The Failure of Biotin or Oxybiotin To Exert a "Procarcinogenic" Effect on Tumor Formation by 4-Dimethylaminoazobenzene

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Oxybiotin, the oxygen analog of biotin (4, 7), possesses biotin-like activity for various microorganisms, the rat, and the chick (4, 8, 15, 17). In this respect, oxybiotin is unique among the vitamins of the B complex, since biological activity is retained in spite of the substitution of the sulfur of the ring by oxygen. It has been demonstrated that the activity of oxybiotin is an intrinsic property of the molecule and is not due to its conversion to biotin (1, 12, 18).

Du Vigneaud et al. (5) have reported that biotin possesses a procarcinogenic effect on tumor formation by 4-dimethylaminoazobenzene. Pursuant to our investigations on the biological activity of oxybiotin, we undertook a comparison of the procarcinogenic activity of this compound to that of biotin under conditions similar to those of du Vigneaud et al. (5). These studies, presented in this paper, failed to afford any evidence for the procarcinogenic activity of either oxybiotin or biotin.

METHODS

Male, weanling albino rats were obtained from the Sprague-Dawley colony and kept in individual, wide-meshed, screen-bottomed cages. During the first 4 weeks, the animals were fed Purina Dog Chow. They were then transferred to the purified basal diet containing 4-dimethylaminoazobenzene (Table 1), which, for some groups, was supplemented with biotin or oxybiotin as described in the following section. The diets, which were fed ad libitum for the duration of the experiment, were freshly prepared every 2 weeks and stored in the refrigerator. The animals were weighed at frequent intervals, and their daily food intakes determined periodically. At the conclusion of the experiment, the surviving rats were sacrificed by decapitation.

Upon death of the animal, a portion of the liver and, when present, of liver tumor was removed and fixed in formalin for histologic studies. The remainder of the liver and liver tumor was dried at 45°C. in vacuo over phosphorus pentoxide. The thoroughly ground tissue (500 mg.) was autoclaved at 15 lbs. for 90 minutes with 15 ml. of 4 N sulfuric acid and filtered. After neutralization with sodium hydroxide, the biotin content of the filtrates was determined by the procedure of Wright and Skeggs (19). A differential assay for the biotin and oxybiotin contents was conducted by the Ilaney’s nickel method of Hofmann et al. (9). Care was taken to utilize only the grossly non-necrotic portion of the liver tumor for both histologic examination and biotin analysis.

RESULTS

In a preliminary experiment extending for 8 months, twenty rats received the basal diet, while an equal number received the same diet supple-

1 Patterned after diet B of du Vigneaud et al. (5).

TABLE 1

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Gm/100 gm of diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Vitamin-free&quot; casein (Labco)</td>
<td>15</td>
</tr>
<tr>
<td>Primex</td>
<td>10</td>
</tr>
<tr>
<td>Sucrose</td>
<td>60</td>
</tr>
<tr>
<td>Egg white (dried)</td>
<td>10</td>
</tr>
<tr>
<td>L-cystine</td>
<td>1</td>
</tr>
<tr>
<td>Salt mixture (Osborne and Mendel)</td>
<td>4</td>
</tr>
<tr>
<td>Choline</td>
<td>0.25</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>0.002</td>
</tr>
<tr>
<td>Thiamine</td>
<td>0.0005</td>
</tr>
<tr>
<td>Pyridoxine</td>
<td>0.005</td>
</tr>
<tr>
<td>Nicotinic Acid</td>
<td>0.002</td>
</tr>
<tr>
<td>L-1-nositol</td>
<td>0.100</td>
</tr>
<tr>
<td>Pantothenic acid</td>
<td>0.001</td>
</tr>
<tr>
<td>2-Methyl-1,4-naphthoquinone</td>
<td>0.0001</td>
</tr>
<tr>
<td>d-tocopherol acetate</td>
<td>0.001</td>
</tr>
<tr>
<td>4-Dimethylaminoazobenzene</td>
<td>0.100</td>
</tr>
</tbody>
</table>

* Each 100 grams of diet contained 4,070 units of Vitamin A and 814 units of Vitamin D supplied as Natola (Parke, Davis & Co.). 4-Dimethylaminoazobenzene was incorporated in the diet by dissolving it with heat in the Primex.
mented with 40 µg of d-biotin/100 gm. No hepatic tumors were present in either group, as determined by gross or microscopic examination. However, significant histopathologic findings in the liver, similar to those noted in experimentally induced cirrhosis, were observed in four animals of each group. The degree of histopathologic change was comparable in the two groups.

Since it seemed probable that the histologic changes observed in the above study were preliminary to the development of hepatic tumors, the experimental period during which the diets containing 4-dimethylaminoazobenzene were fed was extended to 12 months in the subsequent investigation. Sixty animals were distributed into three equal groups (Table 2). One group received only the basal diet, while the remaining two groups received the basal diet plus 40 µg of d-biotin and 800 µg of dl-oxybiotin/100 gm, respectively. This quantity of dl-oxybiotin was used, since previous investigations had shown that dl-oxybiotin is approximately 5 per cent as effective as d-biotin in curing biotin deficiency in the rat (2).

A moderate biotin deficiency developed in the animals receiving only the basal diet, as evidenced by their decreased growth (Table 2) and liver biotin contents (Table 3), as well as by the appearance of a slight degree of alopecia, dermatitis, and cheilosis. The rats fed biotin and oxybiotin-supplemented diets did not show these symptoms. No significant differences in the daily food intakes of the animals in the various groups were observed. The average daily food intake was 14 gm/rat.

The hepatic tumor incidence in the various groups is shown in Table 2. The gross observations were confirmed by microscopic examination. In some animals of each group the tumor resembled a hepatoma, while in others there was obvious adenocarcinoma. In addition, the histopathologic changes observed in the preliminary study were also present in the livers of many animals in this series. These changes were distributed uniformly among the three groups.

The results of the biotin and oxybiotin analyses of livers and hepatic tumors are given in Table 3.

**DISCUSSION**

In reviewing the experiences of the Wisconsin group with 4-dimethylaminoazobenzene, Miller (14) reported a liver tumor incidence of 90–100 per cent at 6 months in rats of the Sprague-Dawley strain receiving certain nonprotective diets. The protective nature of the basal diet employed in the present experiments is evidenced by the extended latent period and lowered incidence of tumor formation. Thus, no tumors were present at 8...
months, and at 12 months the tumor incidence was only 25–35 per cent. These results are in agreement with those of du Vigneaud et al. (5) and of Burk et al. (3), who, utilizing a basal diet practically identical with that employed in our studies, also found no hepatic tumors at 6 months. However, these workers noted a tumor incidence of 60 per cent at 6 months when the basal diet was supplemented with biotin. Despite attempts to reproduce the experimental conditions of du Vigneaud et al. and of Burk et al., this procarcinogenic effect of biotin was not demonstrable in our experiments. Oxybiotin was also devoid of any significant procarcinogenic activity. The evidence for the importance of biotin in carcinogenesis is conflicting. While several workers have observed a procarcinogenic effect (8, 5, 6), others have presented data which lend no support for a particularly significant role of biotin in tumor formation (10, 11, 16, 18).

The lowered liver biotin content of the basal group is in agreement with the findings of Burk et al. (3). The correlation noted by these workers between the protection against hepatic tumor formation and the decreased liver biotin was not found in our experiments, where the tumor incidence did not parallel the liver biotin content. Miller (14) was unable to correlate the liver biotin content with the protective character of various 4-dimethylaminoazobenzene-containing diets.

As seen in Table 3, the biotin content of the hepatic tumors produced by the feeding of 4-dimethylaminoazobenzene was significantly lower than that of liver tissue. A similar observation has been made by other investigators (3, 10, 14, 18). It is of interest that supplementation of the basal diet with biotin markedly increased the biotin content of the tumors (Table 3) without affecting either their incidence, size, or histopathology. West and Woghom (18) have similarly observed a lack of correspondence between the biotin content and growth of rapidly growing transplants of Sarcomas 37 and 180.

CONCLUSION

1. No evidence has been obtained for a "procarcinogenic" action of d-biotin or dl-oxybiotin in a protective diet containing 4-dimethylaminoazobenzene.

ACKNOWLEDGMENTS

We are indebted to Dr. James W. Reagan of the Institute of Pathology of Western Reserve University for the histologic examination of the tissues.

REFERENCES

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