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Carcinogenic Response of the Syrian Golden Hamster Treated at Birth With 7,12-Dimethylbenz(a)anthracene.* (28736)

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High incidences of malignant lymphomas and lung adenomas and a few other tumors have been obtained in mice treated at birth with chemical carcinogens(1,2,3,4,5,6,7,8,9). It becomes apparent from some of these studies that 7,12-dimethylbenz(a)anthracene (DMBA) is particularly powerful in evoking malignant lymphomas in several strains of mice treated at birth.

The effects of DMBA, administered at birth in different species, are being studied in this laboratory. In Lewis rats, it gave rise to subcutaneous sarcomas at the site of injection(10).

The present paper describes the action of DMBA given subcutaneously to newborn Syrian golden hamsters. In adult Syrian golden hamsters subcutaneous administration of DMBA induced sarcomas(11), while cutaneous application resulted in the development of dermal melanocytomas(12).

Materials and methods. Syrian golden hamsters from a colony originally obtained from Abrams Small Stock Breeders, Chicago, Ill., and bred randomly in this laboratory since 1959, were used. Each litter was housed with its mother until it was weaned, and was then separated according to sex. The hamsters were housed in plastic cages on sterilized granular cellulose bedding in groups not exceeding 6 to a cage, fed Rockland diet in pellets and tap water *ad libitum*.

The carcinogen used was 7,12-dimethylbenz(a)anthracene (DMBA),† purified by chromatography on magnesia/Celite. It was dissolved in tri-n-caprylin (trioctanoin),† purified prior to use by vacuum distillation. The newborn animals were given a single subcutaneous injection in the interscapular region with a tuberculin syringe using a 30-gauge needle. Concentrations of DMBA were such that the desired dose was contained in 0.05 ml of tri-n-caprylin. Each DMBA treated animal was checked for a few seconds under a U.V. lamp to assure that no free DMBA had leaked on the skin or was left in the needle track. Those animals which showed fluorescent material on the skin were discarded. The injections were made less than 4 hours after birth of the ani-

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TABLE I. Carcinogenic Response of Golden Hamsters Injected at Birth with DMBA.

No. of survivors at weaning	Survival rate in weeks													No. of animals with: Melanotic tumor			Subcutaneous sarcoma			Malignant lymphoma			Other tumors
	10	20	30	40	50	60	70	80	90	100	110	120	130	140	No.	%	Latent period, wk	No.	%	Latent period, wk	No.	%	Latent period, wk	
100 μg DMBA inj into 60 hamsters at birth																								
23 ♀	23	20	14	10	6	3	—	—	—	—	—	—	—	—	21/111	91.3	17(11–52)	18/18	78.2	22(8–48)	1	—	42	a
17 ♂	16	12	4	2	2	1	—	—	—	—	—	—	—	—	15/46	88.2	14(10–38)	14/14	80.2	15(10–34)	1	—	36	b
Uninjected control																								
47 ♀	47	47	47	46	46	42	37	24	9	5	2	—	—	—	—	—	—	1	2.1	111	—	—	—	c
54 ♂	42	39	39	37	37	36	33	32	27	26	13	7	1	—	—	—	—	—	—	—	2	3.7	99,115	d

a. 1 breast adenocarcinoma
1 papilloma of esophagus
1 angiosarcoma of kidney

b. 1 hemangioma of lymph node
1 angiosarcoma of kidney

c. 1 papilloma of forestomach
1 adenoma of thyroid
1 pulmonary adenomatosis

d. 4 cortical adenomas
1 cholangioma
1 papilloma of forestomach
3 hemangiomas

mals. Three groups of newborn hamsters received a single injection of DMBA at the doses of 1000, 200 and 100 μg respectively. A control group of 60 newborn hamsters was given a single injection of 0.05 ml of tricaprylin and another control group of 47 females and 54 males was kept untreated. The number of injected animals and the number of survivors at weaning (5 weeks) are shown in Table I.

After weaning, experimental and control animals were carefully checked and weighed at weekly intervals and the skin and subcutaneous changes were recorded on graph paper. The animals were allowed to die spontaneously or were killed with ether when found in poor condition. A complete necropsy was performed on all animals. All organs were examined macroscopically and were fixed in 10% buffered formalin. Histological studies were performed routinely on the lymph nodes, thymus, spleen, kidney, sternal bone marrow, lungs and any additional organs which showed gross pathological changes. Sections from these tissues were stained with hematoxylin and eosin and with the addition of special methods, when necessary.

Results. The groups given 200 and 1000 μg of DMBA had a very high mortality rate before weaning time: only 2 females and 1 male survived out of 50 newborns in the 200 μg group and 3 females and 1 male out of 53 in the 1000 μg group. All these hamsters developed multiple melanotic tumors of the skin. Of 3 females in the 1000 μg group, each one had a papilloma of the skin, 2 had 2 subcutaneous sarcomas each, and one had a malignant lymphoma. The number of animals in these groups is too small for further elaboration of these results.

In the tricaprylin-treated control group, 29 hamsters out of 60 (14 ♀ and 15 ♂) survived at weaning time. This group is now in its 77th week of age with 10 ♀ and 10 ♂ surviving, and no tumors of any kind have been observed.

The groups treated with 100 μg of DMBA and the untreated controls are described in Table I. Survival rate after weaning in the

DMBA injected group appears significantly reduced.

The number, incidence and latent periods of the tumors are shown in Table I. Following injection with DMBA, a high incidence of melanotic tumors and of subcutaneous sarcomas and a few malignant lymphomas were obtained.

Macroscopically and cytologically, the melanotic tumors obtained in this experiment were similar to those previously induced with skin applications of DMBA in adult Syrian golden hamsters and they were diagnosed as dermal melanocytomas(12,13,14). In addition to the pigmented tumors, the skin of several carcinogen treated animals exhibited a large number of melanotic spots which were less than 2 mm in their largest diameter. Dermal melanocytomas and melanotic spots appeared spread on the backs of the animals.

The subcutaneous sarcomas were found mainly on the back, ranged from 10 to 40 mm in diameter and were usually necrotic in the center. Histologically these tumors were spindle cell sarcomas, some of them containing numerous giant cells. Mitoses were a rare feature. Metastases, mainly to the lungs and lymph nodes, occurred in 13 cases.

The 3 malignant lymphomas in the DMBA treated groups (one at 1000 μg and two at 100 μg of DMBA) appeared grossly as enlargements of the superficial lymph nodes, associated with hepatomegaly. In these 3 instances the thymus was not grossly recognizable. The cytological diagnosis revealed 2 lymphocytic and one histiocytic malignant lymphomas with microscopic involvement of lymph nodes, liver, kidneys and sternal bone marrow. These 3 lymphomas were found in a total of 47 DMBA treated hamsters at 15, 36 and 42 weeks of age, respectively. In the control group 2 malignant lymphomas of the histiocytic type were found in 101 animals at 99 and 115 weeks of age, respectively. In addition, a few other tumors were observed in treated and in control animals as shown in Table I.

Discussion. These results demonstrate that a single subcutaneous injection of DMBA at birth to newborn Syrian golden hamsters induced high incidences of two distinct types of tumors: subcutaneous sarcomas and dermal melanocytomas.

The sarcomas were induced also in adult hamsters by subcutaneous injection of DMBA(11), while the dermal melanocytomas were obtained also in adult hamsters by skin painting with DMBA(12).

Evaluation of the significance of the few malignant lymphomas observed is somewhat difficult. It should be noted that the latent periods of these tumors were much shorter in the treated groups than in the controls, and that 2 out of 3 lymphomas in the treated groups were of the lymphocytic type that was not seen in the controls.

Two main types of response have been revealed in species treated at birth with DMBA in our laboratory. In the first instance a remote effect of the carcinogen which was primarily seen in Swiss and AKR strains of mice, where the treatment gave rise to tumors distant from the site of application, namely lymphomas and lung adenomas(1,2, 8,9). In the case of Lewis rats(10) and Syrian golden hamsters, on the other hand, DMBA manifested essentially a local action, evoking topically a large number of neoplasms: sarcomas and dermal melanocytomas.

Summary. A single subcutaneous injection of 7,12-dimethylbenz(a)anthracene in tricaprylin was given in the interscapular region to newborn Syrian golden hamsters. Doses of 1000, 200 and 100 μg were administered and tricaprylin treated and untreated controls were also observed. The induction of subcutaneous sarcomas and of dermal melanocytomas was obtained. In addition, a slight acceleration of the appearance of malignant lymphomas was found.

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Renal Excretion of Tetracycline in the Aglomerular Toadfish, *Opsanus tau.** (28737)

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The renal excretion of tetracycline by the dog and man has been claimed to be affected by glomerular filtration alone(1,2). However, Sullivan and Fanelli(3) have adduced evidence in the dog that tritiated tetracycline is excreted by the renal tubules in addition to filtration at the glomeruli when the clearance is corrected for the amount of free drug in the plasma water relative to the creatinine clearance. The mechanism of renal excretion was attributed to nonionic diffusion(4). In the course of this work is was deemed of considerable interest to know whether or not tetracycline could be excreted by the aglomerular kidney which is characteristic of certain marine teleosts. The natural choice, therefore, fell upon the aglomerular toadfish *Opsanus tau* because of its ready availability and extreme hardiness in captivity. This fish is an excellent experimental animal in which to study the process of renal tubular excretion(5).

Methods. Thirty-two quantitative urine collection periods were secured from 20 toadfish weighing 155 to 440 g. All toadfish were obtained from Great South Bay, L. I., and housed in running sea water or in full strength artificial sea water. Tritiated tetracycline‡ (specific activity 5.1 μc/mg) in doses of 5 to 50 mg/kg was administered intramuscularly in a small volume of distilled water after obtaining control blood and urine samples. In most cases a control blood sample was omitted as it was found that radioactive background counts from control toadfish plasma were very uniform from animal to animal. Stress on the hemodynamics of the fish were thereby reduced to a minimum. After a period of 4 to 6 hours during which tetracycline became distributed through the body fluids a polyethylene catheter was inserted into the urinary papilla and secured in place. The free end of the catheter tubing was plugged and anchored to the anal fin by 2 loose ties. Urine collection periods were 5 to 18 hours in duration. A blood sample was secured at the end of the urine collection period by puncture of the caudal vessel. Under these conditions the rate at which the plasma concentration of tetracycline falls is so slow that a blood sample drawn at the end of the period suffices for calculation of urine

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