Potentiation of Benzo[*a*]pyrene Tumorigenesis in the Progeny of Female Hamsters Injected with Freund's Incomplete Adjuvant[1] (35600)

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The injection of chemical carcinogens during late pregnancy has been shown to cause tumors in the progeny. In the case of urethane (1) and diethylnitrosamine (2) the tumors in the progeny were identical in type and location with those produced by these agents in adult animals. Maternal injection of benzo[*a*]pyrene (BP) has recently been found (3) to increase the incidence of pulmonary tumors and croton oil-promoted skin papillomas in the offspring. These effects are usually ascribed to transplacental passage of the agents or their metabolites. However, Tomatis (4) found that the injection of dimethylbenzanthracene into pregnant mice increased the (late) incidence of several different "spontaneous" tumors in both the F1 and F2 descendants. In the latter, transplacental or lacteal transmission of a carcinogen seemed unlikely. Similarly, Shay *et al.* (5) gave methylcholanthrene for 2 months by stomach tube to female rats before pregnancy; both F1 *and F2* offspring had a significantly increased occurrence of a wide variety of benign and malignant tumors. We report here that BP-induced sarcomas in the progeny of hamsters injected during pregnancy with Freund's incomplete adjuvant appeared earlier and grew more rapidly than they did in the progeny of untreated hamsters. This is of particular interest because the mineral oil and emulsifier components of this adjuvant have been carefully screened, in an attempt to exclude polycyclic hydrocarbons and other potential carcinogens (6).

Materials and Methods. Twenty random-bred hamsters, synchronously exposed to pregnancy, were obtained from the Lakeview Hamster Colony, Newfield, N.J. All animals were given Purina laboratory chow with water *ad libitum*. On day 2 of (presumed) pregnancy, 12 animals were injected sc in the right lower back with 1.0 ml of Freund's incomplete adjuvant (Difco 0639-Bayol F 85% and arlacel A 15%) in which 0.5 ml of Eagle's minimum essential medium was emulsified. On day 9, the culture medium was similarly injected, but the adjuvant was omitted. The remaining 8 hamsters were not treated in any way. All 20 of the animals proved to be pregnant, and delivered on day 16. (One untreated mother died soon after delivery).

Progeny. When 2 days old, all offspring of both groups appeared healthy and of uniform size. At the age of 21 days, male and female offspring were caged separately in groups of 3 (or occasionally 2) and given the first of 3 weekly sc injections of BP in the right mid-dorsal region. Each injection consisted of 3 mg of BP (Nutritional Biochemicals) dissolved in 0.25 ml of high quality olive oil. At this time, there were 26 offspring of untreated mothers and 54 offspring of adjuvant-injected mothers, of which 24 and 50, respectively, survived to the date when the first tumor appeared. (Autopsy of the 6 animals that died did not disclose the cause of death). An additional animal in the maternally untreated group died during the period of tumor development; tumor tissue was not found. (This hamster was excluded from the data). Twenty days after the last BP injection, the average weight of the maternally injected group was found to be almost identical to that noted in the control group.

Tumor incidence and development were recorded at appropriate intervals, tumor size being represented by two-dimensional drawings. At 119 days of age (98 days after the first BP injection) a few animals with

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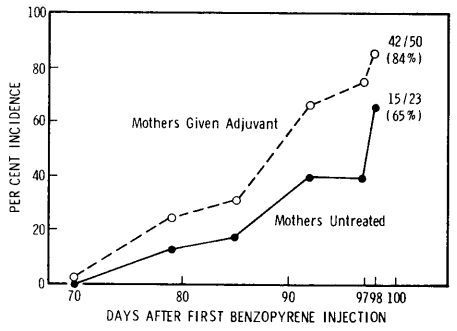

FIG. 1. Cumulative percentage incidence of BP-induced tumors in the progeny of adjuvant-treated and untreated mothers. The points on the graph were obtained by inspection and/or palpation, except for the final points, which show the incidence found at autopsy when the experiment was terminated.

large tumors in the maternally treated group were in critical condition and the experiment was terminated. All animals were killed with ether; their tumors were weighed, and the thoracic and abdominal organs were inspected (without significant findings except for splenomegaly).

Results. Figure 1 shows an accelerated incidence of tumors in the progeny of adjuvant-treated, as compared with untreated, mothers. The sharply increased incidence in the control group between day 97 and the termination date (day 98) reflects the postmortem finding of 6 tumors too small to be seen or palpated (<0.1 g). In both the control and the experimental group, tumors appeared earlier (and at termination were much larger, Table I) in female as compared to male offspring. Since the male to female ratio was 28/22 in the experimental group and 12/11 in the control group, it is clear that the difference in incidence between the two groups would have been even greater if these ratios had been identical.

The data in Table I show that, in both the control and the experimental group, tumors found at autopsy in female hamsters were 2.9 and 2.5 times as heavy, respectively, as those found in males. The values also show that tumors found in the maternally treated group were 3.5 to 4.5 times as heavy as those in the control group. As shown in the last column, this was true for males, females, and males and females, combined. The median tumor weights also correlate well with the average tumor weights, for M, F, and M + F, in both the control and the experimental groups (Table I, Col. 5).

Discussion. The mechanism of potentiation of BP tumorigenesis which we observed in the offspring of adjuvant-treated mothers is unknown. Freund's adjuvants, the mineral oil components of which are complex and uncertain (6) belong to a heterogeneous group of agents which, when given alone, nonspecifically stimulate both humoral and cellular antibodies. Some of these agents, particularly mycobacteria and their extracts, have been found to suppress tumor growth (7–9) to such an extent that optimism for their clinical use has arisen (10).

On the other hand, it has been shown under various experimental conditions that Freund's complete adjuvant, which contains

TABLE I. Effect of Maternal Adjuvant Injection on BP Tumorigenesis in the Offspring.

Maternal treatment	No. and sex of hamsters	Incidence (%)	Av wt of tumors (g)	Median wt	Range (g)	Treated/ control
Adjuvant	28 Male	75	3.5[a]	0.6	<0.1–45	4.4[b]
	22 Female	94	8.6	4.0	0.3–36	3.8
	50 M + F	84	6.1	1.5	<0.1–45	3.6
None	12 Male	50	0.8	0.2	<0.1– 2.7	
	11 Female	82	2.3	0.6	<0.1–11	
	23 M + F	65	1.7	0.3	<0.1–11	

[a] Average weights of tumors found postmortem in each group, excluding animals without tumors.

[b] Average tumor weights in offspring of adjuvant-treated mothers divided by average tumor weights in offspring of untreated mothers.

mycobacteria, potentiates or enhances the development of several types of tumors (9, 11, 12). Freund's *incomplete* adjuvant, given ip 3 and 4 weeks after neonatal injection of hamsters with SV40, markedly accelerated the development of SV40-induced tumors (13). These enhancing effects have usually been considered (12, 13) analogous to the classical phenomenon of immunologic enhancement (prevention by humoral antibody of the interaction between sensitized lymphocytes and the antigens on tumor cells) (14). A similar explanation could well be advanced for the findings reported here, since it is probable that the offspring received mineral oil adjuvant transplacentally from their mothers (3). (It should be noted that on the basis of sequential interplay between humoral and cell-associated antibodies, paradoxical and apparently contradictory results may be explained) (12).

Increased uptake of BP by stimulated reticuloendothelial cells may have contributed to the observed enhancement; however, it is well known that hamster fibroblasts in tissue culture are readily made sarcomatous by direct exposure to BP. Though the only known carcinogenic activity of Freund's incomplete adjuvant is the production of myeloma when injected ip in certain strains of mice (15) the possibility that this oil may have been cocarcinogenic with BP should also be considered.

In man, mineral oil and its emulsions are commonly ingested in large amounts for constipation, and applied to the skin and nasal passages for their soothing effect. In cases of paraffinoma of the lung, mineral oil has been found in the bronchial lymph nodes and in the spleen (16). The possible clinical significance of the observations reported here should perhaps be considered.

Summary. Sarcomas induced by benzo[*a*]pyrene in the mature offspring of hamsters injected sc on the second day of pregnancy with Freund's incomplete adjuvant appeared earlier and grew more rapidly than did similarly induced tumors in the offspring of untreated mothers. Though females were intrinsically more susceptible than males to BP tumorigenesis, the enhancing effect of the adjuvant was approximately equal in male and female offspring.

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