Influence of Low Dose of 2-Acetylaminofluorene on Liver Tumorigenesis in Mice.* (25043)

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Production of hepatomas and other tumors in mice with 2-acetylaminofluorene (AAF) has been observed by Armstrong and Bonser (1,2) and other investigators(4-6). In these experiments, AAF was fed to adult mice for periods ranging from 25 to 77 wks. It has been observed that tumors are produced more readily in younger than in older mice following exposure to carcinogenic hydrocarbons (5-7) or urethan(8), suggesting that administration of AAF to suckling mice might prove especially effective in carcinogenesis. This was done in our experiment, the result being that hepatoma induction was enhanced in some mice.

Materials and methods. Albino mice, 7 to 8 days of age, and related to strain A/He were fed 3 times weekly by stomach tube with 0.05 ml of methocel-Aerosol OT (dioctyl ester of sodium sulfosuccinic acid) suspension containing 1.4% AAF, for total of 10 feedings. Control suckling mice from different litters were similarly fed a suspension containing no AAF. Following each feeding, the mice were returned to original cages. At about 5 weeks of age, they were weaned and segregated according to sex. During treatment, the mothers were maintained on Purina Lab Chow pellets and continuous supply of tap water. Weanlings received the same diet. The experiment was terminated when the young reached one year of age. These mice, as well as those which died earlier, were autopsied and various organs examined for macroscopic tumors. Tissues were excised and fixed in Tellyesniczky's fluid (20 parts 70% ethyl alcohol, 2 parts formalin, 1 part glacial acetic). Tumors recorded were those visible grossly. However, tumor diagnosis in every instance was based on microscopic examination.

Results. (Table I). Hepatomas were observed frequently among male mice treated with AAF in Group 1. Frequently these tumors were multiple, some mice bearing as many as 18 distinct, elevated nodules/liver. Many hepatomas measured 5 to 10 mm in diameter. Both grossly and microscopically, the hepatomas were similar to those described previously by Andervont and Dunn(9). Although 60% of male mice exposed to AAF bore hepatomas (Group 1), the females proved resistant, only one out of 86 (1%) bearing a hepatoma during comparable period (Group 2). A similar sex difference in incidence of induced hepatomas, many tumors among males but none in females, was reported by Leathem(10) following exposure of mice to semi-purified diet containing AAF. A low incidence or complete lack of hepatomas was observed among male and female mice treated with methocel-Aerosol OT suspension alone (Groups 3, 4) or which received no treatment (Groups 5, 6). While exposure to AAF led to increased incidence of hepatomas in Group 1, this carcinogen appeared to have no significant effect on induction of pulmonary tumors. Thus, essentially the same incidence of pulmonary adenomas was observed whether or not the mice received AAF (Groups 1-6).

AAF was administered to mice during 22 days. This brief yet effective period of treatment is considerably less than those periods employed by others(3-6). Also, in the latter experiments(3-6), the compound was given 3 times weekly or continuously, for 25-77 wks. Actual total intake of AAF/mouse is not indicated. However, from concentrations employed and duration of treatment, it is probable that the amounts were many times greater than the 7 mg total dose which each mouse received in present investigation. This 7 mg dose represents to date the smallest quantity of AAF which increased significantly the incidence of hepatomas in mice. However, since this dosage and the 22-day period

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TABLE I. Tumors Induced in Albino Mice with 2-Acetylaminofluorene (AAF), 6 Groups.

Treatment	Total No. mice*	Sex	Observation period (avg days)	Mice with tumors: Hepatomas	Mice with tumors: Pulmonary adenomas
AAF - suspension	62	♂	365	37	17
Idem	86	♀	362	1	27
Suspension only	27	♂	364	1	8
Idem	25	♀	366	0	9
None	29	♂	365	1	10
"	28	♀	365	0	9

* Survivors at time first hepatoma was observed.

of treatment were selected arbitrarily, it is possible that an even smaller amount of AAF, and given over a shorter time, also may be effective. These results demonstrate that 7-8 day old male mouse is highly susceptible to hepatocarcinogenic action of orally administered AAF.

Summary. Administration by stomach tube of a suspension containing 2-acetylaminofluorene to suckling albino mice was followed by development of high incidence of hepatomas in males (60%) but not in females (1%). A similarly low incidence of hepatomas (0%-4%) was observed in untreated mice from same strain or mice treated with suspension alone. The low dose of 2-acetylaminofluorene represents smallest quantity of this compound effective to date in liver tumorigenesis. Also, results indicate that suckling male mouse is especially susceptible to hepatoma induction with 2-acetylaminofluorene.

1. Armstrong, E. C., Bonser, G. M., *J. Path. and Bact.*, 1944, v56, 507.
2. ———, *ibid.*, 1947, v59, 19.
3. Wilson, R. H., DeEds, F., Cox, A. J., Jr., *Cancer Res.*, 1947, v7, 444.
4. Foulds, L., *Brit. J. Cancer,* 1947, v1, 172.
5. Law, L. W., *Science,* 1940, v91, 96.
6. Rous, P., Smith, W. E., *J. Exp. Med.,* 1945, v81, 597.
7. Greene, H. S. N., *Science,* 1945, v101, 644.
8. Rogers, S., *J. Exp. Med.,* 1951, v93, 427.
9. Andervont, H. B., Dunn, T. B., *J. Nat. Cancer Inst.,* 1952, v13, 455.
10. Leathem, J. H., *ibid.,* 1955, v15, 1547.

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Transamination in *Leptospira biflexa.** (25044)

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Few studies on metabolism of leptospirae have been published. Chang(1) reported that sugars were not utilized by *Leptospira icterohemorrhagiae.* Marshall(2) and Helprin and Hiatt(3) studied the effect of serum or fatty acids on oxygen utilization by whole cells of *L. icterohemorrhagiae.* Fulton and Spooner (4) using the same organism analyzed the growth medium for chemical changes and found that small quantities of volatile fatty acids accumulated. An investigation of amino acid metabolism in the leptospirae was undertaken since sugars are not utilized(1). Enzymatic transamination, firmly established in animal tissue and other bacteria(5), was performed with whole cells and cell-free extracts of *L. biflexa.*

Materials and methods. The culture of *L. biflexa* was obtained from A. D. Alexander, Walter Reed Army Medical Center. Leptospirae were grown in 4 l quantities in medium of Cox(6) and harvested by centrifugation

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