

# Certain Effects of Egg White and Biotin on the Carcinogenicity of *p*-Dimethylaminoazobenzene in Rats Fed a Sub-Protective Level of Riboflavin\*

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Considerable interest in the possible cocarcinogenic action of biotin and in the anticarcinogenic influence of egg white and avidin has arisen since the report by du Vigneaud and his group (2) on the effect of these substances upon the rate of hepatoma formation in rats fed *p*-dimethylaminoazobenzene. These investigators found that rats fed the dye in protective diets containing high amounts of riboflavin developed hepatomas when sufficient biotin was given, and that the effect of biotin was neutralized when the diet contained enough egg white to bind the dietary biotin in the biotin-avidin complex. The livers of the protected rats were found to contain on the average only one-third as much biotin as the nontumor parts of the livers in the animals given biotin (1). Chiefly as a result of these experiments several attempts have been made to retard the growth of various types of neoplasia in human patients and laboratory animals by the administration of egg white or avidin (3, 4, 8, 10). None, however, have been successful.

Smith, Lillie, and Stohlman (9) studied the liver damage in rats fed a high level, 0.1 per cent, of *p*-dimethylaminoazobenzene in partially purified diets containing large amounts of various protein sources, and found that 32 per cent of dried egg white prevented liver tumors and greatly reduced the degree of cirrhosis, while 37 per cent of casein gave slight protection and 53 per cent of yeast or 32 per cent of gelatin none at all. These diets contained at least 5 per cent of yeast, and no symptoms of biotin deficiency in the rats fed egg white were mentioned. The suggestion was made that egg white can partially detoxify the azo dye, and that its protective effect may not be due solely to its avidin content.

The present study deals with the effect of egg white on the incidence of hepatomas in rats fed *p*-

dimethylaminoazobenzene in a highly purified diet containing a sub-protective level of riboflavin. Attempts to alter the effect of the egg white by the injection of biotin or by denaturing the avidin in the egg white by heat are reported.

## METHODS

Young adult male albino rats of the Sprague-Dawley strain and 120 to 200 gm. in weight were fed the rations *ad libitum*. They were kept in groups of 7 or 8 in screen-bottomed cages. The dye was fed for 4 months and the livers were examined by laparotomy at the end of this period; the animals were then continued on the same basal diets without the dye for another 2 months. At 6 months a final examination of the livers was made.

The basal diets contained either vitamin-free casein (5), 120 gm., or a similar quantity of domestic dried egg white, unheated or heated; glucose monohydrate, 790 gm.; salts, 40 gm.; corn oil, 50 gm.; thiamine chloride, 3 mgm.; pyridoxine hydrochloride, 2.5 mgm.; calcium pantothenate, 7.0 mgm.; and choline chloride, 30.0 mgm. per kgm. Two milligrams of riboflavin per kgm. was added to the diets containing casein. In the other diets the egg white, heated and unheated, was assayed for riboflavin (fluorometric procedure<sup>1</sup>) and only enough of this vitamin was added to bring the total to 2 mgm. per kgm. of diet. The unheated egg white samples assayed 3.0 and 17.0  $\mu$ gm. of riboflavin per gm., while the latter sample after heating contained 12  $\mu$ gm. per gm. Each rat received 1 drop of halibut liver oil per month throughout the experiment. The carcinogen, *p*-dimethylaminoazobenzene, was fed at a level of 0.06 per cent, and was incorporated in the diets by dissolving it with heat in the corn oil.

The heated egg white was prepared by autoclaving 150 gm. of the dried domestic product with 750 cc. of water in a shallow pan for 90 minutes at 15 lbs. pressure. It was then dried and ground. No assays

<sup>1</sup> These assays were kindly made by Mr. B. S. Schweigert.

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for avidin were made. Since the animals on the heated egg-white diet continued to lose weight over the first 2 months of the experiment, each egg-white diet in this series was supplemented with 2 per cent of purified casein at the expense of glucose for the following 4 months. Each rat of one group fed the unheated egg white received 2  $\mu$ gm. of crystalline biotin in 0.2 cc. of saline solution by subcutaneous injection 3 times a week.

### RESULTS

The results obtained are summarized in Table I. The first series comprised two groups of rats, one fed the diet containing casein (Group 1) and the other given egg white instead (Group 2). The controls (Group 1) responded to the carcinogen in the same manner as observed repeatedly in previous experi-

ments (5, 6, 7). Although the survival at 4 months was slightly lower than usual, the tumor incidence of 82 per cent at 6 months, and the moderate to severe cirrhosis that developed, were typical for this diet. The substitution of egg white for casein (Group 2) completely prevented the formation of tumors by 6 months, and there was no gross cirrhosis in this group at 4 months. Although both groups consumed on the average the same amount of food and carcinogen, the control group gained weight whereas the egg-white group lost some weight, particularly in the last 2 months. After 3 months the first symptoms of biotin deficiency were noted in the rats fed the egg-white diet. The syndrome, which increased in severity throughout the remainder of the experiment, consisted of a generalized loss of hair, erythematous dermatitis, hunched backs, hypersensitiveness, and depositions of red-brown pigment about the eyes, nose, and paws.

The second series of experiments included 2 groups fed casein and unheated egg white as in the first experiment. In addition one group was fed unheated egg white and given repeated injections of crystalline

biotin, while another received heated egg white. The rats in each of these groups ate more food and gained more weight than those in the previous series. Again the rats fed the control diet (Group 3) exhibited a typical incidence of liver tumors, 77 per cent, by 6 months. The rats fed unheated egg white (Group 4) developed a severe biotin deficiency as before, and had a final tumor incidence of only 14 per cent. It is interesting that this group consumed more dye than did the controls.

The injection of 2  $\mu$ gm. of crystalline biotin 3 times a week into each rat of another group fed unheated egg white (Group 5) completely prevented the biotin deficiency syndrome. The animals ate somewhat more food and gained more weight than did their controls (Group 3). However, only 10 per cent of the rats had hepatomas at 6 months; this incidence

TABLE I: EFFECT OF EGG WHITE AND BIOTIN ON THE CARCINOGENICITY OF *p*-DIMETHYLAMINOAZOBENZENE IN DIETS CONTAINING A SUB-PROTECTIVE LEVEL OF RIBOFLAVIN

Group	Diet	Av. weight, gm.			Average daily food intake gm./rat	Survival † at 4 mo.	Tumor incidence ‡		Negative survivors at 6 mos.	Cirrhosis at 4 mo.			Biotin deficiency symptoms
		Start	4 mos.	6 mos.			4 mos.	6 mos.		None	Mod-erate	Severe	
1	Casein	191	193	221	8.0	11/15	4/11	9/11	2	1	8	2	None
2	Egg white	180	185	170	8.0	13/15	0/13	0/13	8	13	0	0	Severe
3	Casein	177	200	220	10.0	13/15	1/13	10/13	0	0	9	4	None
4	Egg white *	182	244	246	11.3	14/15	0/14	2/14	11	12	2	0	Severe
5	Egg white * + injected biotin	175	246	278	10.8	10/15	0/10	1/10	5	5	5	0	None
6	Heated egg white *	181	230	258	10.2	11/15	0/11	2/11	6	8	2	1	None

\* 2 per cent purified casein added at the expense of glucose after the second month (see text).

† Survival = Number at 4 months over number at start.

‡ Tumor incidence = Number with tumors over survival at 4 months.

is comparable to that of group 4, which did not receive biotin. Similar results were obtained when rats were fed egg white that had been heated to destroy the avidin (Group 6). The animals consumed about the same amount of dye as the controls, and deficiency symptoms were entirely lacking. The incidence of hepatic tumors, 18 per cent at 6 months, was similar to that observed in each of the other groups fed egg white in this series. Apparently the 2 per cent of purified casein that was added to all the egg-white diets of this series after the second month had little or no influence on tumor development.

### DISCUSSION

The present experiments demonstrate that the replacement of 12 per cent of casein by egg white in a highly carcinogenic purified diet results in a striking decrease in the rate of hepatoma formation. The egg white exerted its effect equally well whether it was fed as the relatively native protein, or after having been heated sufficiently to destroy the avidin present, or when it was supplemented with crystalline biotin.

In the latter 2 instances sufficient biotin was supplied in the diet (heated egg white contains available biotin) and by intestinal synthesis or through deliberate injection to prevent occurrence of the characteristic deficiency syndrome. It is not known whether still higher levels of biotin would have overcome the protective effect of the egg white, but since no increased tumor incidence was observed when biotin was injected, and particularly when heated egg white was fed, the concept of an altered biotin balance (1) does not appear to explain these results. Instead, the anticarcinogenic action of egg white in these experiments seems to be independent of its avidin content.

The original experiments demonstrating the cocarcinogenic effect of biotin on the induction of hepatomas by *p*-dimethylaminoazobenzene were conducted with diets containing casein and/or egg white and high levels, 10 and 20  $\mu$ gm. per gm. of diet, of riboflavin (2). These diets largely prevented the formation of liver tumors by 150 to 230 days when no excess biotin was given. The tumor incidence was increased to over 50 per cent upon the addition of crystalline biotin or a biotin ester concentrate to the diets at levels of 0.3 to 2.0  $\mu$ gm. per rat per day. This effect was independent of dietary egg white as long as an excess of biotin was present in the diet. If sufficient egg white was fed to bind all the biotin very few tumors were evident at the end of the experiment.

The diets discussed in the present paper contained a level of riboflavin, 2  $\mu$ gm. per gm. of diet, which, when casein is fed, is not sufficient to protect against tumor development by *p*-dimethylaminoazobenzene. Yet egg white, heated or unheated, and egg white with injected biotin protected strongly against the effects of the azo dye. It is possible, of course, that the level of riboflavin in the diet may determine the cocarcinogenic action of biotin on the development of liver tumors due to *p*-dimethylaminoazobenzene. Other compounds, possibly present in natural materials such as egg white and casein, or formed via intestinal synthesis, may likewise affect the cocarcinogenic effect of biotin. Thus, for example, Burk and his associates (1) found that 0.05 per cent of *p*-aminobenzoic acid in the diet overcame the action of biotin and prevented the formation of hepatomas by the azo dye.

#### SUMMARY

Six groups of 15 rats each were fed 0.06 per cent of *p*-dimethylaminoazobenzene in highly purified diets containing a sub-protective level of riboflavin, *e.g.*, 2  $\mu$ gm./gm. diet, and 12 per cent of protein as vitamin-free casein, unheated dried egg white, or heated dried egg white. Each rat of one group fed unheated egg white also received 2  $\mu$ gm. of crystalline biotin by subcutaneous injection 3 times a week. The dye was

fed for 4 months and followed by the dye-free basal diets for another 2 months. The livers were inspected by laparotomy at 4 months and a final tumor count was made at 6 months.

The incidence of liver tumors at 6 months on the casein diets was 77 and 82 per cent, while on the egg-white diets it varied from zero to 18 per cent. Thus neither the injection of biotin nor the heat denaturation of the avidin present in the egg white destroyed the protective effect exerted by this protein. Symptoms of biotin deficiency developed after 3 months on the unheated dried egg-white diets, but did not appear when biotin was given or if the egg white was heated. The protection against hepatoma formation offered by egg white in these experiments appeared to be independent of any obvious biotin-egg-white relationship. It is important to note that these experiments were performed with diets containing a sub-protective level of riboflavin; the previous reports on the cocarcinogenic effect of biotin concerned diets containing highly protective levels of this vitamin.

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