Protective Effect of Riboflavin on the Toxicity of 2-Acetylaminofluorene in Rats.* (17607)

ARTHUR W. WASE AND JAMES B. ALLISON

From the Bureau of Biological Research, Rutgers University, New Brunswick, N. J.

The carcinogen, 2-acetylaminofluorene, has been shown by Griffin *et al.*(1) to produce a reduction in liver protein and riboflavin in the rat. The depleting effects of this drug on the body stores of proteins and riboflavin in dogs have also been demonstrated.(2,3) Other carcinogens have been shown to produce riboflavin deficiencies in rats, the vitamin protecting the animal from the toxic effects of the chemical agent possibly by entering into its detoxication.(4,5) The following experiments were done to determine the protecting effects of dietary protein and riboflavin on the toxicity of 2-acetylaminofluorene in the rat.

Methods. Various amounts of riboflavin and 2-acetylaminofluorene were added to the following diet for rats, the values being expressed as percentages: casein 18, sucrose 13.4, glucose 20.2, dextrin 18.2, lard 24.2, Wesson's salt mixture 1.7,(6) agar 3.3, and cod liver oil 1. These constituents were made into a gel with water equal to 1.7 times the dry weight. The vitamin supplements as mg/kg of wet weight of diet were choline chloride, 1000; inositol, 100; thiamine, 2; ascorbic acid, 4; pyridoxine HCl, 1.6; 40 each of Ca pantothenate, niacin, p-aminobenzoic acid and α-tocopherol and 0.2 mg each 2-methyl,1,4-naphthoquinone, biotin and folic acid. The protein-free diet was made by replacing the casein with an equivalent number of calories in the form of glucose. The diet was pair fed to adult male Long Evans rats (300 g), each animal receiving 30 g per day. The rats were divided into 6 goups of 6 animals each (Table I). After 8 weeks of feeding, the rats were observed for skin lesions and the livers were analyzed for riboflavin.(7)

Results. The results of feeding different amounts of riboflavin to rats with or without 2-acetylaminofluorene in the diet are recorded in Table I. The data in Table I demonstrate that rats receiving 3 γ of riboflavin per day in the synthetic diet containing 18% casein did not show any evidence of a deficiency during the feeding period of 8 weeks. The riboflavin content of the livers averaged 16 γ/g of tissue. When 0.03% 2-acetylaminofluorene was added to the diet, skin lesions developed and the riboflavin content of the liver decreased to 9.5 γ/g of tissue. Thirty γ of the vitamin per day prevented the appearance of symptoms of deficiency in rats fed the carcinogen, the liver riboflavin being the same as the controls. In the absence of proteins in the diet, however, even 50 γ of riboflavin per day did not protect the rats from the toxic effects of 2-acetylaminofluorene. It is possible that the sparing action of protein may be in part through an effect of the protein on riboflavin. Reisen, Schweigert and Elvehjem,(8) for example, have demonstrated that an increase in the amount of protein ingested increased the riboflavin retention in the liver of the rat.

Summary. The carcinogen, 2-acetylaminofluorene, produced a riboflavin deficiency in rats, reducing the liver riboflavin below control values. Increasing the riboflavin content of the diet prevented the appearance of the de-

* These studies were done under a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

1. Griffin, A. C., Cook, H., and Luck, M. J., *Cancer Research*, 1949, v9, 617.

2. Allison, J. B., Wase, A. W., and Wainio, W. W., *Fed. Proc.*, 1949, v8, 179.

3. Allison, J. B., Wase, A. W., Leathem, J. H., and Wainio, W. W., in press.

4. Miller, J. A., *Ann. N. Y. Acad. Sci.*, 1947-48, v49, 19.

5. Griffin, A. C., Clayton, M. S., and Baumann, C. A., *Cancer Research*, 1949, v9, 82.

6. Wesson, L. G., *Science*, 1932, v75, 339.

7. Arnold, A., *Cereal Chemistry*, 1945, v22, 455.

8. Reisen, W. H., Schweigert, B. S., and Elvehjem, C. A., *J. Biol. Chem.*, 1946, v10, 387.

TABLE I.
Skin Lesions and Riboflavin Content of the Liver Associated with Various Amounts of Riboflavin (R) and 2-acetylaminofluorene (AAF) in the Diet.

Group	R, γ/30 g diet	AAF %	Casein, %	Skin lesions	Liver riboflavin, γ/g tissue
1	3	0	18	—	16.0 ± 1*
2	3	.03	18	++++	9.5 ± 1
3	0	0	18	++	12.7 ± 0.9
4	0	.03	18	+++	9.3 ± 2
5	30	.03	18	—	16.0 ± 1.5
6	50	.03	0	+	9.9 ± 1

* Standard error.

ficiency, keeping the liver riboflavin at normal levels. In the absence of protein in the diet, however, the increased riboflavin did not prevent the development of the deficiency.

Received December 14, 1949. P.S.E.B.M., 1950, v73.

Toxicity of Eugenol: Determination of LD50 on Rats.* (17608)

HERBERT A. SOBER,† FRANKLIN HOLLANDER, AND EVA K. SOBER
From the Gastroenterology Research Laboratory, The Mount Sinai Hospital, New York City.

As a result of the demonstration that eugenol (4-allyl-2-methoxyphenol) is a very effective mucigogue and desquamatory agent in the stomach,(1) this substance has been used for the study of mucus secretion and gastric cytology in man, without gastric resection or gastroenterostomy.(2) Our technic involves administration of an aqueous emulsion of the eugenol by stomach tube, and its reaspiration after about 15 minutes. Prior to such clinical application, however, it was deemed necessary to estimate the limits of safe dosage for this substance. Such a study, conducted on dogs without any gastric operation,(3) revealed a maximum safe dosage of about 0.2 g/kg body weight, when an aqueous emulsion was administered in this same manner without reaspiration. A dose of 0.25 g/kg caused no fatalities, but induced vomiting in 2 of 7 experiments. There was no gross or microscopic evidence of any cumulative effect after repeated administration of this dose—10 times in the course of 3 weeks. At dosage levels around 0.5 g/kg, reactions to the irritant, including vomiting and ataxia, were manifest and in 2 of 6 experiments, the dog died within 24 hours. Hence, a more precise determination of the toxicity, carried out on a large number of animals, was necessary. Such a study on rats is presented herein.

Experimental Procedure. About 200 albino rats, each weighing approximately 200 g, were used in this investigation. The animals, kept in individual wire cages, were fasted for 24 hours before the eugenol was administered and for about 4 hours thereafter. A total of 91 animals were included in the final dosage-mortality analysis. The individual dosage groups were so constituted that each contained equal numbers of males and females. Eugenol (Merck, U.S.P.) was administered undiluted in a single dose, in a manner essentially the same as that reported by Shay(4)

* This investigation was supported by a research grant from the National Cancer Institute of the National Institutes of Health, U. S. P. H. S.

† Present address: National Cancer Institute, National Institutes of Health, Bethesda, Md.

1. Hollander, F., and Lauber, F. U., PROC. SOC. EXP. BIOL. AND MED., 1948, v67, 34.

2. Hollander, F., Hess, M., and Sober, H. A., *J. Nat. Cancer Inst.*, 1947, v7, 365.

3. Lauber, F. U., and Hollander, F., *Gastroenterology*, in press.

4. Shay, H., and Gruenstein, M., *J. Lab. Clin. Med.*, 1946, v31, 1384.