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Inhibitory Effect of Sodium Cyclamate and Sodium Saccharin on Tumor Induction by 2-Acetylaminofluorene in Rats (37999)

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The nonnutritive sweeteners cyclamate and saccharin have been reported to induce bladder tumors in rats and mice (1–4). In a preliminary experiment studies were conducted to determine whether the concurrent administration of the carcinogen 2-acetylaminofluorene (AAF) with sodium cyclamate or sodium saccharin would result in a more rapid occurrence of bladder tumors in female rats than occurred with either of these nonnutritive sweeteners alone. The findings indicated that AAF not only did not induce a more rapid onset of bladder tumors in rats fed sodium cyclamate or sodium saccharin but conversely that the latter nonnutritive sweeteners resulted in a marked decrease in the incidence of mammary and ear duct tumors in rats administered AAF. The present experiment was undertaken to confirm and extend the latter observation.

Materials and Methods. Forty-eight female rats of the Horton–Sprague–Dawley strain[1] were selected at an average body weight of 56.8 g (range 47–65 g) and were divided into four comparable groups of 12 animals each. Group I was fed a natural food stock ration;[2] Groups II, III, and IV were fed the stock ration plus the following supplements: Group I, 300 mg AAF[3] per kg of diet; Group III, 300 mg AAF per kg of diet + 5% sodium cyclamate;[4] and Group IV, 300 mg AAF per kg of diet + 5% sodium saccharin.[5] AAF, sodium cyclamate, and sodium saccharin were incorporated in the diets in place of an equal amount of stock ration. Animals were placed in metal cages with raised screen bottoms (three rats per cage) and were provided the test diets and water *ad lib.* Rats were weighed weekly and were examined at this time for the presence of palpable tumors. Surviving animals were autopsied after 40 wk of feeding and were examined for the presence of grossly visible liver tumors. These were graded on a scale of 0–4. Portions of the liver as well as the entire urinary bladder were placed in 10% neutral formalin for fixation; and paraffin sections were prepared, stained with hematoxylin and eosin, for microscopic examination.

Results. Gain in body weight was retarded in all rats fed AAF compared to that of rats fed the stock ration without AAF. For the first 20 wk of feeding little if any difference in body weight occurred between the three groups fed AAF. Thereafter, rats fed the diet containing sodium cyclamate (Group III) showed a slight loss in body weight in contrast to the continuing weight increment of rats in the other groups. Data on body weight are summarized in Table I.

In agreement with previous findings (5–7) AAF when fed at a level of 300 mg per kg of diet resulted in a high incidence

[1] Obtained from Horton Laboratories, Inc., Oakland, CA.

[2] Purina Laboratory Chow in meal form, Ralston Purina Company, St. Louis, MO.

[3] *N*-2-Fluorenylacetamide, Eastman Kodak Co., Rochester, NY.

[4] The sodium cyclamate employed in the present experiment was kindly provided by Dr. Ronald G. Wiegand of Abbott Laboratories, North Chicago, IL.

[5] Saccharin Sodium, Abbott Laboratories, North Chicago, IL.

TABLE I. Body Weight of Rats in the Various Groups During the Course of the Experiment (12 Animals per Group).[a]

Dietary group	Average body wt (g) after following weeks of feeding			
	10	20	30	40
Group I	239.8	279.7	309.1	331.8
Group II	193.9	234.1	248.1 (11)	252.2 (6)
Group III	193.8	226.5	223.0	206.5 (10)
Group IV	192.0	222.6	241.9 (11)	248.8 (6)

[a] The average initial body weight of rats in the various groups was 56.8 g. The values in parentheses indicate the number of animals which survived and on which data are based when this number was less than the original number per group.

of palpable mammary and ear duct tumors. The first such tumor was noted after 10 wk of feeding, and 11 of the 12 rats in Group II (91.7%) had developed such tumors by the end of the 40th wk of feeding with an average time of onset of 28 wk. These tumors ranged in size from that of a small pea to a large walnut. The number of palpable tumors per tumor-bearing rat at death or when animals were sacrificed after 40 wk of feeding ranged from one to four with an average of 2.2 per rat. In contrast to the above only two rats (16.7%) fed the diet containing sodium cyclamate (Group III) developed palpable mammary or ear duct tumors (one after 30 and the other after 37 wk of feeding), and these consisted of a single tumor per rat. The incidence of tumors of rats fed the diet containing sodium saccharin (Group IV) was intermediate between Groups II and III. Six of the 12 rats (50%) in Group IV developed palpable mammary or ear duct tumors by the end of the 40th wk of feeding with an average time of onset of 29 wk and with an average of 1.3 palpable tumors per tumor-bearing rat. No tumors were noted in any of the rats in Group I. A number of deaths occurred in tumor-bearing rats during the course of the experiment. These occurred on the average 6–7 wk after the first appearance of palpable mammary or ear duct tumors in all groups fed AAF. Of the 24 palpable tumors in Group II, 3 were ear duct tumors and 21 mammary tumors; of the two palpable tumors in Group III, one was an ear duct tumor and one a mammary tumor; of the eight palpable tumors in Group IV, two were ear duct tumors and six mammary tumors. No data are available as to the histological appearance of mammary and ear duct tumors of rats in the various groups.

Although previous studies indicated that the incidence of liver tumors in Sprague–Dawley female rats fed AAF is low compared to their high incidence in males (7, 13) five of the six surviving rats in Group II in the present experiment had liver tumors of grade 3 or 4 in severity as judged by gross examination.[6] None of the rats in Groups III and IV had liver tumors that exceeded grade 1 in severity by such examination. Histologically hepatoma were observed in all rats fed AAF. On a scale of 0–4, three rats in Group II had hepatoma of grade 4 severity (Fig. 1), two rats had hepatoma of grade 3 severity, and one rat had a hepa-

[6] Rats of the Horton–Sprague–Dawley strain are descendants of Sprague–Dawley rats that had been maintained for a number of generations at the Horton Laboratories. To what extent rats in this closely inbred colony may now differ from the original Sprague–Dawley strain has not been determined. It is possible that the high incidence of liver tumors in female rats of the Horton–Sprague–Dawley strain fed AAF may be due to a strain difference in response.

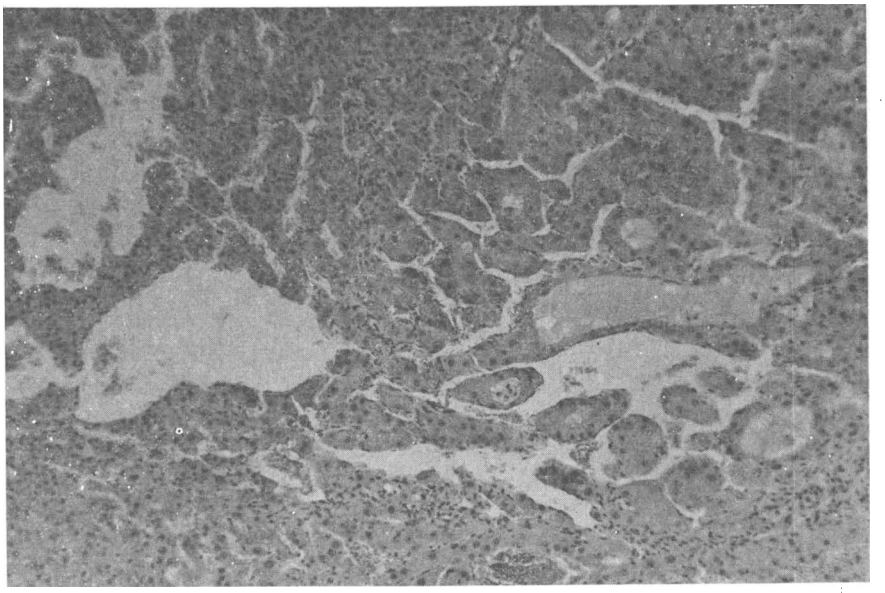

FIG. 1. Photomicrograph of liver of rat in Group II with grade 4 hepatoma. Note loss of normal architecture, nuclear hyperchromasia, and the presence of atypical and anaplastic tumor cells. Hematoxylin and eosin stain, ×225.

toma of grade 1 severity. Hepatoma of grade 4 severity were considered malignant on the basis of loss of normal architecture, nuclear hyperchromasia, and the presence of atypical and anaplastic tumor cells. Grade 3 hepatomas were considered potentially malignant but had not progressed to the severity of a grade 4 lesion. Of the 10 sur-

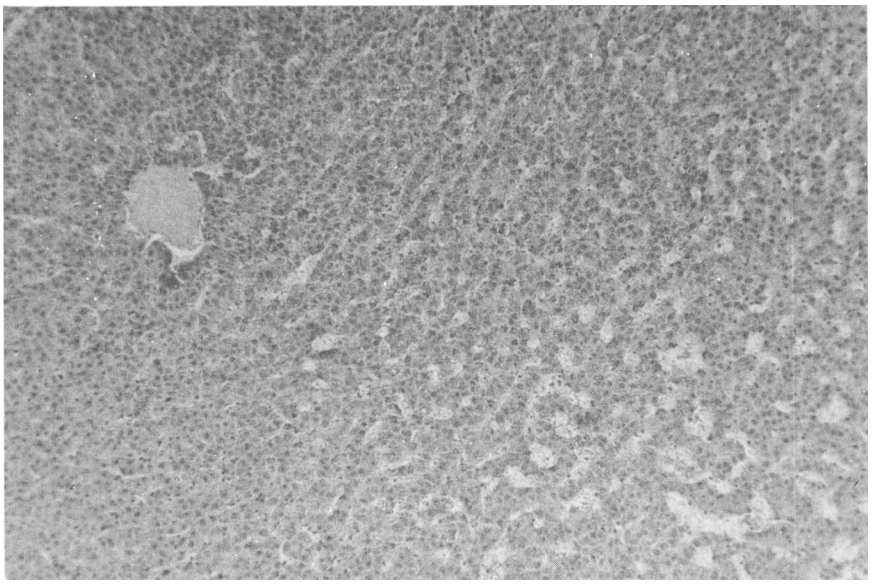

FIG. 2. Photomicrograph of liver of rat in Group III with grade 1 hepatoma. Note loss of normal hepatic cord arrangement adjacent to central vein but with maintenance of normal appearance of hepatic cells. Hematoxylin and eosin stain, ×225.

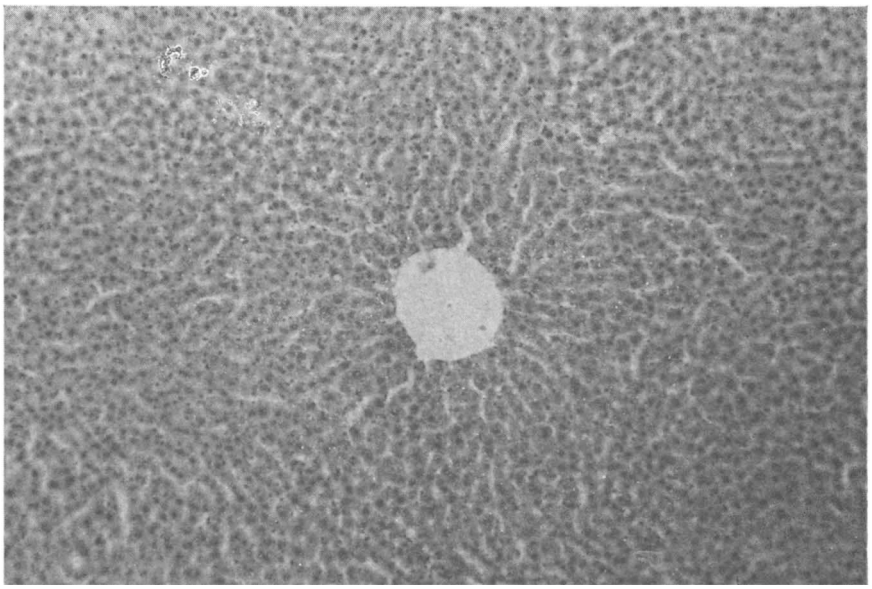

FIG. 3. Photomicrograph of liver of rat in Group I. Note normal hepatic parenchyma consisting of normal hepatic cells arranged in cords radiating from the central vein. Hematoxylin and eosin stain, ×225.

viving rats in Group III, the hepatoma ranged from 0.5 to 1.0 in severity. Hepatomas of grade 1 severity were benign and were characterized by microscopic focal nodular hepatomas dispersed in the normal hepatic parenchyma. These nodules consisted of normal-appearing hepatic cells with a loss of normal hepatic cord arrangement (Fig. 2). Of the six surviving rats in Group IV, the hepatoma ranged from 1.0 to 1.5 in severity. No tumors were observed either grossly or histologically in any of the rats in Group I (Fig. 3). Microscopic examination of the urinary bladders indicated that the mucosal lining was hyperplastic in all rats fed AAF and was particularly so for rats in Group IV with one animal in the latter group exhibiting squamous metaplasia and precancerous changes of the mucosal epithelium. Malignant lesions of the urinary bladder, however, were not observed in any of the rats.

Discussion. Present findings indicate that the administration of sodium cyclamate and to a lesser extent sodium saccharin resulted in a marked reduction in the incidence of palpable mammary and ear duct tumors and the size of liver tumors in rats fed a stock ration containing AAF. Inhibition of AAF tumor formation has also been obtained by the administration of methylcholanthrene (8, 9), acetanilide (10), chloramphenicol (11), and more recently phenobarbital (12). No data are available as to the *modus operandi* whereby sodium cyclamate and sodium saccharin exerted their inhibitory effect on tumor induction in the present experiment. Studies by Miller *et al.* have shown that the carcinogenic effects of AAF are due not to this substance per se but rather to its conversion to *N*-hydroxy-2-acetylaminofluorene, a metabolite of AAF (13), and the subsequent sulfonation of the latter by hepatic arylsulfotransferase to *N*-sulfoxy-2-acetylaminofluorene, which is considered to be the "ultimate carcinogen" (14). If the above reactions fail to occur, administration of AAF will not have a tumor-inhibitory effect. Any substance which interferes with the occurrence of one or both of the above reactions might, therefore, have a tumor-inhibitory effect. In the case of phenobarbital administration it has been shown that the latter increases the urinary excretion of *N*-hydroxy-AAF in rats fed AAF (15),

which may account at least in part for its protective effect. There is some evidence that the inhibitory effects of phenobarbital and methylcholanthrene on tumor formation may also result from their stimulation of liver enzymes that detoxify carcinogens (16–18). No data are available as to whether any of the above mechanisms were operative in respect to the protective effects of sodium cyclamate and sodium saccharin in the present experiment. Food consumption was not determined for rats in the various groups in the present study. Inasmuch as the weight increment of rats fed sodium cyclamate (Group III) and to a lesser extent sodium saccharin (Group IV) was slightly retarded compared to that of rats in Group II, animals in Groups III and IV may have ingested fewer calories and less AAF than was the case for rats in Group II. The possibility has not been excluded that the lower tumor incidence of rats in Groups III and IV may have been due, at least in part, to a diminished intake of calories and/or AAF.

Summary. Studies were conducted on the effects of orally administered sodium cyclamate and sodium saccharin on the tumor incidence of female rats fed the carcinogen 2-acetylaminofluorene (AAF). Eleven out of 12 rats (91.7%) fed a natural food stock ration supplemented with 300 mg AAF per kg of diet developed palpable mammary and ear duct tumors during an experimental period of 40 wk in contrast to a tumor incidence of two out of 12 rats (16.7%) in rats fed a similar ration supplemented with 5% sodium cyclamate and six out of 12 rats (50%) fed a diet containing 5% sodium saccharin. Administration of sodium cyclamate and sodium saccharin also resulted in a marked reduction in the size of liver tumors of rats fed AAF as judged by gross and microscopic examination of the livers of surviving rats after 40 wk of feeding.

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