SHORT COMMUNICATIONS

THE EFFECTS OF INJECTED BIOTIN AND DETHIOBIOTIN ON BUTTER YELLOW CARCINOGENESIS IN RATS FED A PROTECTIVE DIET*

By M. H. Briggs†

Following the discovery of the carcinogenicity of the azo dye butter yellow (*N*,*N*-dimethyl-*p*-aminoazobenzene), a number of investigators (Nakahara, Mori, and Fujiwara 1938*a*, 1938*b*) established that feeding yeast or liver extracts to rats afforded them considerable protection against tumor formation by the dye. Working with purified diets, two further groups (Gyorgy, Poling, and Goldblatt 1941; Kensler *et al.* 1941) demonstrated that excess casein and riboflavin together were also highly protective against the action of the dye. However, du Vigneaud *et al.* (1942) have reported that the daily addition of 2 μg of crystalline biotin to such a diet destroys its protection. This finding was confirmed in more extensive experiments by Burk *et al.* (1943), though Kline, Miller, and Rusch (1945) demonstrated that the dietary casein could be replaced by egg white and injections of 6 μg of biotin weekly without affecting the protection.

More recently Axelrod and Hofmann (1953), using a protective diet similar to the previous studies, were unable to demonstrate any procarcinogenic action by either biotin or oxybiotin. The present experiments were therefore undertaken to investigate the effects of biotin and dethiobiotin injections on rats fed a protective diet containing butter yellow.

Experimental

Thirty-six male albino weanling rats of the Sprague-Dawley strain were obtained from Holtzman Inc. and were singly caged in wide-mesh, screen-bottomed cages. For the first 4 weeks they were fed the basal diet (see Table 1) without injections. After this time the animals were divided into three equal groups of 12 rats. Group 1 received injections of 5 μg of *d*-biotin weekly; group 2 received 50 μg of *d*-biotin, while group 3 received 50 μg of *d*-dethiobiotin. All solutions were prepared in sterile saline and injections were given subcutaneously twice weekly in two equal doses.

Throughout the experiment the rats were frequently weighed and the consumption of food and water (which were fed *ad libitum*) was determined every 2 weeks. The mean weight of each group at the beginning of the experiment was 66 g. At the end of the experiment the mean weights were: group 1, 225 g; group 2, 232 g; and group 3, 189 g. The growth on dethiobiotin is greater than would have been predicted from the results of Rubin, Drekter, and Moyer (1945).

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† Department of Chemistry, Victoria University of Wellington, Wellington, N.Z.

After 140 days from the beginning of the injections all animals were still alive and apparently healthy, though group 2 animals had brown pigment in the fur on their heads and backs. No tumors were detectable by palpation. The animals were killed and an immediate post-mortem macroscopic examination was made of

TABLE 1
COMPOSITION OF THE BASAL DIET

Constituent	Concentration (g/100 g diet)	Constituent	Concentration (g/100 g diet)
Vitamin-free casein	15	Pyridoxine	0·0005
Dried egg white	10	Nicotinic acid	0·002
"Crisco"	10	*i*-Inositol	0·1
Sucrose	60	Calcium pantothenate	0·001
L-Cystine	1	2-Methyl-1,4-naphthoquinone	0·0001
Osborne-Mendel salt mixture	4	*dl*-α-Tocopherol acetate	0·001
Choline	0·25	Butter yellow	0·1
Riboflavin	0·002	Vitamin A	4070 i.u.
Thiamine	0·0005	Vitamin D	814 i.u.

all major organs. The liver and kidneys of each animal were removed and half of each organ preserved for microscopic examination. The other half was assayed for total biotin by the *Lactobacillus arabinosus* method of Wright and Skeggs (1944) and also the yeast method of Hertz (1943).

TABLE 2
BIOTIN CONTENTS OF RAT TISSUES

Group	Tissue	Total Biotin* (μg/g fresh tissue)
1	Liver	0·53
	Kidney	0·29
2	Liver	0·59
	Kidney	0·30
3	Liver	0·51
	Kidney	0·26

*Mean of 12 determinations.

Results

No tumors were discovered in any animal on macroscopic or microscopic examination. No other abnormalities of any type were found, though cirrhosis and bile-duct hyperplasia were particularly looked for. The biotin contents of the

livers and kidneys of each group are given in Table 2. The values recorded are derived from the yeast assay which consistently gave biotin values about 5 per cent. higher than the *L. arabinosus* assay. These biotin values are comparable with those of normal organs and are much higher than the biotin contents of butter yellow hepatomas reported by Pollack *et al.* (1942) and West and Woglom (1942).

The present experiment demonstrates that neither biotin nor dethiobiotin, injected at the levels stated above, can destroy the protection against butter yellow carcinogenesis afforded to rats by dietary casein and riboflavin supplements. The difference between this result, together with that of Axelrod and Hofmann (1953), and the earlier findings may be due to variations in the purity of the dietary constituents.

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References

Axelrod, A. E., and Hofmann, K. (1953).—*Cancer Res.* **13**: 442.

Burk, D., et al. (1943).—*Cancer Res.* **3**: 130.

du Vigneaud, V., et al. (1942).—*Science* **95**: 174.

Gyorgy, P., Poling, C. E., and Goldblatt, H. (1941).—*Proc. Soc. Exp. Biol., N.Y.* **47**: 41.

Hertz, R. (1943).—*Proc. Soc. Exp. Biol., N.Y.* **52**: 15.

Kensler, C. J., et al. (1941).—*Science* **93**: 308.

Kline, B. E., Miller, J. A., and Rusch, H. P. (1945).—*Cancer Res.* **5**: 641.

Nakahara, W., Mori, K., and Fujiwara, T. (1938*a*).—*Gann* **32**: 465.

Nakahara, W., Mori, K., and Fujiwara, T. (1938*b*).—*Gann* **33**: 406.

Pollack, M. A., et al. (1942).—*Cancer Res.* **2**: 748.

Rubin, S. H., Drekter, L., and Moyer, E. H. (1945).—*Proc. Soc. Exp. Biol., N.Y.* **58**: 352.

West, P. M., and Woglom, W. H. (1942).—*Cancer Res.* **2**: 324.

Wright, L. D., and Skeggs, H. R. (1944).—*Proc. Soc. Exp. Biol., N.Y.* **56**: 95.