FURTHER OBSERVATIONS ON THE LIPOTROPIC NEED FOR INOSITOL

BY M. L. MACFARLAND AND E. W. MCHENRY

(From the Department of Public Health Nutrition, School of Hygiene, University of Toronto, Toronto, Canada)

(Received for publication, July 15, 1948)

Inositol was originally shown to be a lipotropic agent in rats maintained under special conditions (1); the animals were depleted of fat and of B vitamins by maintenance for 3 weeks on a fat-free, high carbohydrate diet, and during a subsequent week fat synthesis was promoted by a supplement of B vitamins and a beef liver fraction. Under these conditions the rats developed markedly fatty livers containing large amounts of cholesterol. These fatty livers were produced despite the provision of choline, both in the liver fraction and as a supplement. The accumulation of fat and cholesterol in the liver was prevented by administration of inositol. The lipotropic action of inositol in rats has been confirmed by Engel (2), by Forbes (3), and by Handler (4), and has been demonstrated in humans by Abels et al. (5).

Several explanations of the production of fatty livers resistant to choline but responsive to inositol have been offered. In early work in this laboratory, crude preparations of biotin appeared to be equivalent to the beef liver fraction in producing the effect; the term “biotin fatty liver” was used to describe the fatty liver responsive to inositol. Subsequent investigation with pure biotin showed that this concept was erroneous (6); the biotin explanation has been fully criticized by Best et al. (7). In several studies in our laboratory, attempts were made to define further the dietary conditions necessary to cause fatty livers characterized by a high cholesterol content (resulting from synthesis) and by resistance to choline. By means of the same basal regimen, it was found that, when thiamine was the only supplement, the fatty livers were completely responsive to choline (8). With the addition of the other B vitamins increasing resistance to choline and responsiveness to inositol were observed (8); the full effect was secured only when beef liver fraction was furnished (6). Handler (4) concluded that the beef liver fraction could be replaced by biotin plus folic acid and has also stated that the effect was due to a stimulation of food consumption.

In this paper further efforts to explain the production of fatty livers showing resistance to choline and responsiveness to inositol are reported. While it was found that the beef liver fraction could be partially fractionated, further work along this line appeared to be unnecessary because of results obtained with pure substances.
Methods

The rats used were Wistar strain animals of both sexes reared in the colony of the Connaught Medical Research Laboratories. After attaining an average weight of 90 to 100 gm., they were provided with a fat-free, high carbohydrate diet containing no source of B vitamins (9). At the end of 3 weeks, having decreased in body weight by approximately one-third, the animals were divided into strictly comparable groups of generally ten rats, except in some cases when nine were used. During the subsequent week, various supplements were administered to different groups. Each rat was given the basal B vitamin supplement daily by subcutaneous injection and received the following amounts: thiamine hydrochloride 25 γ, riboflavin 25 γ, pyridoxine hydrochloride 40 γ, calcium pantothenate 100 γ, p-aminobenzoic acid 100 γ, and nicotinic acid 100 γ. The additional supplements under investigation were provided orally, mixed with the food, and the dosages were as follows: choline 20 mg., inositol 25 mg., folic acid 5 γ, biotin 5 γ. When abnormal quantities of B vitamins were given, the following amounts were provided in the food in addition to the injected basal amounts: thiamine 75 γ, riboflavin 430 γ, calcium pantothenate 2.4 mg., and nicotinic acid 6.5 mg.

The beef liver fraction, similar to that used previously (10), was obtained from the Connaught Medical Research Laboratories and the procedure of Hutchings et al. (11) was adopted in the preparation of norit eluate fractions from it. Crude liver fraction was diluted, adsorption on Pfanstiehl norit A was carried out at pH 3.0, and after washing the norit with 50 per cent ethanol, it was eluated twice at 70° with ammonia-ethanol solution. The combined eluates were finally concentrated in vacuo to the original volume of the liver fraction. The filtrate obtained after separation of the norit was neutralized and concentrated to appropriate volume. The daily dosage of these preparations was 2 cc. per rat.

At the end of the test week each animal received an intraperitoneal injection of nembutal, the livers were removed, and the crude fatty acids of the livers and carcasses were determined by methods described in previous publications (9, 12). In all cases, analyses were made on the pooled livers and pooled carcasses for each group. The total cholesterol content of the petroleum ether extracts was estimated by a modification of the procedure of Schoenheimer and Sperry (13). Data for carcass fats are not included in the reported results because they are not pertinent to the discussion. All data for liver lipides are results obtained by the analysis of tissues pooled for each group of animals. The figures presented have been selected as typical from a much larger series of similar observations.
Results

Typical effects on liver lipides caused by feeding various beef liver preparations in one of several experimental series are given in Table I. Attention is drawn to the characteristic effect of the liver fraction in causing livers high in fat and in cholesterol despite the supply of choline, both in the fraction and as an additional supplement. Throughout these studies, the criterion of potency has been the ability to produce fatty livers rich in cholesterol when choline was supplied. It is apparent that the eluate fraction exhibited effects very similar to those obtained when the original material was provided. The filtrate preparation contained some activity and it is obvious that adsorption had not caused complete fractionation. The activity of inositol, particularly on liver cholesterol, is clearly evident.

Since Handler had reported (4) the joint efficacy of biotin and folic acid in simulating the action of the liver fraction, the effects of feeding this combination were examined. The results of a typical experiment in this series are reported in Table I.

It is clear that the administration of folic acid as sole replacement for liver fraction did not result in the development of fatty livers with the

### Table I

<table>
<thead>
<tr>
<th>Supplements</th>
<th>Liver Crude fatty acids</th>
<th>Cholesterol</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg. per cent</td>
<td>per cent of Group I</td>
<td>mg. per cent</td>
</tr>
<tr>
<td>Original liver fraction, choline</td>
<td>2170</td>
<td>21.1</td>
<td>100</td>
</tr>
<tr>
<td>(Group I)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Original liver fraction, choline, inositol</td>
<td>456</td>
<td>5.8</td>
<td>21</td>
</tr>
<tr>
<td>Liver eluate, choline</td>
<td>2078</td>
<td>25.3</td>
<td>96</td>
</tr>
<tr>
<td>filtrate, choline</td>
<td>1170</td>
<td>15.6</td>
<td>54</td>
</tr>
<tr>
<td>Folic acid, choline</td>
<td>635</td>
<td>11.1</td>
<td>29</td>
</tr>
<tr>
<td>&quot; &quot; biotin, choline</td>
<td>993</td>
<td>14.8</td>
<td>46</td>
</tr>
<tr>
<td>Additional B vitamins, choline</td>
<td>1303</td>
<td>18.3</td>
<td>60</td>
</tr>
<tr>
<td>&quot; &quot; &quot; biotin, folic acid, choline</td>
<td>2185</td>
<td>25.7</td>
<td>101</td>
</tr>
<tr>
<td>Additional B vitamins, biotin, folic acid, choline, inositol</td>
<td>546</td>
<td>9.1</td>
<td>25</td>
</tr>
</tbody>
</table>

*Values in these columns are calculated by the equation,
Average weight of liver fatty acids or cholesterol in particular group/ 
Average weight of liver fatty acids or cholesterol in Group I × 100
LIPOTROPIC NEED FOR INOSITOL

characteristics of the "liver fraction fatty liver." The lipide accumulation obtained when biotin and folic acid were both provided with choline did not contain as much fat nor as high a proportion of cholesterol as was obtained with the use of liver fractions. The present evidence does not substantiate Handler's conclusion (4) that the action of liver fraction in these experiments can be duplicated by administering biotin and folic acid without other additional measures.

Although the basal B vitamin supplement would be expected to be adequate under most conditions, it seemed reasonable to investigate the possibility that the additional quantities of vitamins provided by the liver fraction were related to the effects obtained. Consequently, the activity of further dietary supplements (equivalent to the vitamin content of the liver preparation and given in detail under "Methods") of thiamine, riboflavin, calcium pantothenate, and nicotinic acid were tried. The results, reported in Table I, show that feeding of additional amounts of these four factors failed to produce the same effects as the liver preparations, although the quantity of fatty acids was more similar than with other supplements. However, when biotin and folic acid, as well as additional thiamine, riboflavin, pantothenate, and nicotinic acid were provided, the liver lipide responses were very similar to those observed subsequent to liver supplementation. The results of one of five similar experiments are reported in Table I. Even with generous provision of choline, fatty livers containing a high proportion of cholesterol were obtained. The final line in Table I shows the lipotropic effect of inositol on the fatty liver produced by giving

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Supplements* in addition to basal B vitamins</th>
<th>Weight gain in test week</th>
<th>Food consumption</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>gm.</td>
<td>gm. per day of test</td>
<td>gm.</td>
</tr>
<tr>
<td>1</td>
<td>Biotin, folic acid, choline</td>
<td>30</td>
<td>10.8</td>
<td>7.1</td>
</tr>
<tr>
<td>2</td>
<td>Biotin, folic acid, additional B vitamins, choline</td>
<td>26</td>
<td>10.2</td>
<td>7.6</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>38</td>
<td>12.1</td>
<td>9.5</td>
</tr>
</tbody>
</table>

* The animals were fed ad libitum with the exception of the members of Group 2; they were permitted only as much food as the members of Group 1 on the corresponding day of the test.
M. L. MACFARLAND AND E. W. MCHENRY

biotin and folic acid in conjunction with abnormal amounts of other B vitamins.

Under the special conditions of our experiments, the animals exhibit a distinct need for dietary inositol for lipotropic purposes. Because Handler (4) has suggested that this augmented need for inositol is related to temporary stimulation of food consumption, an experiment was carried out in which the animals receiving biotin, folic acid, and the additional quantities of the other B vitamins were given only as much food as animals developing fatty livers which were much more responsive to choline; that is, animals without the augmented amounts of B vitamins. The results are described in Table II. A comparison of Groups 1 and 2, which had approximately the same amount of food, indicates that restriction of food consumption in Group 2 did not eliminate the effects of the additional vitamin factors on the liver lipides, but it is evident that food intake also had an effect in producing the high cholesterol fatty liver resistant to choline.

DISCUSSION

Under the conditions of our experiments, fatty livers are produced in a few days by the rapid synthesis of fat, presumably from carbohydrate. When thiamine is the only B vitamin supplement, the liver fat can be maintained at a normal level by supplying one lipotropic agent, choline (8). The addition of other B vitamins causes fatty livers which are not completely responsive to choline but which are amenable to choline and inositol. The most marked resistance to choline was observed previously (10) when a beef liver fraction was added to the vitamin supplements; this observation was made when pure biotin and folic acid were not available. The hypothesis that the liver fraction was active because of its biotin content was found to be untenable (5). The suggestion of Handler (4) that biotin and folic acid, given with customary amounts of other B vitamins, will simulate the liver fraction has not been confirmed by us. The question as to whether the activity of the liver fraction is due to an unidentified constituent or to its supply of extra amounts of a number of B vitamins appears to have been settled in favor of the latter explanation and further fractionation of the liver preparation seems unnecessary. All of our observations indicate that a fatty liver, at least of the type produced by in vivo fat synthesis, is made resistant to choline and responsive to inositol by increasing the intake of B vitamins, both in kind and in quantity.

No clear explanation is available as to why liver fat can be demobilized by choline alone under some circumstances and why inositol must be provided under other conditions. Presumably both substances are lipotropic because they promote the formation of phospholipides. We suggested (6) previously that different fatty acids may be involved; no further informa-
tion on this point is available. In every experiment in this laboratory
inositol has exerted a greater effect on liver cholesterol than has choline and
we have observed instances in which inositol has markedly reduced liver
cholesterol without any definite effect on fatty acids. Handler (4) has
suggested that a large increase in food intake, with a surge in fatty acid
synthesis, may be the factor causing choline resistance and inositol re-
sponsiveness. Pair feeding tests, of which a typical one is reported above,
show that food consumption is a contributing factor but there is also a
specific effect from the B vitamin supplements.

SUMMARY

Fatty livers occurring in choline-fed rats and susceptible to inositol
have been produced in animals maintained on a fat-free, high-carbohydrate
diet by supplying (a) crude beef liver fraction, (b) liver fraction eluate, or
(c) biotin and folic acid with abnormal amounts of other B vitamins.
Biotin and folic acid did not exhibit this effect unless the amounts of other
B vitamins were abnormal. The action of the first two supplements is
apparently explained by the third. The combined supplements have a
specific effect which is augmented by an increased food consumption.

BIBLIOGRAPHY

   and Med., 54, 157 (1943).
   368 (1946).
    (1941).
FURTHER OBSERVATIONS ON THE
LIPOTROPIC NEED FOR INOSITOL
M. L. MacFarland and E. W. McHenry


Access the most updated version of this article at
http://www.jbc.org/content/176/1/429.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail
alerts

This article cites 0 references, 0 of which can be
accessed free at
http://www.jbc.org/content/176/1/429.citation.full.h
tml#ref-list-1