ON THE LIPOPOTROPIC ACTION OF PROTEIN*

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The lipotropic action of protein, which was first recognized in 1935 (1, 2), has been the subject of many investigations (3–6). These have centered largely around the question of whether the lipotropic activity of a protein can be attributed entirely to its content of cystine and methionine. Evidence has been presented on both sides of the controversy that has arisen (6–11), and, although some factors of a general nature that affect the lipotropic activity of a diet have been elucidated (6, 12, 13), methionine is the only amino acid that has been shown to cause a reduction in the deposition of fat in the livers of animals fed diets deficient in choline (11). Attempts to evaluate the information available on this subject are complicated by the variety of experimental conditions (the differences in the age and weight of the rats, the amount and type of dietary protein, the amount and type of dietary fat, the rate of growth of the animals, the method of feeding, and the nutritive value of the entire diet) that have been used, and the claims for a lipotropic action of proteins beyond that of the methionine contained in them still appear to rest on rather tenuous grounds.

When it became apparent that protein (14) and the amino acids, threonine (14, 15) and glycine (16), were effective in reducing the deposition of fat in the livers of young rats fed low protein diets containing choline, the idea was entertained that these results might be linked to the earlier observations on the lipotropic action of protein. Since much of the evidence for a lipotropic action of proteins, other than that of the methionine contained in them, had been obtained from studies in which diets relatively high in methionine or other lipotropic factors had been used, it appeared that this secondary effect of protein on fat deposition in the liver might be most clearly demonstrated in animals fed low protein diets containing close to the required amount of choline or choline precursors.

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PROTEIN AND FAT DEPOSITION

EXPERIMENTAL

Methods

Male weanling rats of the Sprague-Dawley strain weighing from 40 to 50 gm. were divided into similar groups of six animals each and were maintained in individual cages with raised screen bottoms. The animals were fed ad libitum and were weighed weekly during the experimental periods of 2 weeks.

The composition of the basal diet was as follows: sucrose 81.4, casein 9.0, corn oil 5.0, Salts 4 (17) 4, DL-methionine 0.3, and DL-tryptophan 0.1 per cent. Vitamins were included to provide, in mg. per 100 gm. of ration, thiamine hydrochloride 0.5, riboflavin 0.5, niacin 1.0, calcium pantothenate 2.0, pyridoxine 0.25, biotin 0.01, folic acid 0.02, vitamin B₁₂ 0.002, inositol 10.0, and choline chloride 150. 2 drops of halibut liver oil diluted with corn oil and fortified with vitamins E and K to provide vitamin A (400 i.u.), vitamin D (4 i.u.), 0.04 mg. of 2-methyl-1,4-naphthoquinone, and 4.0 mg. of α-tocopherol were administered orally each week.

All changes in the basal diet were compensated for by adjustments in the percentage of sucrose.

After 2 weeks the animals were sacrificed for the determination of liver fat. Each animal was stunned and decapitated and the liver was excised and stored at -4°. Fat was determined by ether extraction of the dried and ground liver (18).

Results

The results presented in Table I include both growth rates and liver fat values.

It is apparent that neither 0.15 per cent choline chloride nor 1.0 per cent DL-methionine (Group 1 versus Groups 2, 7, 15) prevented fat from accumulating in the livers of young rats fed 9 per cent casein diets. In fact choline and this high level of methionine together (Group 17) were only slightly more effective than was either one alone. In each of the three experiments, fat accumulated to the extent of 8 to 13 per cent (wet weight) in the livers of control animals (Groups 2, 7, 15), values which are of course well below those of 15 to 30 per cent found when animals are fed diets severely deficient in choline, but which are nevertheless higher than values reported as normal.

An increase in the level of casein to 20 per cent (Group 9) or the inclusion of gelatin (Group 3) reduced fat deposition only slightly when methionine was low and choline was omitted from the diet, but a comparison of Groups 2 and 17 with Groups 4 and 20 shows that the inclusion of gelatin in a 9 per cent casein diet containing choline resulted in a decrease in liver
fat deposition from 25 to 13 per cent dry weight (approximately). Similar reductions were observed when the casein level was increased from 9 to 18 per cent or over, in diets containing either methionine or choline (Group 1 versus 5; 2 versus 6; 7 versus 10, 11, 13; 15 versus 20; 17 versus 21), but no further reduction occurred when the protein content was increased above 18 per cent (Groups 13 and 14). When threonine was substituted for the additional protein in diets containing either 0.15 per cent choline or 1.0 per cent methionine plus 0.15 per cent choline (Group 7 versus 8; 15 versus 16; 17 versus 18), fat deposition was likewise reduced. In each instance in which the effect of protein or threonine was apparent, the diet contained either close to the stated requirement of choline, or methionine in quantities considered sufficient to replace choline. Since the diets of Groups 7, 8, and 10, and those of all groups in Experiment 3, contained

### Table I

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Group No.</th>
<th>Casein per cent</th>
<th>Methionine per cent</th>
<th>Choline chloride per cent</th>
<th>Supplement</th>
<th>Rate of growth*</th>
<th>Liver fat*</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Dry weight</td>
<td>Wet weight</td>
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<td>1</td>
<td>9</td>
<td>0.3</td>
<td></td>
<td></td>
<td>14.6 ± 0.7</td>
<td>28.6 ± 1.6</td>
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<tr>
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<td>9</td>
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<td></td>
<td>14.2 ± 0.9</td>
<td>27.3 ± 2.9</td>
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<tr>
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<td>3</td>
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<td>6% gelatin</td>
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<td>32.3 ± 1.2</td>
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<td>4</td>
<td>9</td>
<td>0.3</td>
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<td>0.15</td>
<td>23.9 ± 1.5</td>
<td>15.8 ± 2.3</td>
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<td>5</td>
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<td>34.2 ± 2.3</td>
<td>16.3 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>18</td>
<td>0.3</td>
<td></td>
<td></td>
<td>34.9 ± 2.2</td>
<td>10.0 ± 0.6</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>9</td>
<td>1.0</td>
<td></td>
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<td>12.9 ± 0.7</td>
<td>29.2 ± 1.4</td>
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<tr>
<td></td>
<td>8</td>
<td>9</td>
<td>1.0</td>
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<td>0.36% DL-threonine</td>
<td>17.7 ± 0.8</td>
<td>17.9 ± 0.9</td>
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<td>29.8 ± 1.6</td>
<td>18.9 ± 3.5</td>
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<td></td>
<td>24.0 ± 0.4</td>
<td>18.9 ± 1.2</td>
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<td></td>
<td>37.0 ± 1.3</td>
<td>9.8 ± 0.5</td>
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<tr>
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<td>12.1 ± 0.6</td>
<td>26.3 ± 2.0</td>
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<tr>
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<td>9</td>
<td>1.0</td>
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<td>17.4 ± 0.8</td>
<td>15.5 ± 0.8</td>
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<td>0.15</td>
<td>11.1 ± 0.6</td>
<td>22.6 ± 2.6</td>
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<tr>
<td></td>
<td>18</td>
<td>9</td>
<td>1.0</td>
<td></td>
<td>0.15</td>
<td>17.6 ± 0.9</td>
<td>10.9 ± 0.4</td>
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<tr>
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<td>9</td>
<td>1.0</td>
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<td>0.15</td>
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<tr>
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<td>21</td>
<td>18</td>
<td>0.7</td>
<td></td>
<td>0.15</td>
<td>34.9 ± 1.4</td>
<td>9.3 ± 0.3</td>
</tr>
</tbody>
</table>

* The values represent the mean ± standard error of the mean for six animals.
very close to the same amount of methionine, the effects noted in com-
parisons involving these groups cannot be attributed to variations in the
methionine content of the diets.

When groups fed high casein diets containing methionine and lacking
choline (Groups 5, 11, 13, 20) are compared with those fed similar diets
containing choline (Groups 6, 12, 14, 21), it is apparent that methionine,
even at a level of 1.7 per cent (Group 11), did not cause quite as great a
reduction in the deposition of liver fat as did 0.15 per cent choline.

DISCUSSION

These results indicate that the effect of additional protein or threonine
in reducing fat deposition in the livers of young rats fed 9 per cent casein
diets, supplemented with methionine and containing apparently ample
amounts of choline (14, 15), can also be demonstrated when choline in the
diet is replaced by 1.0 per cent methionine. Since in an earlier study (16)
no effect of threonine was detected when choline was omitted from diets
containing 0.3 per cent DL-methionine, it seems that this effect of protein or
threonine is demonstrable only when the diet contains close to the stated
requirement of known lipotropic factors.

Earlier studies on the lipotropic effect of protein assume new interest
in the light of this observation. Best and Ridout (8) found that 5 per cent
meat powder, plus methionine and cystine equivalent to that contained in
30 per cent casein, was less effective than was 30 per cent casein. Although
the meat powder diet should have provided ample methionine, it would
have been deficient in other amino acids such as threonine.

Beeston et al. (19) observed that fat deposition in the livers of mature
rats fed diets containing 5 per cent casein and 0.1 to 0.2 per cent choline
was from 7 to 8 per cent (wet weight), or approximately double that found
in the livers of similar animals fed high casein diets. The latter diets would
have provided ample threonine and other amino acids.

Both Tucker et al. (9) and Treadwell et al. (10) have suggested that the
effect of protein on fat deposition in the liver can be explained solely on the
basis of the methionine and cystine content of the protein. In their work,
levels of casein up to only 25 per cent, an amount insufficient to provide
optimal methionine, were fed, and reductions in liver fat to only 10 per
cent (wet weight) were observed. Since Beveridge et al. (6) have shown
that casein is more effective than the methionine and cystine it contains
when it is fed at levels above 22 per cent, it would appear that their
diets provided insufficient methionine or choline to permit the secondary
effect of protein on fat deposition to become apparent.

Eckstein (11) was also unable to demonstrate any lipotropic effect of
essential amino acids other than methionine when he used choline-free
diets. Such diets have been used in previous studies (20–24) in which most of the known amino acids have been tested for lipotropic activity, and in these also effects of amino acids would probably be masked (16).

In the work of Beveridge et al. (6), which was alluded to earlier, the greater effectiveness of casein over its equivalent in methionine and cystine was evident only when the diet contained about 0.5 per cent of methionine. Since mature rats were used in their study and since the animals were pair fed and in most instances lost weight, that amount of methionine probably permitted close to the maximal lipotropic effect.

Although young animals were used in the study reported in this paper, and while mature rats were used in most of the earlier studies, fat accumulated in the livers of mature rats receiving choline when the casein content of the diet was reduced to 5 per cent (25). Threonine was much more effective than additional choline in reducing this accumulation of fat. Also high fat diets were used in most of the earlier studies discussed, but threonine is effective in reducing fat deposition in the livers of rats fed diets containing 20 per cent fat (16).

When all of these facts are considered, it appears that the secondary effect of protein in reducing the deposition of fat in the liver is a reflection of the amino acid-deficient diets used in most of these studies. Threonine (14, 15) or threonine and glycine (16) are required to insure minimal fat deposition in the liver when low casein diets are fed. With other proteins other amino acids may be required (15). Evidence is currently accumulating in our laboratory (unpublished results) that glycine is more effective than threonine when albumin diets (which were commonly used by Channon and his associates) are fed.

The importance of these results in studies of lipotropic factors is difficult to evaluate. It would appear from a consideration of previous results (26) that complications from the secondary effect of protein are unlikely when diets low in casein are used without supplements of sulfur amino acids for studies of the lipotropic action of choline and some choline precursors. However, when sulfur amino acids are provided, as is the case in studies of the choline-sparing action of methionine, the balance of amino acids in the diet could affect the results. Even with higher protein diets, the amino acid balance of the diet may be of significance (unpublished results).

Another point of interest in this study is that no further decrease in liver fat was obtained when methionine was increased above 1.2 per cent nor when the level of casein was increased to 40 per cent in choline-free diets. In every instance, however, when choline was included with high methionine or high protein, a further small reduction in liver fat occurred. Treadwell (27) has studied the methionine requirement for both growth and lipotropic activity and has stated that 1.2 per cent methionine is suffi-
cient for maximal lipotropic activity; nevertheless, the inclusion of choline in one of his diets resulted in some further decrease in liver fat. Rose et al. (28) have reported similar results. It thus appears that some effect of choline on fat deposition can be demonstrated even in the presence of large amounts of methionine or protein. No attempt has been made to compare choline and methionine on a quantitative basis. Such a study, currently under way at Toronto, has proved to be very complex.

From the results, the conclusion that there are two distinct effects of dietary protein on fat deposition in the liver appears justified; first, the effect of methionine, which has been well established (6, 11), in sparing choline by providing methyl groups for choline synthesis, and secondly, the effects of other amino acids, partial deficiencies or imbalances of which cause fat to accumulate in the liver.

**SUMMARY**

The effects of choline, methionine, and additional protein on fat deposition in the livers of rats fed low protein (casein) diets have been studied. Neither choline nor methionine in amounts sufficient to meet the stated requirements of the rat for these compounds for growth and the prevention of fatty infiltration prevented some excess of fat from accumulating in the liver. Only when either the protein or the threonine content of the diet was increased was the fat content of the liver reduced to what is considered the normal range.

It is suggested that this secondary lipotropic effect of protein (the sparing of choline by methionine being the primary effect) is not a choline-sparing action but results from the provision of certain amino acids, deficiencies of which (with low casein diets, threonine deficiency) cause fat to accumulate in the liver. This effect is apparent only when the diet contains either choline or methionine in amounts approaching what is considered to be the requirement.

**BIBLIOGRAPHY**


1 Personal communication from Dr. C. C. Lucas, Banting and Best Department of Medical Research, University of Toronto.
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