CXXXII. THE EFFECT OF PROTEINS IN THE PREVENTION OF DIETARY FATTY LIVERS

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RATS fed on low protein diets of high fat content with or without small amounts of cholesterol develop intensely fatty livers; when caseinogen is used as the dietary protein, the degree of fat infiltration diminishes on increasing the amount of this protein present in the diet, irrespective of any action of choline [Channon & Wilkinson, 1935; Beeston *et al.* 1935]. Further work has shown that a daily intake of 1 g. caseinogen has an effect equivalent to that of 7–8 mg. choline [Beeston & Channon, 1935; Beeston *et al.* 1936]. Best *et al.* [1936] obtained similar results regarding this extensive action of caseinogen on liver fat deposition in preventive experiments. While we are continuing our studies of the action of pure amino-acids as one method of elucidating the mechanism of this action of caseinogen [Beeston & Channon, 1936; Channon *et al.* 1938], information as to the relative lipotropic actions of other proteins is desirable for two reasons. Firstly, it would enable the intensity of action of different proteins to be studied in relation to their amino-acid composition, and perhaps provide evidence of value from this point of view; secondly, it is important to determine which proteins are most satisfactory from the experimental point of view. Diets low in protein tend to cause weight losses even in the short term studies often used in this type of work, and a balance has to be struck between the desirable high fat percentage in the liver and ensuing weight losses. The work described in this paper is, therefore, a study of the actions of a number of plant and animal proteins in preventing fat deposition in the liver, caseinogen being used as the standard.

EXPERIMENTAL

Preparation of the proteins

Edestin was crystallized from 5% NaCl extracts of hempseed, washed twice with water, twice with alcohol and with ether; the dried powder was extracted twice with hot alcohol and with ether. Yield 75–80 g./kg. hempseed.

Fibrin obtained from the slaughter-house was water-washed until colourless, and dried in alcohol; the powdered product was extracted with water, cold alcohol, hot alcohol and finally with ether.

Gliadin. Repeated extraction with 73% alcohol (3, 3 and 2 l.) of wheat gluten flour (1 kg.), concentration *in vacuo* of the extracts and cooling in the refrigerator gave a jelly of gliadin, which was dehydrated by successive treatments with acetone; the finely ground product was extracted with cold absolute alcohol and with ether. Yield 275–290 g./kg. gluten flour.

Zein. Maize gluten meal (1 kg.) was extracted repeatedly with hot 80% alcohol (4, 2 and 2 l.), the extract concentrated to 2.5 l., and mixed with 2.5 l. of ether. The gelatinous precipitate was dissolved in warm 90% alcohol and

allowed to drip slowly from a separating funnel into running water; the precipitated zein was collected, washed in water, air-dried and purified by extraction with ether. Yield 130–150 g./kg. maize gluten meal.

Beef muscle protein. Lean meat from which fat had been carefully dissected was minced, dried in alcohol and ground; the powder was repeatedly extracted with hot alcohol and hot ether.

Gelatin. "Cox's Instant Powdered Gelatin" was extracted twice with alcohol and once with ether.

Whale muscle protein and Gromax, commercial preparations of whale meat kindly supplied by Messrs Lever Bros. and Unilever, Ltd., were purified by repeated extraction of the crude material with boiling alcohol and with ether; after the first few extractions the product was ground to a fine powder.

Albumin was a commercial preparation from eggs. *Caseinogen* was an alcohol-extracted preparation supplied by Glaxo, Ltd. Neither of these materials was further extracted since they were found to be essentially choline-free.

Nitrogen, moisture and ash determinations were made on each of the materials prepared. The latter was approximately 4% for the beef and whale muscle proteins, 26% for the Gromax, 1.5% for the gelatin and less than 0.5% for the other proteins.

Choline was estimated in each of the products by hydrolysis, acetylation and assay on isolated rabbit intestine; the whale meat protein contained 34 mg. and 53 mg. choline/100 g. in the two batches, the Gromax 18 mg./100 g., but in all the other proteins used the choline content was less than 9 mg./100 g. and in many cases less than 4 mg./100 g.

The animal experiments

In Exp. 1 the control group of rats received a diet which would cause "cholesterol" fatty livers, consisting of caseinogen (alcohol-extracted) 5, beef dripping 30, cod liver oil 1, cholesterol 2, marmite 5, salts 5 and glucose 52 parts. Seven other groups received a similar diet save that 10 parts of different proteins were substituted for 10 parts glucose. There were 9 or 10 female rats in each group, and the period of feeding was 21 days. At the end of the experiment, the livers were pooled, and the fatty acids + unsaponifiable matter prepared by the saponification method. The results are recorded in Table I, in which "fat" in the heading of the last column but one refers to the sum of the fatty acids and unsaponifiable matter: it will, on this particular diet, contain about 3 parts of cholesterol but this will vary little from group to group. The difference between the percentages and amounts of "fat" of the control group and of the other groups will, therefore, to all intents and purposes represent a change in the glyceride content of the liver caused by the inclusion in the diet of 10% of each particular protein.

In Exp. 2 the control group of animals received a diet consisting of caseinogen (alcohol-extracted) 8, beef dripping 39, cod liver oil 1, glucose 47, salt mixture 5 parts; in addition each animal received 12.5 μ g. aneurin and 25 μ g. lactoflavin per day. Ten other groups received the same diet, save that 7 parts of different proteins were substituted for 7 parts of glucose. All the groups contained 10 rats, and were fed for 21 days. The animals were killed by guillotine and the individual livers worked up by the saponification method for fatty acids and unsaponifiable matter. The results are recorded in Table II as mean values for the ten individual rats of each group, and hence figures in the last column for the weight of fat in the liver of the 100 g. rat may differ slightly from the product of the two previous columns which record the liver as a percentage of the

final body weight and the percentage of fat in the fresh liver. The term "fat" means the sum of the fatty acids and the unsaponifiable matter, as it does in all the experiments.

In Exps. 3 and 4 (Table III, A and B) the control diet was the same as in Exp. 2 save that the basal protein was egg albumin instead of caseinogen, while the lactoflavin was omitted. In Exp. 3 the period of feeding was 17 days, and in Exp. 4, 18 days. The livers were investigated in Exps. 3-6 as described for Exp. 2. Details of the diets used in Exps. 5 and 6 are recorded later in the text.

RESULTS

In Exp. 1 the proteins were used as 10 % supplements, since in the work of Beeston *et al.* [1935] 10 % of caseinogen had proved very effective on the "cholesterol" fatty liver when supplementing a basal 5 % of the same protein.

Table I. *Exp. 1. The effect of protein supplements on lipid deposition in the liver of rats receiving a diet containing fat and cholesterol*

Group	Protein of diet	No. of animals	Final body wt. g.	Change in wt. $\pm$ % initial body wt.	Liver % final body wt.	"Fat" % fresh liver	"Fat" in liver of 100 g. rat
1	5 % caseinogen	10	149	-4.6	5.22	32.55	1.699
2	5 % caseinogen + 10 % caseinogen	10	164	+3.7	4.52	22.14	1.001
3	5 % caseinogen + 10 % edestin	10	168	+5.7	4.73	23.76	1.124
4	5 % caseinogen + 10 % fibrin	10	165	+6.7	5.14	27.63	1.420
5	5 % caseinogen + 10 % gelatin	9	157	-2.1	5.54	35.07	1.943
6	5 % caseinogen + 10 % gliadin	10	171	+9.1	5.15	30.33	1.562
7	5 % caseinogen + 10 % Gromax	10	164	+4.1	4.37	27.12	1.185
8	5 % caseinogen + 10 % zein	9	145	-4.2	4.75	27.15	1.290

Table I shows that the addition of 10 % of caseinogen has lowered the liver-fat percentage from 32.55 in the control group to 22.14, and decreased the weight of fat in the liver of the 100 g. rat by 0.698 g. The effect of this percentage of caseinogen, therefore, while not so great as in the work quoted above, is sufficiently pronounced for deductions to be made concerning the relative actions of the other protein supplements. It should be pointed out that, in this paper, more weight has been attached to the values for liver fat/100 g. rat than to the percentages of fat in the fresh liver, though these latter figures have, of course, been considered. Edestin and Gromax have had actions of approximately equal intensity to that of caseinogen; in a second group zein and fibrin may be classed together; gliadin appears to have had little if any effect, while gelatin has caused an increase in the amount of fat to 1.943 g. which is about 15 % greater than the control value.

In Exp. 2 the observations of Exp. 1 were extended to a study of the "fat" fatty liver, and the amount of caseinogen, the basal protein of the diet, was increased to 8 % in order to avoid as far as possible weight losses, which tend to occur on diets containing 5 % caseinogen and 40 % fat. At the same time, egg albumin and the proteins of whale and beef muscle were also investigated, all the proteins being fed as 7 % supplements.

The percentage and weight of fat in the control group, 27.12 and 1.664 g., are both very satisfactory from the point of view of studying the comparative effects of the different proteins. The addition of 7 % caseinogen has lowered the percentage of fat to 17.92 and decreased the weight per 100 g. rat to 0.843 g., which is more than half of the control value. The results in Table II place the

Table II. *Exp. 2. The effects of protein supplements on fat deposition in "fat" fatty livers*

Group	Protein in diet	Final body wt. g.	Change in wt. $\pm$ % initial body wt.	Liver % final body wt.	Fat % fresh liver	Fat in liver of 100 g. rat g.
1 (Control)	Caseinogen 8	180	+ 3	5.99	27.12	1.664
2	Caseinogen 8 + albumin 7	200	+ 11	4.11	19.73	0.880
3	Caseinogen 8 + beef muscle 7	197	+ 7	4.49	20.31	0.994
4	Caseinogen 8 + caseinogen 7	200	+ 10	4.28	17.92	0.843
5	Caseinogen 8 + edestin 7	197	+ 8	4.80	20.82	1.078
6	Caseinogen 8 + fibrin 7	202	+ 13	4.40	19.70	0.906
7	Caseinogen 8 + gelatin 7	192	+ 5	4.31	24.70	1.094
8	Caseinogen 8 + gliadin 7	188	+ 2	4.53	21.29	1.007
9	Caseinogen 8 + Gromax 7	188	+ 1	3.86	17.71	0.738
10	Caseinogen 8 + whale muscle 7	201	+ 8	3.59	14.57	0.570
11	Caseinogen 8 + zein 7	184	+ 1	4.07	18.53	0.801

protein supplements in three classes as regards the order of their effect in preventing fat deposition:

(a) proteins having an intensity of action similar to that of caseinogen—albumin, fibrin, Gromax and zein;

(b) proteins, the effect of which is greater than that of caseinogen—proteins of whale muscle;

(c) proteins having a less intense action than that of caseinogen—beef muscle protein, edestin, gelatin and gliadin.

It is clear from Tables I and II that certain of the proteins have not given consistent results in these two experiments, and this matter will be discussed later.

As a result of Exps. 1 and 2 it was felt that this method of experimentation in which supplements of 7 or 10 % protein were used would not provide adequate evidence concerning proteins of low lipotropic activity. It seemed, therefore, desirable to carry out Exps. 3 and 4 in which the proteins were added as 20 % supplements. The greater effect which should be observable with these larger supplements would serve the further purpose of eliminating the uncertainty

Table III. *The effect of protein supplements in decreasing liver fat*

Group	Protein in diet	No. of animals	Final body wt. g.	Change in wt. % initial body wt.	Liver % final body wt.	Fat % fresh liver	Liver fat per 100 g. rat g.
A. Exp. 3							
Control	8 % albumin	9	177	- 4	5.56	28.84	1.652
2	8 % albumin + 20 % albumin	10	200	+ 10	3.75	7.87	0.301
3	8 % albumin + 6 % caseinogen	10	200	+ 6	5.20	24.94	1.382
4	8 % albumin + 13 % caseinogen	9	200	+ 11	3.91	12.87	0.524
5	8 % albumin + 20 % caseinogen	10	206	+ 11	3.58	8.29	0.289
6	8 % albumin + 20 % edestin	10	202	+ 7	3.35	9.06	0.332
7	8 % albumin + 20 % gelatin	10	173	- 4	4.94	22.29	1.160
8	8 % albumin + 20 % gliadin	9	206	+ 8	3.69	12.51	0.463
9	8 % albumin + 20 % Gromax	10	189	+ 3	3.40	12.19	0.430
10	8 % albumin + 20 % zein	10	192	+ 1	4.50	22.55	1.124
B. Exp. 4							
Control	8 % albumin	10	166	- 2	5.18	26.17	1.494
2	8 % albumin + 20 % beef muscle	10	196	+ 17	4.04	8.53	0.353
3	8 % albumin + 20 % fibrin	9	179	+ 4	4.49	12.41	0.573
4	8 % albumin + 20 % whale muscle	10	192	+ 11	3.79	7.18	0.275

caused by variations in the different groups, due to unknown factors which cannot be controlled. In Exps. 3 and 4, 8% albumin was used instead of caseinogen, because it had been our general experience, as discussed later, that at low levels such as this albumin caused more fatty livers than did caseinogen.

The results of Exp. 3 show very clearly the successive decreases in the amount of liver fat caused by increasing the caseinogen content of the diet, for the figures of the control group, 28.84% and 1.652 g., of liver fat are progressively decreased until, when the diet is supplemented by 20% caseinogen, there remains in the liver only 8.29% and 0.289 g. This series of figures is typical of those obtained in a large number of similar experiments. Exp. 3 also shows that in the conditions used albumin and edestin have actions equal to that of caseinogen; in our experience the slightly higher figures which are obtained with these two proteins, 0.301 and 0.332, as compared with 0.289 g. with caseinogen, are not to be interpreted as significantly different. The results with gliadin, however, in which the liver fat is 12.51% and 0.463 g., show that it is less effective than caseinogen, edestin and albumin. The results show clearly also that both zein and gelatin have positive, though considerably slighter actions, for the amounts of fat in the liver with these two proteins, 1.160 and 1.124 g. respectively, are some 30% less than that of the control group 1.652 g., there being a fall both in the liver size and in the fat percentage in the livers. The interesting point that supplements of these two deficient proteins have an action on liver fat is elaborated later. In Exp. 4 the control figures 26.17% and 1.494 are very similar to those of Exp. 3. The results of this experiment show that the whale muscle protein is the most effective of the three proteins used, its action being as great as those of caseinogen, edestin and albumin in Exp. 3; beef muscle protein has an action which may not be significantly less, while the action of fibrin is definitely less intense. Although it might have been preferable to have included in Exp. 3 a further group with a supplement of caseinogen, the similarity of the control figures in Exps. 3 and 4 makes it reasonable to regard these two experiments as being essentially one. If this be done, the different protein supplements can be arranged in three groups as regards the intensity of their actions in preventing fat deposition in the liver, (a) the most active—albumin, caseinogen, edestin, the proteins of whale muscle and possibly of beef muscle, (b) proteins with a marked action but less than those of group (a)—gliadin, Gromax and fibrin and (c) proteins with a definite though considerably less intense action—gelatin and zein.

The finding that albumin had an effect as great as that of caseinogen was surprising in view of our previous experience in which this protein at low levels caused more fatty livers than caseinogen. Thus, in an early experiment carried out by Dr A. W. Beeston, a comparison was made of the effects of 5% albumin and of 5% caseinogen in diets containing 40% fat. The percentages of "fat" in the fresh livers and the actual weights in the liver of the 100 g. rat were: caseinogen 26.34%, 1.58 g.; albumin 33.77%, 2.28 g. At the same low level, therefore, the amount of fat in the liver is some 50% greater when albumin is the protein. Two further experiments, one on the "fat" and the other on the "cholesterol" fatty liver, gave results of the same order.

"Cholesterol" fatty liver			"Fat" fatty liver		
Protein in diet	Glyceride in liver %	Glyceride in liver of 100 g. rat	Protein in diet	Fat in liver %	Fat in liver of 100 g. rat g.
8% caseinogen	32.19	2.13	5% caseinogen	21.30	1.077
8% albumin	36.20	2.82	5% albumin	29.52	1.712

The results of these three experiments in which the use of albumin in place of caseinogen resulted in the deposition in the liver of 30–70 % more fat, led us to compare the actions of these two proteins at a series of levels. The usual 40 % fat diet was used for 17 days, when the individual livers were worked up by the saponification method, the mean results being recorded in Table IV.

Table IV. *Exp. 5. Fat contents of the livers of rats receiving a diet containing 40% fat with varying percentages of albumin or caseinogen*

Group	Protein % of diet	No. of animals	Average initial body wt. g.	Change in wt. $\pm$ % initial wt.	Liver % final body wt.	Fat in liver %	Fat in liver of 100 g. rat g.
1 A	8 % caseinogen	9	183	- 5.6	4.75	20.51	1.000
1 B	8 % albumin	10	179	+ 3.3	5.86	29.10	1.758
2 A	14 % caseinogen	9	178	- 0.1	4.87	22.56	1.099
2 B	14 % albumin	10	182	+ 6.2	6.19	24.64	1.564
3 A	21 % caseinogen	10	182	+ 2.8	4.42	8.62	0.390
3 B	21 % albumin	8	175	+ 5.1	4.91	10.28	0.505

The results in Table IV present an anomaly in that the amount of fat in the livers of the rats receiving 14 % caseinogen, 1.099 g., is slightly greater than that of those receiving 8 % caseinogen, 1.000 g. This is the only example of such an occurrence which we have encountered in a very large series, for the effect of increasing the caseinogen content of the diet by 5 % has invariably resulted in a fall in the amount of liver fat, a typical series being recorded in Table III. This happening, moreover, does not affect the interpretation of the results in Table IV. These, together with those already recorded, show that at all protein levels of 14 % or less, albumin produces considerably more fatty livers than does caseinogen, but that when its concentration is increased above this it manifests a very marked action on liver fat. Thus, whereas an increase in the albumin content of the diet from 8 to 14 %, an increased intake by the animal of about 0.5 g. per day, has lowered the weight of fat from 1.758 to 1.564 g., a decrease of only 0.194 g., a further increase to 21 % has caused it to decrease from 1.564 to 0.505 g.

A study was next made in more detail of the action of gelatin, since this protein is deficient in certain amino-acids. Four groups of animals received for 17 days a diet consisting of albumin 8, beef dripping 40, cod liver oil 1, salt mixture 5: the control group received 46 parts of glucose and in groups 2, 3 and 4 supplements of 6, 13 and 20 % of gelatin were added with a corresponding decrease in the glucose. All the diets contained aneurin such that each rat received 10 μ g. per day. The livers were worked up individually for fatty acids and unsaponifiable matter by the saponification method. The mean results are recorded in Table V.

Table V. *Exp. 6. The effect of gelatin on the deposition of liver fat*

Group	Protein in diet	No. of rats	Initial body wt. g.	% change in body wt.	Liver % final body wt.	Fat % fresh liver	Fat in liver of 100 g. rat g.
1	8 % albumin	10	183	+ 1	5.5	27.56	1.642
2	8 % albumin + gelatin 6 %	10	180	+ 1	5.3	28.44	1.555
3	8 % albumin + gelatin 13 %	10	182	+ 8.2	5.2	25.83	1.402
4	8 % albumin + gelatin 20 %	9	178	- 2.7	5.3	22.10	1.215

Table V confirms the result of Exp. 3 in that the addition of 6, 13 and 20 % gelatin has caused a progressive decrease in the percentage of fat in the liver from 27.56 to 22.10, and in the absolute weight from 1.642 to 1.215 g., and with these confirmatory results there seems to be no doubt that gelatin supplements exert a definite but limited action in decreasing liver fat.

DISCUSSION

The results of the experiments described above are summarized in Table VI, in which the decreases of liver fat in the 100 g. rat caused by the protein supplements are expressed as percentages of the corresponding decrease due to an equal supplement of caseinogen.

Table VI. *Comparative effects of different proteins in decreasing liver fat*

Exp.	...	...	1	2	3 and 4	5 and 6	
Protein basal %	...	...	Caseinogen 5	Caseinogen 8	Albumin 8	Albumin 8	Albumin 8
Protein supplement %	...	...	10	7	20	6	13
Albumin	—	—	—	95	99	40	102
Beef muscle	—	—	—	82	96	—	—
Caseinogen	—	—	100	100	100	100	100
Edestin	—	—	82	71	97	—	—
Fibrin	—	—	40	92	77	—	—
Gelatin	—	—	-35	69	36	43	24
					34 (Exp. 6)		
Gliadin	—	—	20	81	87	—	—
Gromax	—	—	74	113	90	—	—
Whale muscle	—	—	—	133	102	—	—
Zein	—	—	59	105	39	—	—

Although Exp. 1 differed from the others in that the diet produced a "cholesterol" fatty liver, it is considered justifiable to include its results in this table and to compare them with those of the "fat" fatty liver experiments, since all the factors known to influence fat deposition exert an influence on both types of fatty liver.

While the results in Table VI are consistent with respect to some of the protein supplements, in other cases the lipotropic activity appears to vary with the nature of the basal protein. Thus beef muscle protein and edestin appear to be more effective, whale muscle protein, zein and perhaps gelatin less effective, when fed with albumin than when added to caseinogen. Further the amount of basal protein also appears to be of importance since fibrin, gelatin, gliadin, Gromax and zein all show conspicuously greater actions when fed as 7 % supplements to 8 % caseinogen in Exp. 2 than when fed as 10 % supplements to 5 % caseinogen in Exp. 1. The possible significance of these variations is mentioned below. No comment is offered on the increase in liver fat to a value greater than that of the control caused by gelatin in Exp. 1, shown as a negative value in Table VI. Deviations of this type in groups of animals have been encountered before, and it is not possible to decide whether this result is fortuitous or otherwise; the results of Exps. 2-6 suggest the former explanation.

Comment on the actions of two proteins in particular is necessary. Albumin when fed either as a supplement to caseinogen or alone is equal to that protein in activity at a level of 21 or 28 %. The considerable data presented earlier regarding its action at 5 or 8 % show that it invariably produces more fatty livers than caseinogen at these lower percentages. One result of its action at an intermediate value, 14 %, occurs in Exp. 5, where the addition of 6 % albumin

to a basal 8% albumin has had little, if any, effect on the fat deposition. This result may be suspect because of the caseinogen value in the same experiment, but we think it probable that the figure is reliable. The fact that albumin has an activity less than that of caseinogen at 5 or 8%, but equal to it at 20% or more, may mean that its action is really less than that of caseinogen throughout, and that this fact is only observable at the lower levels where considerable differences occur. Alternatively, if the result with 14% albumin in Exp. 5 is correct, it implies that there is a sudden increase in the intensity of its lipotropic action at higher levels, a fact which may be connected with the amino-acid make-up of the protein. Regarding this protein no published data are available, although Best *et al.* [1936] reported that unextracted egg white had an action similar to that of caseinogen.

Of the two deficient proteins studied the series of results in Exp. 6 shows that gelatin fed as a supplement has a definite though considerably less marked action, for the result of the 20% group in Exp. 6 confirms that of Exp. 3, and the intermediate values at 6 and 13% in Exp. 6 provide further evidence. The published figures of an experiment by Best *et al.* [1936] led them to suggest that gelatin had little if any action.

In considering all the data we feel that greater reliance should be placed on the results of Exps. 3 and 4 in which the added proteins constituted 20% of the diet, since the higher percentage will emphasize differences in their action, and the more fatty livers resulting from the use of albumin as the basal protein will increase the sensitivity. It may be concluded that all the proteins are active to a greater or lesser extent irrespective of whether they supplement albumin or caseinogen. Lipotropic activity is most marked in the cases of Gromax and whale muscle protein; and if the lesser activity of Gromax be correlated with its higher ash content (26%) it becomes probable that these two whale meat preparations are practically equal in this respect. In view of the fact, recorded earlier, that these two proteins contained appreciable quantities of choline, it must be pointed out that even in Exps. 3 and 4, where they were fed in greater amounts the daily intake of choline from this source did not exceed 1 mg./rat. From evidence accumulated in these laboratories it is considered very improbable that this amount of choline is responsible for any serious proportion of the activity found for either the Gromax or the whale muscle protein. These proteins then may be regarded as having actions equal to, if not exceeding, that of caseinogen. Beef muscle protein and edestin appear to have activities very nearly as great as that of caseinogen although Best *et al.* [1936] report the activity of beef muscle protein, prepared by essentially the same method, to be considerably less. Beeston *et al.* [1936] showed that edestin was active when fed alone but did not compare its action with that of caseinogen. Definitely less active are fibrin and gliadin, while the least activity is shown by gelatin and zein.

It is appropriate at this point to emphasize that the albumin and gelatin used were commercial products, and though it would, unquestionably, have been preferable to have used pure preparations, neither such materials nor facilities for their preparation were available to us.

It is possible that the lipotropic action of proteins is due to the action of their constituent amino-acids, since the administration of cystine and methionine in diets has been found respectively to increase and decrease liver fat [Beeston & Channon, 1936; Tucker & Eckstein, 1937; Channon *et al.* 1938]. If the lipotropic action of a protein is thus the algebraic sum of factors tending to increase or to decrease liver fat, then clearly both the amount and nature of the basal protein will exert a profound influence on the behaviour of supplements of

any other protein. Such considerations might account for the differences pointed out between the results of Exp. 1 and those of Exp. 2, and for the contrasts between Exps. 1 and 2 in which caseinogen was the basal protein, and those of Exps. 3, 4, 5 and 6 in which albumin was the basal protein. If the action of one or more factors does not increase beyond a certain limit, as the results of Beeston & Channon [1936] with cystine imply, then the effectiveness of protein supplements will not necessarily increase regularly on increasing the amount of the supplement; the results with low and high percentages of albumin are explicable on this basis. Exps. 3 and 4 where the total protein intake was 28 % of the diet, provide the further conclusion that if the lipotropic factors are amino-acids, one or more of them must be essential amino-acids; if this were not so all diets of high protein content should be of the same activity, the lipotropic substance being synthesized by the animal. It must be borne in mind, however, that the apparent activities of gelatin and zein could also be due to provision of a considerably greater nitrogen intake. On diets low in protein the substances responsible for the lipotropic action may be preferentially used for other purposes, and only become available for such action when the nitrogen intake is considerably increased. The necessity, for instance, for providing ample nitrogen in diets when testing the essential nature of amino-acids has been stressed by Rose [1937].

Lastly, from the point of view of further studies on the fatty liver problem, these results show that commercial egg albumin at low levels causes very fatty livers without any weight loss, and that in order to provide more protein nitrogen it is possible to add gelatin, for a reasonable proportion of this protein will not significantly decrease the liver fat. Maclean *et al.* [1937] and McHenry [1937] have used beef muscle protein in liver fat studies since this protein gives a better growth response in young animals, and according to Best *et al.* [1936] has a smaller lipotropic action than caseinogen.

SUMMARY

1. The lipotropic activities of various choline-free protein preparations have been compared with that of caseinogen, by feeding them both as sole dietary protein and as supplements to albumin and to caseinogen.
2. All the proteins investigated have apparent lipotropic actions, in order of decreasing intensity—Gromax and whale muscle protein, caseinogen, albumin, beef muscle protein and edestin, fibrin and gliadin and, lastly, gelatin and zein.
3. Albumin has been investigated in greater detail, and though of an activity similar to that of caseinogen at high levels, it consistently gives more fatty livers when fed at low levels.
4. The influence of protein supplements on liver fat appears to depend on the amount and nature of the basal protein.
5. Suggestions are put forward as to the possibility of the lipotropic activity of proteins being due to constituent amino-acids.
6. The possibility of avoiding weight losses in fatty liver studies by using diets containing low proportions of albumin supplemented with gelatin is pointed out.

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