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Ninety Day Subchronic Toxicity of *N*-2-Fluorenylacetamide (2-FAA) In C57BL/6j and BALB/cStCr1BR Mice (38164)

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Armstrong and Bonser (1) showed that intubation of 2-FAA (*N*-2-Fluorenylacetamide) thrice weekly into CBA strain mice required dosing for 53 wk before bladder tumors developed. Wilson *et al.* (2) reported that feeding 0.062 or 0.125% of 2-FAA in the diet to C57 strain mice for 248 days produced similar lesions but 5 of 16 animals died before tumors developed. Foulds (3) also reported tumors in R3 strain mice receiving 2-FAA in the diet, 0.03% for 5 wk then 0.05% for 20 wk. 2-FAA induced mortality was highest in the first few weeks of administration. Miller *et al.* (4) found tumors from feeding 0.05% 2-FAA in the diet for 10 mo but 16% of the animals died from the chemical prior to tumor development. Levi *et al.* (5) fed 0.05% of 2-FAA in the diet for 4–12 wk and produced urinary bladder hyperplasia but no tumors or deaths. However, Wood (6) using IF and R3 mouse strains reported high death rates in both strains at 39 wk prior to the development of a significant number of bladder tumors. Moreover, Armstrong and Bonser (7) employing CBA, IF, R3, White Label, and Strong strains of mice and administering 6 mg/mouse/wk observed differences in tumor incidence between strains and sexes. They concluded that such differences may have been related to the fact that some strains and the male mouse were less resistant to the toxic effects of 2-FAA. Inasmuch as there was no data on the subchronic (90 day) toxicity of 2-FAA and because such information was essential for the design of life-time carcinogenic study, we have investigated the subchronic toxicity of the chemical in male and female mice of the C57BL/6j and BALB/cStCr1BR strains.

Methods. One thousand two-hundred mice of each strain, equally divided between the sexes, were used in each experiment. They were maintained under minimum disease conditions in quarters thermostatically controlled to 72 ± 5°F with free access to food and water. The mice weighed from 17 to 31 g. Both strains and sexes were randomized as to animals, cages, and racks. The groups were composed of 80, 160, 240, and 240 animals equally divided by sex and fed 2-FAA at concentrations of 0.05, 0.025, 0.01, 0.005, and 0.001%, respectively. Dietary content of 2-FAA in Purina meal was determined by the methods of Bowman and King (8). There were 240 controls. The animals were introduced into the experiments at the rate of 120 per week for 10 wk and removed at the same rate after 90 days. All animals were weighed weekly as individuals on an electronic balance. Observations of their physical condition were made at the same time and data were stored in a Modular Computer Systems III/5 minicomputer. All animals were necropsied and the following tissues examined histopathologically after staining with hematoxylon–eosin: lungs, heart, aorta, thymus, skeletal muscle, kidney, adrenal, liver, spleen, gall bladder, pancreas, cerebrum, cerebellum, spinal cord, stomach, colon, ileum, duodenum, 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 unidentified mass.

Results. No observable signs of toxicity were produced in either strain or sex of mice fed 2-FAA. However, 13 mice of the C57BL/6

and 10 mice of the BALB/c strains were lost from causes unrelated to feeding the chemical. At the 0.05% concentration of 2-FAA there was a slight nonsignificant retardation in weight gain throughout both 90-day feeding experiments. Furthermore, this effect was sex related; the C57BL/6 male mice lost more weight than the female, but this effect was reversed in the BALB/c strain.

A dose dependent urinary bladder epithelial hyperplasia was observed in both strains and sexes of mice fed 2-FAA at dietary levels of 0.001–0.01 per for 90 days. The 0.01–0.05 levels gave a 100% response. The hyperplastic responses were almost identical in both experiments (Fig. 1). In both experiments the response was greater in males than in the females. The various degrees of response are shown in Fig. 2. A metaplasia of the urinary bladder epithelium was observed in both sexes of the BALB/c strain with male response double that of the female. It was dose related because the incidence increased with an increasing concentration of 2-FAA in the diet. One BALB/c male mouse developed urinary bladder carcinoma after 90 days of consuming 0.05% 2-FAA (Fig. 3D). Another male of this strain developed a similar urinary bladder carcinoma after consuming 0.025% 2-FAA for 90 days.

Urinary bladder concretions were observed in the males of both strains in all groups; including the controls, and were not dose related. Adrenal spindle cell hyperplasia was observed in both males and females of both strains and was more pronounced in the latter. It also was not dose related.

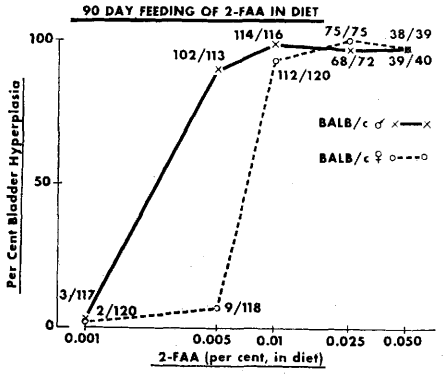

FIG. 1. Total urinary bladder hyperplasia in BALB/c mice as a function of 2-FAA concentration in the diet. Numbers signify the number of positives over the total number of animals for each point. The C57BL/6 strain showed a similar response. Note the greater sensitivity of the males to 2-FAA.

Discussion. The present investigation of 90 day subchronic toxicity in C57BL/6 and BALB/c strain mice of both sexes indicated no toxicity from feeding 2-FAA at concentrations covering the range of 0.001–0.05%. This confirms the results reported by Levi *et al.* (5), but not those obtained by others (3, 4, 6) who observed deaths attributable to the chemical. Such differences in toxicity are probably related to the different strains of mice used by various investigators, inasmuch as the dietary levels of 2-FAA were within the range we employed. Mouse urinary bladder epithelial hyperplasia produced by feeding 2-FAA at concentrations varying from 0.03 to 0.05% had been reported previously by others (1, 3, 5–7, 9). We have confirmed these earlier studies and, in addition, have demonstrated a dose-response relationship between the concentration of 2-FAA fed and the number of animals showing urinary bladder epithelial hyperplasia. Moreover, we have shown that carcinoma of this organ occurred in BALB/c male mice receiving the 0.025 and 0.05 concentrations of 2-FAA. Furthermore, it has been shown that both sexes of this strain developed a squamous metaplasia of urinary bladder epithelium after consuming concentrations of 0.005–0.05% of 2-FAA for 90 days.

Several sex related responses were also observed: squamous metaplasia greater in BALB/c males; urinary bladder concretions in males of both strains; adrenal spindle cell hyperplasia greater in females of both strains; urinary bladder hyperplasia greater in males of both strains; weight retardation greater in C57BL/6 males and BALB/c females. Such observations support the report of Armstrong and Bonser (7) regarding differences in response related to both strain and sex of the mouse used. The bladder concretions found confirm the observations reported by Haley *et al.* (10) for acute exposure to 2-FAA.

Summary. Subchronic (90 day) toxicity of *N*-2-fluorenylacetamide has been investigated in male and female C57BL/6j and BALB/cStrCr1BR strain mice. No signs of toxicity

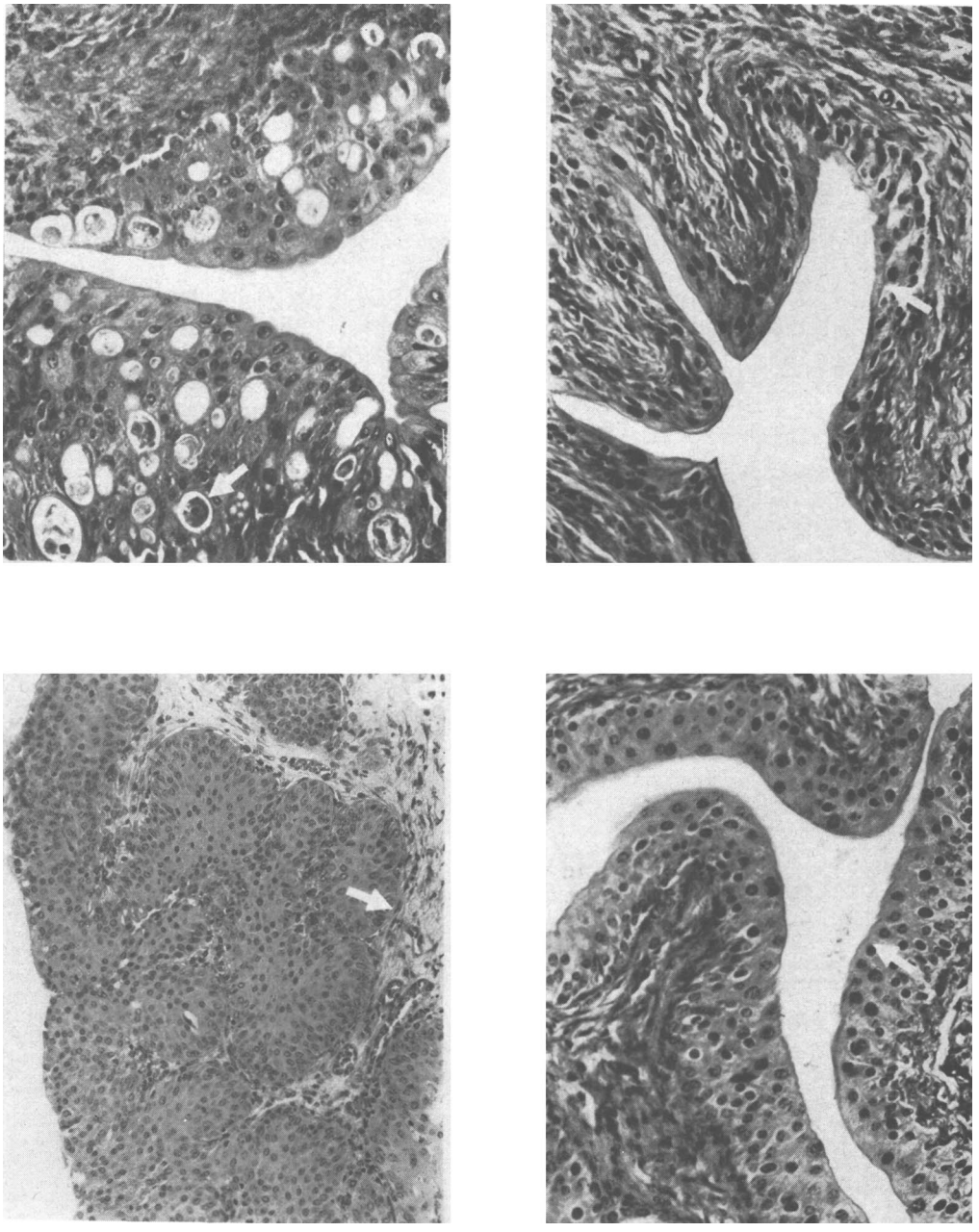

FIG. 2. 2-FAA induced changes in the urinary bladder epithelium of BALB/c male mice, Hand E stain, × 400.

A.—Normal transitional epithelium of mouse urinary bladder, arrow indicates three cell layers in thickness.

B.—Hyperplasia, arrow indicates five cell layers in thickness with superficial layer of large foamy cells wholly or partially absent.

C.—Squamous metaphasia of urinary bladder epithelium (arrow).

D.—Carcinoma *"in situ"* of the urinary bladder epithelium, arrow indicates intact basement membrane.

were observed with dietary levels ranging from 0.001–0.05% 2-FAA. A dose dependent urinary bladder epithelial hyperplasia was seen in both strains with the males being more susceptible than the females. Squamous metaplasia of the urinary bladder was observed in the BALB/c mice with the male response being double that of the female. One urinary bladder carcinoma was found in a BALB/c male at the 0.025 and 0.05% 2-FAA levels. Adrenal gland spindle cell hyperplasia was more pronounced in the females of both strains. Males of both strains had urinary bladder concretions. No other significant histopathological changes were observed.

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Volume **145**, No. 3 (1974), in the article, "Composition of Myelin Proteins in Murine Genetic Myelin Dysgenesis: The Quaking Mutant," by Mary J. Druse and Edward L. Hogan, pp. 747-751:

Page 750, line 20 should read: of this high molecular weight protein.....