THE B VITAMINS AND FAT METABOLISM

III. THE EFFECTS OF VITAMIN B_e UPON LIVER AND BODY FAT

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Previous work has shown that rice polish concentrate, used as a dietary supplement in conjunction with thiamine and riboflavin, caused a marked increase in the body fat of rats (1). Bender and Supplee (2) found that, under such conditions, this concentrate had a supplemental effect upon body weight. They correlated the weight changes with the presence or absence of dermatitis, interpreting their results to mean that the factor in rice polish concentrate affecting growth was vitamin B_e. When crystalline vitamin B_e became available, it was possible to determine whether this factor influenced weight and body fat in rats.

Methods

Young white rats of the Wistar strain, reared in the Connaught Laboratories colony, were employed. They were kept in individual screen bottom cages with water freely available. The animals were weighed daily. Groups of ten rats, five male and five female, were used and, unless otherwise stated, all results given are averages for such groups.

The rats were fed basal diets devoid of the vitamin B complex, low in choline, which is known to exert a marked influence upon liver fat, and low in casein because of the lipotropic action of this protein. The composition of the basal diets is given in Table I. After a 3 week depletion period the following supplements, dissolved in normal saline solution, were injected subcutaneously for a period of 1 week: thiamine hydrochloride (Merck) 12.5 micrograms, riboflavin (Hoffmann-La Roche) 10.0 micrograms, nicotinic acid (Eastman Kodak Company) 0.1 mg., vitamin B_e (kindly donated by Merck and Company and by E. R. Squibb and Sons)
20 micrograms, choline (British Drug Houses) 10 mg. per rat per day. Extensive work in this laboratory has shown that all of these supplements have the same effect when injected as when given by mouth.

The animals were killed by stunning, the livers removed, and the total crude fatty acids in the bodies determined according to the method previously published (1). Owing to the much smaller amount of tissue in the liver, certain modifications of this method were made. The liver from each animal was placed in a tared 200 cc. round bottom flask with an extended neck, and the flask reweighed. To each were added 15 cc. of 60 per cent potassium hydroxide solution. The flasks were heated on a boiling water bath for 45 minutes. The flasks were removed from the bath and 15 cc. of 95 per cent ethyl alcohol were added to each. After a reflux condenser was attached, the flask was reheated for 1 hour. The condenser was then removed and heating continued until the alcohol was evaporated. To each was added sufficient 40 per cent sulfuric acid solution to make the contents acid to litmus. The flasks were chilled and to each were added 25 cc. of petroleum ether and sufficient water to make the total volume 200 cc. From this point the procedure was the same as that used for the bodies.

**EXPERIMENTAL**

**Series I**—This experiment was designed to determine whether vitamin B₆ caused changes in body weight and in liver and body fat when supplied alone or in conjunction with various combinations of some of the known factors of the vitamin B complex and

### Table 1

**Composition of Basal Diets**

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Diet 1</th>
<th>Diet 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein (Labco vitamin-free)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Sucrose</td>
<td>84</td>
<td>54</td>
</tr>
<tr>
<td>Triolein</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>Salt mixture (Steenbock-Nelson Salts 40 (3))</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Agar</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Cod liver oil concentrate</td>
<td>0.015</td>
<td>0.015</td>
</tr>
</tbody>
</table>
The rats were fed a fat-free basal ration (Diet 1) *ad libitum* for 3 weeks, after which all groups, except one reserved as a control, received supplements and the same basal diet for 1 week. The average weight of the rats decreased from 96 to 71 gm. during the depletion period. The weight changes during the supplement period and the total crude fatty acids in the liver and body at the end of the experiment are given in Table II.

### Table II

_Effects of Vitamin B Supplements after Depletion Period_

<table>
<thead>
<tr>
<th>Series No.</th>
<th>Supplements</th>
<th>Average individual weight changes in 4th wk.</th>
<th>In body</th>
<th>In liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>gm. per cent</td>
<td>gm. per cent</td>
<td></td>
</tr>
<tr>
<td>I. Fat-free diet</td>
<td>None, 3 wks. depletion</td>
<td>0</td>
<td>2.9</td>
<td>7.8</td>
</tr>
<tr>
<td></td>
<td>&quot; end of experiment</td>
<td>-10</td>
<td>1.9</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>Vitamin B₆</td>
<td>+5</td>
<td>3.6</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>&quot; + choline</td>
<td>+4</td>
<td>3.5</td>
<td>8.1</td>
</tr>
<tr>
<td></td>
<td>&quot; + &quot; + vitamin B₆</td>
<td>+3</td>
<td>3.4</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>&quot; + &quot; + flavin</td>
<td>+3</td>
<td>4.3</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>&quot; + &quot; + &quot; + vitamin B₆</td>
<td>+13</td>
<td>6.1</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>Choline + vitamin B₆</td>
<td>-8</td>
<td>2.0</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>None, 3 wks. depletion</td>
<td>0</td>
<td>1.7</td>
<td>3.2</td>
</tr>
<tr>
<td>II. Fat-free diet</td>
<td>Nicotinic acid</td>
<td>-11</td>
<td>1.5</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>Thiamine</td>
<td>+4</td>
<td>3.9</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td>&quot; + choline</td>
<td>+6</td>
<td>4.4</td>
<td>9.1</td>
</tr>
<tr>
<td></td>
<td>&quot; + &quot; + nicotinic acid</td>
<td>+6</td>
<td>4.2</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>&quot; + &quot; + flavin</td>
<td>+5</td>
<td>4.3</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>&quot; + &quot; + &quot; + nicotinic acid</td>
<td>+8</td>
<td>4.5</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>&quot; + &quot; + &quot; + vitamin B₆</td>
<td>+12</td>
<td>4.8</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>nicotinic acid</td>
<td>+12</td>
<td>3.6</td>
<td>3.6</td>
</tr>
<tr>
<td>III. High fat diet</td>
<td>None</td>
<td>-5</td>
<td>3.2</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>Thiamine</td>
<td>+7</td>
<td>7.3</td>
<td>15.0</td>
</tr>
<tr>
<td></td>
<td>&quot; + choline</td>
<td>+7</td>
<td>6.8</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>&quot; + vitamin B₆</td>
<td>+8</td>
<td>6.9</td>
<td>18.7</td>
</tr>
<tr>
<td></td>
<td>&quot; + &quot; + choline</td>
<td>+8</td>
<td>6.0</td>
<td>4.8</td>
</tr>
</tbody>
</table>
Series II—In this series the effect of nicotinic acid upon liver and body fat was investigated. It was employed because it is a constituent of the rice polish concentrate which we previously used. Nicotinic acid was given alone and with various combinations of other B vitamins and choline. The method used was the same as in Series I.

Series III—As a supplement to a diet free from fat and choline, thiamine will produce a greater than normal amount of fat in the liver (Series I). A high fat diet free from choline and supplemented with thiamine yields a still higher value for liver fat (4). In a study of vitamin B₆ deficiency in rats Halliday (5) found significantly heavier livers containing a higher percentage of total fatty acids in the deficient animals. The addition of choline remedied this condition to a large extent but even massive doses failed to bring the liver weight and total fatty acid content quite to normal. Series III was designed to show whether choline plus vitamin B₆ was more effective than choline alone in producing a normal amount of liver fat in rats receiving a high fat diet. The rats were depleted of their body stores of the vitamin B complex by feeding Diet 1 for 3 weeks. During the subsequent supplement period of 1 week Diet 2, containing 30 per cent triolein, was fed. Groups of twenty rats were used.

DISCUSSION

In Series I and II previous reports of the synthesis of fat from carbohydrate when thiamine was the only supplement were confirmed. Vitamin B₆ in conjunction with thiamine, choline, or the two together had no effect upon the amount of liver or body fat. When vitamin B₆ was combined with thiamine, choline, and riboflavin, the percentage of fat in the body was raised and the body weight was increased. Since we have been unable to determine the amount of vitamin B₆ present in the rice polish concentrate used in earlier work, we have been unable to compare the effects of the concentrate and of pure vitamin B₆. In Series II nicotinic acid had no consistent effect upon liver fat but had some effect in increasing body fat. Body weight was not altered by this substance.

In all three series, when choline was included as a supplement,
the percentage of fat in the liver was normal. This would indicate that choline was able to control the level of liver fat whether fat was supplied in the diet or whether it was formed by synthesis from carbohydrate when thiamine was furnished. The administration of vitamin $B_6$ with thiamine, in the absence of choline, has in no case resulted in a lowering of the high liver fat values ordinarily obtained with thiamine alone. In combination with choline, vitamin $B_6$ has apparently exerted no supplementary effect upon liver fat. These experiments offer no explanation for Halliday's finding that, in vitamin $B_6$-deficient rats, choline did not reduce the liver fat to normal.

In a recent paper Hastings, Muss, and Bessey (6) noted a decrease in liver metabolism associated with fatty infiltration when rats were kept on a basal diet without added yeast extract. When yeast extract was administered, neither effect was evident. The basal diet used by these workers would have been practically free of choline. The yeast extract, as prepared by them, would have contained a considerable amount of choline. The presence of choline would offer a possible explanation for the ability of yeast extract to prevent the development of fatty livers.

György and Goldblatt (7) have reported liver injury occurring in some rats kept on a diet deficient in the vitamin B complex and supplemented with thiamine, riboflavin, and a purified vitamin $B_6$ preparation. Here, again, the absence of choline in the basal diet and its presence in the two preventive substances, yeast and Peters' eluate fraction of yeast, may be significant.

SUMMARY

1. The administration of vitamin $B_6$ in conjunction with thiamine, riboflavin, and choline to rats fed a fat-free diet causes a slight increase in body fat and an increase in body weight.

2. Nicotinic acid slightly augments the effect of vitamin $B_6$ upon body fat but not upon body weight.

3. Neither vitamin $B_6$, nicotinic acid, nor riboflavin will prevent the deposition of fat in the liver which results when thiamine is administered. The amount of liver fat is normal if choline is administered, either alone or with any combination of the above factors.
BIBLIOGRAPHY

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