PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 152, 156-159 (1976)

Dose-Response Hyperplasia and Neoplasia from Feeding *N*-2-Fluorenylacetamide (2-FAA) to BALB/c Mice for Varying Time Intervals (39350)

THOMAS J. HALEY, JOHN FARMER, W. E. JAQUES, C. FRITH, R. W. SPROWLS, AND G. SCHIEFERSTEIN

Department of Health, Education, and Welfare, Food and Drug Administration, National Center for Toxicological Research, Jefferson, Arkansas 72079

A dose-response-related urinary bladder hyperplasia in both sexes of BALB/c strain mice fed *N*-2-fluorenylacetamide at dietary concentrations ranging from 10 to 500 ppm for 90 days was reported by Haley *et al.* (1). After consuming 500 ppm of the carcinogen for 90 days, one male mouse developed urinary bladder carcinoma. Another male mouse receiving 250 ppm also developed a similar carcinoma. Clayson *et al.* (2) also observed bladder hyperplasia after feeding the carcinogen at concentrations of 300 ppm for 2 weeks. They suggested that there is a reasonable correlation between early hyperplasia and the final development of carcinoma. However, feeding *N*-2-fluorenylacetamide, 100 ppm, for 455 days resulted in only 3 of 33 mice showing bladder hyperplasia and no carcinoma (Clayson and Bonser, 3). Moreover, 1-month treatment with the chemical resulted in bladder hyperplasia which was present when the experiment was concluded. The males had a greater response than the females. Haley *et al.* (4) obtained a dose-response related urinary bladder hyperplasia in BALB/c mice fed *N*-2-fluorenylacetamide concentrations varying from 8 to 86 ppm for 3 months. Their males were also more responsive than their females. No carcinomas were produced in that experiment. In contrast to Clayson and Bonser (3), no lethality from the chemical was observed. Upon the basis of the above reports, two questions arise: (i) Is the presence of *N*-2-fluorenylacetamide required to prolong urinary bladder hyperplasia once it is induced? (ii) Will continuous feeding of the chemical for 12 months convert the hyperplastic state into carcinoma and at what time interval? We have investigated these questions with large numbers of BALB/c mice and a serial sacrifice design.

Methods. BALB/c strain mice, 3360, equally divided between the sexes were used. The animals were maintained under minimum disease conditions in quarters thermostatically regulated to 24° with free access to food and water. The mice weighed from 20 to 30 g. The sexes were randomized as to cages and racks. The mice were divided into two experiments of 1680 mice each; one group received the carcinogen for 3 months, then were fed control diet for the ensuing 9 months, while the second group received the carcinogen for 12 months. In each experiment the groups were composed of 240, 480, 480, 240, 160, and 80 animals, equally divided by sex and fed dietary concentrations of *N*-2-fluorenylacetamide of 0, 8, 24, 45, 59, and 86 ppm, respectively. *N*-2-fluorenylacetamide dietary content was determined by the methods of Bowman and King (5). Sacrifice periods were 3, 5, 7, 9, and 12 months, and 1/5 of the animals were sacrificed at each time interval. All mice were necropsied and the following tissues were examined microscopically after staining with hematoxylin-eosin: lungs, heart, aorta, thymus, anterior femoral muscle, kidneys, adrenal, liver, spleen, gallbladder, pancreas, cerebellum, spinal cord, stomach, colon, ileum, duodenum, cervical lymph node, salivary glands, lacrimal gland, eye, harderian gland, thyroid, trachea, esophagus, skin, tongue, sternum, testes, epididymus, preputial gland, seminal vesicle, coagulating gland, ovary, uterus, mammary gland, urinary bladder, prostate, pituitary, and any abnormal tissue masses. All data, including physical condition, were stored in a Modular Computer Systems III/5 minicomputer, and the histopathological information was coded on mark sensitive forms and entered into the system with an optical reader. All animals were weighed weekly as individuals on an electronic balance interfaced with a minicomputer.

To determine the possible activation or

interaction of *N*-2-fluorenylacetamide with murine leukemic viruses, assays for Sendai, Polyoma, and murine leukemia virus (MuLv) were conducted at 5-, 7-, 9-, and 12-month sacrifice intervals. Severs (6) and Parker *et al.* (7) methods were used for Polyoma and Sendai viruses and the Klement *et al.* (8) and the Rowe *et al.* (9) methods for MuLv.

Results. General observations. The previously observed calcareous pericarditis (4) was observed in the controls and treated animals with the condition more pronounced in the males than in the females. There were no significant effects on body weight in any group in either experiment. Acanthosis, ulceration, and acute and chronic inflammation of the skin was observed only in the males in both experiments and was probably related to aggressiveness. Table I lists the toxic effects (pooled data) produced by feeding *N*-2-fluorenylacetamide over 12 months with sacrifices at 3-, 5-, 7-, 9-, and 12-month intervals, and it can be seen that the changes were more pronounced in the males. Similar changes were obtained in the experiment where the chemical was fed for only 3 months and the liver changes suggest that permanent damage had already occurred at this time. The liver changes confirm (Clayson and Bonser (3). In both experiments, no significant changes were observed in the aorta, thymus, anterior femoral muscle, kidney, adrenal, spleen, pancreas, cerebellum, spinal cord, stomach, colon, ileum, duodenum, cervical lymph nodes, eye, thyroid, trachea, esophagus, tongue, sternum, testes, epididymus, preputial gland, seminal vesicle, coagulating gland, ovary, uterus, mammary gland, prostate, or pituitary.

Urinary bladder hyperplasia and neoplasia. When 2-FAA was removed from the diet after feeding for 3 months, the hyperplasia in males decreased to almost zero (Fig. 1). The females at 59 and 86 ppm showed hyperplasia in 10/15 and 8/8 mice at 9 months and an increase at 12 months. No carcinoma was observed. However, when 2-FAA was fed for 12 months a dose-related hyperplasia occurred in the males. Of importance is the appearance of hyperplasia in the lowest dose group of the males (Fig. 1). Thus it appears that total dose or continual dosage, not the rate of delivery, is the important factor in hyperplasia development. A pronounced difference in response in females is also evident and it is probably related to the degree of metabolic conversion of 2-FAA by this sex (15). The relationship of dose to urinary bladder carcinoma in males is shown in Table II. No urinary bladder carcinoma was found in the females. Apparently a pronounced and fully developed hyperplasia is essential for the development of neoplasia. The above results do not appear to be related to activation of oncogenic viruses by 2-FAA, or dose of 2-FAA to the proportion of mice with positive virus counts, or to the proportion of virus positive mice at each stage of hyperplasia or neoplasia when analyzed statistically. However, virus counts do increase with age.

Other neoplastic changes. Table III lists

TABLE I. TOXIC EFFECTS FROM FEEDING *N*-2-FLUORENYLACETAMIDE TO BALB/c MICE (POOLED SACRIFICE DATA).

	Fed only 3 months				Fed up to 12 months			
	Control		Treated		Control		Treated	
Effect	♂	♀	♂	♀	♂	♀	♂	♀
Liver fatty metamorphosis	8/111*	0/115	45/674	2/707	8/109	0/119	54/677	0/696
Liver fibrosis	2/111	0/115	16/674	2/707	0/109	0/119	15/677	0/696
Liver necrosis	6/111	2/115	30/674	2/707	3/109	0/119	31/677	7/696
Liver acute inflammation	8/111	3/115	30/674	8/707	2/109	2/119	31/677	8/696
Liver chronic inflammation	8/111	2/115	37/674	5/707	3/109	0/119	43/677	12/696
Liver nodular hyperplasia	4/111	0/115	17/674	1/707	2/109	0/119	18/677	0/696

* Number responding over total number in group.

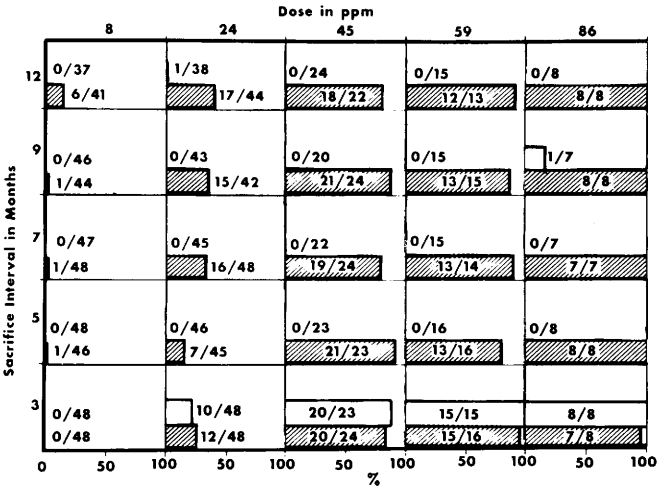

FIG. 1. Bladder hyperplasia in male BALB/c mice fed 2-FAA. Clear area = fed only 3 months; shaded area = fed until sacrificed.

TABLE II. INCIDENCE OF URINARY BLADDER NEOPLASIA IN BALB/c STRAIN MALE MICE FED *N*-2-FLUORENYLACETAMIDE FOR VARYING INTERVALS.

Sacrifice interval (months)	Dose of *N*-2-fluorenylacetamide (ppm)					
	0	8	24	45	59	86
3	0/23*	0/48	0/48	0/24	0/16	0/8
5	0/23	0/46	0/44	0/22	0/16	0/8
7	0/20	0/47	0/47	0/24	0/14	0/7
9	0/20	0/44	0/42	0/24	0/15	3/8
12	0/23	0/41	0/44	1/22	3/13	4/8

* Number responding out of total number in group.

TABLE III. TOTAL NEOPLASIA OCCURRING IN OTHER TISSUES OF BALB/c MICE FED *N*-2-FLUORENYLACETAMIDE (POOLED SACRIFICE DATA).

	Fed only 3 months				Fed up to 12 months			
	Control		Treated		Control		Treated	
Neoplasia	♂	♀	♂	♀	♂	♀	♂	♀
Lung alveolar cell adenoma	10/112*	1/115	52/689	26/710	11/109	7/120	41/677	32/695
Hepatocellular carcinoma	2/110	0/115	5/675	0/709	0/109	0/119	7/677	0/700
Parotid gland adenoma	1/109	0/115	14/662	1/682	1/106	0/116	14/653	3/685
Lacrimal gland adenoma	1/90	2/86	19/596	10/495	5/75	0/67	17/477	3/373
Harderian gland adenoma	1/110	0/115	10/671	11/710	4/109	8/120**	8/677	10/696**
Submaxillary gland adenoma	4/109	0/113	6/671	2/701	3/106	1/116	11/672	3/683

* Number responding over total number in group.

** Significant $\chi^2 = 10.67$; $P = 0.01$; all others not significant.

the other neoplasias produced by feeding 2-FAA for 3 and 12 months. Again a pronounced sex difference is evident, with the males being more susceptible in most cases. There was no significant difference between the treated and the controls.

Discussion. The results obtained by feeding *N*-2-fluorenylacetamide for periods of 3 or 12 months indicate that the presence of the chemical is essential for sustaining urinary bladder hyperplasia and possibly causing the induction of neoplasia of this organ. These data confirm the correlation between bladder hyperplasia and neoplasia suggested by Clayson *et al.* (2), and mammary gland hyperplasia and neoplasia reported by Fisher *et al.* (10). The present results, contrary to those of Clayson and Bonser (3),

showed that both bladder hyperplasia and neoplasia can be produced by feeding 2-FAA at lower doses for shorter time periods. Moreover, chemical lethality did not occur, thus confirming the results of Levi *et al.* (11) and Haley *et al.* (1), but not those obtained by others (12–14). Our observations regarding the differences in response, i.e., hyperplasia and neoplasia related to sex, support the report of Armstrong and Bonser (15) regarding differences in response related to both strain and sex of the mouse used.

Summary. Hyperplasia induced by *N*-2-fluorenylacetamide requires the continuous presence of the chemical for its maintenance. Feeding the chemical at levels of 8 to 86 ppm results in a dose-response related urinary bladder hyperplasia and, after 9 to 12 months, in bladder neoplasia at the higher dose levels. Neoplasia of the lungs, liver, parotid gland, lacrimal gland, harderian gland, and submaxillary gland was also observed. Measurement of oncogenic viruses revealed no synergism with 2-FAA and no relationship between virus counts and neoplasia. Calcareous pericarditis was observed and most general toxicological effects occurred in the liver. A pronounced sex difference in response was observed with the males giving greater responses than the females in all instances.

1. Haley, T. J., Schieferstein, G., Harmon, J. R., Dooley, K. L., Jaques, W. E., Frith, C., and Famrer, J. H., Proc. Soc. Exp. Biol. Med. **146,** 648 (1974).
2. Clayson, D. B., Lawson, T. A., Santana, S., and Bonser, G. M., Brit. J. Cancer **19,** 297 (1965).
3. Clayson, D. B., and Bonser, G. M., Brit. J. Cancer **19,** 311 (1965).
4. Haley, T. J., Schieferstein, G., Jaques, W. E., Frith, C., and Sprowls, R. W., J. Pharm. Sci. **63,** 1946 (1974).
5. Bowman, M. C., and King, J. R., Biochem. Med. **9,** 390 (1974).
6. Sever, J. L., J. Immunol. **88,** 320 (1962).
7. Parker, J. C., Tennant, R. W., Ward, T. G., and Rowe, W. P., J. Nat. Cancer Inst. **34,** 371 (1965).
8. Klement, V., Rowe, W. P., Hartley, J. W., and Pugh, W. E., Proc. Nat. Acad. Sci. USA **63,** 753 (1969).
9. Rowe, W. P., Pugh, W. E., and Hartley, J. W., Virology **42,** 1136 (1970).
10. Fisher, E. R., Shoemaker, R. H., and Sabnis, A., Lab. Invest. **33,** 33 (1975).
11. Levi, P. E., Knowles, J. C., Cowen, D. M., Wood, M., and Cooper, E. H., J. Nat. Cancer Inst. **46,** 337 (1971).
12. Foulds, L., Brit. J. Cancer **1,** 172 (1947).
13. Miller, E. C., Miller, J. A., and Enomoto, J., Cancer Res. **24,** 2018 (1964).
14. Wood, M., Eur. J. Cancer **5,** 41 (1969).
15. Armstrong, E. C., and Bonser, G. M., J. Pathol. Bacteriol. **59,** 19 (1947).

Received December 10, 1975. P.S.E.B.M. 1976, Vol. 152.