (control), 6.4 (choline) and 12.2 % (derivative), a reduction of 18.4 units % with choline and of 12.6 units % with the derivative. Although this is true for the average of all 24 animals in each group, it must again be pointed out, as in previous experiments, that the average figure for the does (control 23.7, choline 5.9 and derivative 9.5 %) was again smaller and the derivative apparently more effective than in the case of the bucks (25.9, 6.9 and 14.6 % respectively). This also appears from the diagram to be independent of the amount of food ingested.

Therefore although the problem of the large individual variation so often encountered in these and in similar experiments remains unsolved, the action of the derivative has again been confirmed.

One further point of interest is that the figure shows that the curve of the liver fat of the choline animals follows in a general way that of the corresponding derivative animals in that it tends to rise and fall at the same time, although in a far more limited degree.

A final experiment was now performed to ensure that there was no abnormal excretion of fat in the faeces of the rats receiving the derivative. Two groups of six animals, each containing three bucks and three does, were fed for 15 days on the 40 % fat diet, one group receiving the diet only, the other being given the derivative in addition. The results showed that both groups of animals excreted 0.22 g. faecal fatty acids per rat per day.

In view of the findings that the derivative exercises an effect in so small a daily dose, it is proposed to repeat some earlier experiments which were abandoned at that time on account of the toxicity of the products. In these experiments trimethylamine, trimethyl- γ -hydroxypropylammonium hydroxide and tripropyl- β -hydroxyethylammonium hydroxide were used in the hope that these alterations might throw light on the nature of the active group. Further experiments are also in progress with the derivative used in the present work, with a view to attempting to utilise the preventive action of this compound on liver fat as a means of investigating its mode of action by the methods indicated earlier in this paper.

SUMMARY.

1. The preventive effect of triethyl- β -hydroxyethylammonium hydroxide in controlling the percentage of fat in the livers of rats was studied in seven separate experiments.

2. This compound was shown to have a preventive action, although the effectiveness of its action was less than that of choline.

3. Furthermore it was found to have a greater action in the control of liver fat in the case of does than it had in the case of bucks.

4. The large individual variations found in rats of the same groups remains unexplained. Attempts to control these variations by use of litter-mates for the different groups and of controlled food consumption did not throw light on the variations, which appear to be independent of food intake in the conditions of these experiments.

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