of C3H spleen cells. In addition there was an increased incidence of death in the latter strain combination. The early death of adrenalectomized (C3H × C57Bl/1)F1 recipients of C3H parental strain spleen cells was prevented by the administration of cortisone acetate.

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Carcinogenic Effect of N-Hydroxy-N-2-Fluorenylacetamide, 2′,4′-Dimethylacetanilide, and 2′,4′,6′-Trimethylacetanilide on Liver in Suckling Mice. (31065)

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Adult mice are resistant to liver tumorigenesis when exposed to carcinogenic hydrocarbons or urethan despite the fact that treatment with these agents will produce or enhance tumorigenesis in a variety of other organs(1,2). On the other hand, the liver is highly susceptible to the development of hepatomas when these agents or N-2-fluorenylacetamide (FAA, 2-acetylaminofluorene) are administered orally during the suckling period (3-6). In contrast, adult mice are much less prone to hepatotumorigenesis with FAA(7,8). In the present experiment, advantage was taken of the sensitivity of suckling mice to liver tumorigenesis by testing the aromatic compounds 2′,4′-dimethylacetanilide, and 2′,4′,6′-trimethylacetanilide. These chemicals are suspected of possessing weak tumorigenic activity(9,10). In addition, the well known liver carcinogen N-hydroxy-N-2-fluorenylacetamide (N-OH-FAA)(11), was included as a positive control. This compound is considered to be a proximate carcinogen derived from FAA(12). It already had been demonstrated that as little as 7 mg of FAA was sufficient to induce a high yield of hepatomas when administered orally to suckling mice(4). Since FAA is less potent than N-OH-FAA in liver tumorigenesis(12), it seemed of interest to determine the relative activity of the latter carcinogen in suckling mice when a moderate or low dose was employed.

Materials and methods. C57BL6/A_JF_1 hybrid mice of both sexes were employed. Litters born in this laboratory were divided at random among the different groups. The infants were 1 week old at the start of treatment and were weaned and separated by sex at 4-5 weeks. Animals were housed in stainless steel cages, in air-conditioned quarters, and were fed a diet of Wayne Lab Blox Chow and water *ad libitum*. The mice were examined daily and those found dead autopsied as quickly as possible. Cases of advanced postmortem changes or cannibalism were discarded and do not appear in the data. Approximately the same number of mice (Table I) were lost in each group. Surviving mice were killed at the end of the experiment and grossly visible tumors or lesions recorded and then excised for tissue processing. Tumor diagnoses were confirmed by histologic examination. The data include only those surface hepatomas that measured at least 1 mm in diameter.

All mice were treated by stomach tube, receiving 0.05 ml of inoculum per dose. The tube was introduced directly into the esopha-

TABLE I. Induction of Hepatomas in B6AF$_1$ Hybrid Suckling Mice.

Group No.	Mice: At start No.	Mice: Effective total* No.	Sex	Treatment†	Total dosage (mg)	Mice with hepatomas: Incidence (%)	Tumors per mouse‡ (avg No.)	Largest tumor diameter§ (avg mm)	Mean age at death (wk)
1	37	27	♂	.1% methocel-	—	0	—	—	73
	44	36	♀	Aerosol O.T.—10×		0	—	—	75
2	48	40	♂	.5% N-OH-FAA—10×	2.5	45	2.4	4.0	61
	49	39	♀			0	—	—	62
3	34	24	♂	.5% N-OH-FAA—2×	.5	50	1.0	3.9	69
4	47	37	♂	2% acetanilide—10×	10.0	0	—	—	70
	49	41	♀			0	—	—	72
5	42	33	♂	.5% 2′,4′-dimethyl-	2.5	6	.06	3mm; 10mm	72
	34	23	♀	acetanilide—10×		0	—	—	73
6	44	35	♂	1% 2′,4′,6′-trimethyl-	5.0	3	.03	3mm	73
	41	35	♀	acetanilide—10×		0	—	—	72

* Mice autopsied and alive at 50 weeks when first hepatoma was observed.
† Agents suspended in 0.1% methocel-Aerosol O.T. and given orally 3 times weekly except for Group 3 which received agent twice on same day.
‡ Based on total number of mice in group including non-tumor bearers.
§ Calculated for mice with hepatomas only; for males in Groups 5 and 6, individual measurements of hepatomas are given.

gus with no evidence of regurgitation being noted during or immediately after treatment. Suckling mice were returned to their respective cages soon thereafter. Except for one group which received 2 treatments on the same day, the others were treated 3 times weekly. The experiment included 6 groups given the following treatments: Group 1: 0.1% methocel-Aerosol O.T. alone—10×, Group 2: 0.5% N-OH-FAA*—10×, Group 3: 0.5% N-OH-FAA*—2×, one treatment at 9:00 a.m.; the other at 3:00 p.m. on the same day, Group 4: 2% acetanilide* in 0.1% methocel-Aerosol O.T.—10×, Group 5: 0.5% 2′4′-dimethylacetanilide*—10×, Group 6: 1% 2′4′6′-trimethylacetanilide*—10×.

A preliminary series of tests was conducted with the above agents including the negative control acetanilide to determine the maximum tolerated concentration for each. Concentrations higher than those employed proved toxic.

On the basis of previous results in the development of hepatomas in mice with FAA (4), it was considered sufficient to terminate Group 2 after a mean observation period of 60 to 61 weeks. The mean observation period was extended for the other experimental groups.

Results. The first hepatoma was observed in a male mouse in Group 2 autopsied at 50 weeks. Thus, the effective totals for each group in Table I include only those mice surviving an equivalent period of time. Males and females treated with vehicle only (Group 1) had no hepatomas whereas males treated 10 or 2 times with N-OH-FAA (Groups 2 and 3), exhibited a high incidence of these tumors. Average number of hepatomas per mouse and relative tumor diameter for these groups are given in Table I. Based on the latter 2 parameters, together with the fact that the mean age at death was less for animals given the higher dose of

* N-OH-FAA was synthesized by the method of Miller et al (12); acetanilide and 2′,4′-dimethylacetanilide were obtained from Eastman Organic Chemicals, Rochester, N. Y.; 2′,4′,6′-trimethylacetanilide was prepared by acetylation of the trimethylaniline which was procured from Aldrich Chemical Company, Milwaukee, Wis.

N-OH-FAA, it is apparent that a greater tumorigenic effect was produced in the liver of the male mice of Group 2 than in those given the lower dose of this carcinogen (Group 3). The females in Group 2 had no hepatomas in marked contrast to the high susceptibility demonstrated for the males. No tumors were observed in the acetanilide-treated controls (Group 4). The mice in Groups 5 and 6 treated with dimethyl- or trimethylacetanilide bore only an occasional hepatoma after an observation period comparable to that used with N-OH-FAA (Group 3). Grossly and microscopically, the hepatomas resembled those produced by FAA(4) and the spontaneous and induced hepatomas described in detail by Andervont and Dunn (13).

While an occasional tumor of the lungs and lymphoid tissues was noted, the number was well within normal limits for untreated $B6AF_1$ hybrid mice or hybrids treated with vehicle only and warrant no added comment. Occasional clear cysts varying in size from 2 to 5 mm were seen in the livers of some of the mice treated with N-OH-FAA. However, no hemangiomas or blood cysts were present.

Discussion. In a previous study, oral administration of FAA to immature albino mice beginning at 1 week of age resulted in a high incidence of hepatomas among the males whereas immature females were much less responsive(4). The total dose of 7 mg represented the smallest quantity of FAA effective in hepatotumorigenesis in mice. In the present investigation, one group was exposed to a total dose of 2.5 mg of N-OH-FAA and observed for 60 weeks (Group 2). Another group received a total of 0.5 mg of N-OH-FAA and was observed for 68 weeks (Group 3). Compared to this, the observation period was 52 weeks in the case of FAA(4). Also, the F_1 hybrid was unrelated to the strain used earlier. Thus, a direct comparison of results between the two experiments is not justified. However, while no hepatomas were found at the observation period selected in both the solvent and acetanilide controls (Groups 1 and 4), administration of N-OH-FAA resulted in a high tumorigenic response. Thus, as little as 0.5 mg given in one day was sufficient to produce hepatomas in 50% of the mice, and it is probable that an even smaller dose also would have been effective especially if a strain more susceptible to liver tumorigenesis had been employed, e.g., strain C3H. These findings demonstrate that N-OH-FAA is an extremely potent liver carcinogen when fed to immature male mice, but is not in females. This is in line with the results of Miller *et al*(11) who fed N-OH-FAA to female STS mice continuously for 10-14 months before obtaining a hepatocarcinogenic response. Also, this represents the lowest dose of N-OH-FAA or any other aromatic amine derivative producing liver tumors in experimental laboratory animals.

Lindstrom *et al*(10) reported a hepatotoxic effect when large amounts of 2,4-xylidine were fed to rats, while Morris and Wagner(9) found trimethylaniline to be weakly carcinogenic when fed to rats continuously for 18 months. In the present study, an occasional hepatoma was observed among male mice treated with 2′4′-dimethylacetanilide or 2′4′6′-trimethylacetanilide (Groups 5, 6). If the latter two compounds are carcinogenic in mice, it would appear that they are weakly so, at best. However, since the mice were exposed to these agents for only about 3 weeks, more definitive results could be obtained using a more prolonged period of treatment and a strain more susceptible to liver tumorigenesis. In addition, more extensive exposure to the agents would favor their metabolic conversion to the active N-hydroxy derivatives(12).

Summary. Suckling $C57BL_6/A_JF_1$ hybrid mice were fed the following compounds suspended in methocel-Aerosol O.T. by stomach tube beginning at 1 week of age and continuing for approximately 3 weeks: N-hydroxy-N-2-fluorenylacetamide (N-OH-FAA), 2′4′-dimethylacetanilide, and 2′4′6′-trimethylacetanilide. Acetanilide and the vehicle were similarly administered as controls. Approximately 50% of the male mice, but none of the females, developed hepatomas in groups fed a total of 2.5 mg over several weeks, or 0.5 mg of N-OH-FAA in one day, average number of tumors per liver being greater in the higher dosage group. An occasional hepatoma was

observed among males exposed to the other 2 test compounds while none appeared in either of the control groups. The data demonstrate that a minute amount of N-OH-FAA had a potent tumorigenic effect in the liver when tested in infant male mice.

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Insulin Hypoglycemia Enhanced by Beta Adrenergic Blockade.* (31066)

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"Inderal" (AY 64043,† I.C.I.‡ 45,520, propranolol) is 1-isopropylamino-3-(1naphthyloxy)-2-propranol hydrochloride and is an adrenergic beta receptor antagonist now undergoing clinical trial (1). In connection with work on triglyceride transport in the rat(2) we had occasion to administer "Inderal" together with insulin. The ensuing hypoglycemia was much more severe than when either agent was administered alone. "Inderal" is but one of a number of compounds of similar pharmacological properties now coming into use and which may well be administered to patients also taking insulin. Our data are reported in the hope that it may alert physicians to the possibility of an undesirably low hypoglycemia following production of beta adrenergic blockade in diabetic patients.

Male rats of the Long-Evans strain, weighing about 300 g, were starved for 14 hours after which they received 3 ml or corn oil *per os*. They then were divided into 6 groups. Group I (10 rats) served as controls and were untreated. Group II (10 rats) received 50 mg of propranol *per os*. Group III (5 rats received 0.5 unit of regular insulin intramuscularly. Group IV (5 rats) received 0.5 unit of insulin and 50 mg of propranolol. Group V received 1.0 unit of regular insulin. Group VI received 1.0 unit of insulin and 50 mg of propranolol *per os*. The rats of all groups were bled 6 hours after treatment. Some of these same rats also were bled either 1 or 3 hours after treatment. Glucose levels in heparinized plasma from the blood samples were determined using glucose oxidase (3).

The results (Table I) indicated that although administration of propranolol alone did not significantly alter the plasma glucose level of the rat, when it was administered together with insulin, it markedly intensified the hypoglycemic effect of the latter. Indeed when the blocking agent was administered with 1 unit of insulin, the average plasma

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