

# The Effect of Diet Containing Dried Egg Albumin upon *p*-Dimethylaminoazobenzene Carcinogenesis\*

Paul N. Harris, M.D.

(From the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis 6, Indiana)

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In 1941 Miller, Miner and Rusch (4) reported briefly that a diet containing 12 per cent of dried egg white retarded liver tumor production by *p*-dimethylaminoazobenzene, and remarked that egg white seemed to offer more protection than did casein. Since no additional information upon this subject had appeared, the experiment herein described was begun. Subsequently, Kline, Miller and Rusch (3) showed that the protection displayed by a diet containing 12 per cent of dried egg white was not due to avidin. Since their experiment was conducted differently from ours and was terminated at the end of six months, it seems worth presenting our data.

azobenzene (1, 2), the rats received the carcinogen-containing diet *ad libitum* until it was determined by palpation that a liver tumor was present. The animals were then killed and specimens taken for histologic examination.

The composition of the diets used is given in Table I.

## RESULTS

The results are shown graphically in Fig. 1, in which diets 39 and 40 (each containing 15 per cent of dried egg albumin) are compared with low protein diet 15R (91 per cent rice) and diet 16R (diet 15 plus 3 per cent of Liver Extract, Lilly). These four groups of

TABLE I. COMPOSITION OF DIETS

	Basal diets	
	No. 2	No. 6
Dried egg albumin*	0.0	150.0
Primex	50.0	50.0
McCollum's salt mixture No. 185 (Modified)	40.0	40.0
Carotene	0.01	0.01
Vitamin D Concentrate in Cottonseed Oil (400,000 U./gm.)	0.005	0.005
Thiamin	0.005	0.005
Riboflavin	0.001	0.0015
Vitamin B <sub>6</sub>	0.003	0.003
Nicotinic acid	0.005	0.01
Distilled natural tocopherols	0.01	0.01
Calcium pantothenate	0.0056	0.0056
Choline chloride	0.0	1.0
Starch	0.0	759.0
Ground polished rice	910.0	0.0

\* "Powdered Hen Egg Albumin," obtained from Henningsen Brothers, 99 Hudson Street, New York City.

<i>Diet 15R</i>	<i>Diet 39</i>
4850 gm. second basal diet	4850 gm. sixth basal diet
150 gm. carcinogen solution	150 gm. carcinogen solution
(2% in cottonseed oil)	(2% in cottonseed oil)
<i>Diet 16R</i>	<i>Diet 40</i>
4700 gm. second basal diet	4700 gm. sixth basal diet
150 gm. Liver Extract, Lilly	150 gm. Liver Extract, Lilly
150 gm. carcinogen solution	150 gm. carcinogen solution
(2% in cottonseed oil)	(2% in cottonseed oil)

## METHODS

As in our other experiments with *p*-dimethylamino-

animals were contemporaneous and received the same concentration of carcinogen. As in our other papers, the cumulative tumor percentage is plotted against time in days, and death of a tumor-free animal is indicated by a short line perpendicular to the appropriate curve.

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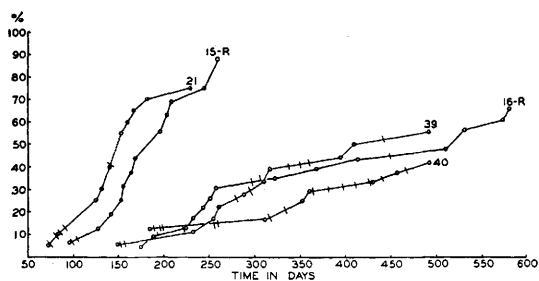


FIG. 1

We did not run an experiment with a diet differing from the albumin diet only in replacement of albumin by casein, but comparison of curves for diets 1 and 21 from our earlier data (1) with diet 39 gives a rough idea of the relative efficacy of casein and egg white in protecting against *p*-dimethylaminoazobenzene carcinogenesis. The curve for diet 21 is reproduced in Fig. 1 of this paper, and those for diets 1 and 21 are given in Figs. 18 and 24 of our earlier paper (1). Certain differences between these diets should be noted. Diets 1 and 21 contained 50 per cent more carcinogen than did 29, and consisted of 20 per cent casein. Diet 21 contained 1.5  $\mu$ gm. of riboflavin per gm., and diet 1 contained 5  $\mu$ gm. per gm. The albumin diet was patterned after diet 21, and was intended to contain 1.5  $\mu$ gm. of riboflavin per gm., but an assay of the egg white revealed a riboflavin content of 21.6  $\mu$ gm. per gm., making the riboflavin content of diet 39 actually 4.7  $\mu$ gm. per gm., a level comparable with that of diet 1. In our original experiments (1), the curves for diets 15 and 21 nearly coincided. Presumably, if diet 21 had been repeated with a concentration of 0.6 mgm. of carcinogen per gram, the resultant curve should have approximated that of diet 15R.

Even after allowances are made for more rapid carcinogenesis on those diets containing 0.9 mgm. of carcinogen per gm., it is evident that the 15 per cent albumin diet afforded striking protection against carcinogenesis as compared with diets low in riboflavin and protein (diet 15R), low in riboflavin but containing 20 per cent casein (diet 21), and containing adequate riboflavin and 20 per cent caesin (diet 1). However, albu-

min offered no more protection than did the addition of 3 per cent of liver extract (diet 16R) to diet 15R. Incorporation of liver extract (diet 40) into diet 39 appeared to result in additional protection against the carcinogen. In view of the relatively large number of deaths of tumor-free animals on diet 40 as shown in Table II and Fig. 1, the significance of this difference is doubtful. However, there was a striking difference in the incidence of cirrhosis as revealed histologically in diets 39 and 40. Of 11 livers from diet 39 examined microscopically, 3 showed no cirrhosis, and of 12 livers from diet 40, ten showed no cirrhosis and two showed slight cirrhosis. There were also fewer deaths among rats on diet 40 during the latent period of carcinogenesis.

DISCUSSION

No attempt was made to control the biotin or avidin content of the albumin diets since it was considered improbable that these two factors were significant. This opinion has been substantiated by Kline, Miller and Rusch (3). Furthermore, although biotin does have a procarcinogenic effect under proper conditions, our experience (2) leads us to believe that if a biotin-containing diet gave a tumor incidence comparable to that of diets 15R or 21, the removal of biotin would not alone suffice to cause the tumor incidence to approximate that of diet 39.

SUMMARY

Development of liver tumors in rats fed a diet containing 15 per cent of dried egg albumin and 0.06 per cent of *p*-dimethylaminoazobenzene was greatly retarded as compared with the development upon a diet favorable to tumor development. The degree of protection was greater than that provided by casein and riboflavin, and seemed slightly enhanced by addition of 3 per cent of liver extract.

REFERENCES

- HARRIS, P. N., KRAHL, M. E., and CLOWES, G. H. A. *p*-Dimethylaminoazobenzene Carcinogenesis with Purified Diets Varying in Content of Cysteine, Cystine, Liver Extract, Protein, Riboflavin, and Other Factors. *Cancer Research*, 7:162-175. 1947.
- HARRIS, P. N., KRAHL, M. E., and CLOWES, G. H. A. The Effect of Biotin upon *p*-Dimethylaminoazobenzene Carcinogenesis. *Cancer Research*, 7:176-177. 1947.
- KLINE, B. E., MILLER, J. A., and RUSCH, H. P. Certain Effects of Egg White and Biotin on the Carcinogenicity of *p*-Dimethylaminoazobenzene in Rats Fed a Sub-Protective Level of Riboflavin. *Cancer Research*, 5:641-643. 1945.
- MILLER, J. A., MINER, D. L., RUSCH, H. P., and BAUMANN, C. A. Diet and Hepatic Tumor Formation. *Cancer Research*, 1:699-708. 1941.

TABLE II

Diet No.	Date begun	Total rats used	Effective total	No. of rats with tumors	50% Tumor incidence, days
15R	May 18, 1944	45	16	14	183
16R	"	35	23	15	455
39	May 29, 1944	25	18	10	410
40	"	25	24	10	never
21	Sept. 11, 1941	30	20	15	153