TABLE III. Hemagglutination of Fresh or Stabilized Bovine RBC by Reovirus Type 3 Strains.

Strain	Hemagglutination titer: Fresh RBC	Stabilized RBC	Ratio of titers, fresh/stabilized bovine RBC
Calf 151J	32	16	2
" 808	32	16	2
Dearing	256	64	4
Jones	128	16	8
Nelson	64	8	8
Calf 376H	256	16	16

filtrate(1), and because the receptors on human erythrocytes for all types of reovirus are reportedly resistant to the action of RDE (4), the interaction of several strains of reovirus 3 with human erythrocytes was investigated. Treatment of human erythrocytes for 1 hour at 37° with a *V. cholerae* filtrate had no effect on the weak agglutination of human erythrocytes by Dearing, Dearing variant, BVA6, and Abney strains, or on the stronger agglutination by calf 376H strain. Such treatment rendered bovine erythrocytes inagglutinable by reovirus 3.

Discussion. The present evidence indicates that the ability to agglutinate bovine erythrocytes is a general characteristic of type 3 reovirus, and that reovirus types 1 and 2 lack this characteristic. In the interaction of type 3 reovirus with bovine and sheep erythrocytes, neuraminic acid-containing receptors of the erythrocytes are involved(1). Furthermore, such receptor substances are also involved in the interaction of reovirus 3 with ovomucin and serum inhibitors. In contrast, there is evidence that reovirus 1 and 2 do not react with neuraminic acid-containing receptors(4,5,6,7). It appears also that such receptors do not play an important role in the agglutination of human erythrocytes by reovirus 3. With all 3 types, intact sulfhydryl groups on the virus, or disulfide linkages are involved in the hemagglutinating activity as well as infectivity(1,8,9).

Summary. 1. Reovirus type 3 strains, originally isolated from different animal species, all agglutinate bovine erythrocytes; only some agglutinate human erythrocytes. 2. None of the strains of reovirus types 1 and 2 agglutinate bovine erythrocytes; they all agglutinate human erythrocytes.

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Accelerated Development of Malignant Lymphomas in AKR Mice Injected at Birth with 7, 12-Dimethylbenz(a)Anthracene.* (27680)

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AK strain mice have a high spontaneous incidence of malignant lymphoma(1). A viral component has been demonstrated in these lymphomas, which can be transmitted by cell-free extracts to newborn C_3H mice (2), and virus-like particles have been observed with the electron microscope in lymphoid organs of this strain(3,4,5). The development of malignant lymphomas can be accelerated by injection of a cell-free filtrate of AK mouse spontaneous lymphoma into newborn AK mice(6,7).

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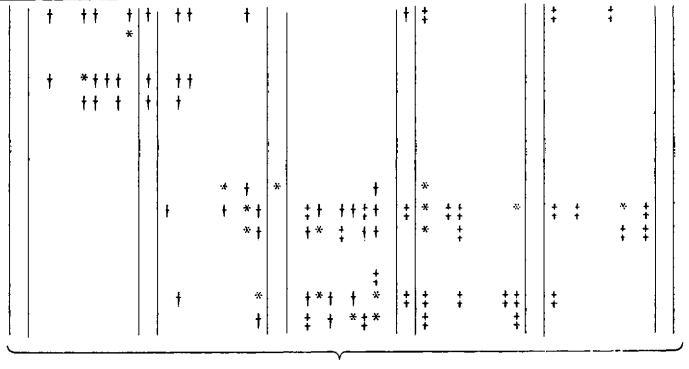
TABLE I. Distribution of Tumors in AKR Mice Treated at Birth with 100 μg DMBA and Untreated.

Treatment	No. of surv. at weaning	Survivors at weeks 10	20	30	40	50	60	Animals with mal. lymphomas No.	%	No. animals with other neoplasms	Age at necropsy (wk)
100 μg DMBA	21 ♀	18	11	5	4	2	—	13	76.4	6[a]	
	22 ♂	21	13	5	—	—	—	13	61.9	6[b]	
Untreated controls	46 ♀	46	46	32	15	6	—	35	77.7	—	
	41 ♂	41	40	32	12	2	—	22	55.0	2[c]	

[a] 3 with lung adenomas at the 43, 51 and 55th wk.
1 with hemangioma of ovary at 22nd wk.
1 with subcutaneous sarcoma at 13th wk.
1 with subcutaneous sarcoma at 25th and with a granulosa cell tumor of ovary at 29th wk.

[b] 2 with lung adenomas at 15th and 22nd wk.
3 with subcutaneous sarcomas at 18th, 19th and 23rd wk.
1 with subcutaneous sarcoma at 17th and with a squamous cell carcinoma of skin at 17th wk.

[c] 1 with anal hemangioma at 28th wk.
1 with malignant abdominal tumor, involving the spleen at 34th wk.

* Animal with mal. lymphoma unclassifiable.
† " " " " stem-cell type.
‡ " " " " lymphocytic type.

An acceleration of the development of malignant lymphomas in this strain can be also obtained using chemical carcinogens. Repeated skin applications or single subcutaneous injection of 7,12-dimethylbenz(a)anthracene (DMBA) to 4-8-months-old Aka mice resulted in an earlier development of lymphomas and in local tumors at the site of treatment(8). Intraperitoneal injections of urethan to AKR mice, starting at the age of 4 weeks, also accelerated the development of lymphomas and in addition induced some hemangiomas(9).

The induction of malignant lymphomas and certain other tumors by injection of chemical carcinogens at birth has been achieved in Swiss mice with DMBA(10,11, 12), benzo(a)pyrene, 3-methylcholanthrene and urethan(11,13), in CBA and 101 inbred mice also with DMBA(14), in C_{57}bl mice with repeated doses of urethan starting at birth(15) and in C_3H/P and C_{57}bl/6JN mice with benz(a,h)anthracene and 3-methylcholanthrene(16).

The present experiment records the effect of a single subcutaneous injection of DMBA to newborn AKR mice.

Materials and methods. AKR inbred mice originally obtained through the courtesy of Dr. Kurt Stern, University of Illinois, Chicago, and since 1961 bred in our laboratory by brother-to-sister mating, were used. They were housed in plastic cages with granular cellulose bedding in groups of 10 according to sex, and were fed Rockland Diet in pellets and tap water *ad libitum*. The carcinogen 7,12-dimethylbenz(a)anthracene (DMBA),† was purified by chromatography on magnesia/Celite and dissolved in tri-n-caprylin,† purified by vacuum distillation.

Eighty-eight mice, less than 24 hours old, were injected subcutaneously in the back with a single dose of 100 μg DMBA in 0.05 ml tri-n-caprylin. Forty-five mice died shortly after the treatment because of acute toxicity of DMBA or cannibalism. At weaning time the remaining 43 mice were separated into 2 groups of 21 females and 22 males. As a control, 46 females and 41 males were kept untreated. Experimental and control animals were checked weekly and observed until spontaneous death or killed when in poor condition. Complete pathological study was done on all animals. In a few instances the histological diagnosis was limited by autolytic changes.

Results. Survival rate, number and types of tumors and their latent period are recorded in Table 1. One female at the 17th week in the treated group, and 1 male with enlarged lymph nodes at the 35th week in the control group were lost without necropsy. Macroscopically and cytologically the malignant lymphomas observed in all groups were found identical to those previously described in Swiss mice in this laboratory(11). Total incidence of malignant lymphomas was found to be similar in the treated and the untreated groups. However in the treated groups 45% of the malignant lymphomas occurred within the 20th week, while in the control groups no lymphoma was found before the 21st week. All the lymphomas observed in the treated group before the time of appearance of the first lymphoma in controls were found to be histologically of the stem-cell type.

At the site of DMBA injection 6 animals developed subcutaneous fibrosarcomas, 2 of which metastasized to the lungs. In the treated groups a total of 11 mice developed lung adenomas, while none were found in the controls.

Discussion. Subcutaneous injection of 100 μg of DMBA in tri-n-caprylin into newborn AKR mice notably accelerated the appearance of lymphomas. It is of some interest that of the various types of lymphoma observed in this strain only those classified histologically as stem-cell lymphomas were affected by the treatment. This is essentially in keeping with prior observations on the effects of carcinogenic chemicals on newborn mice(10,11). The other effect of the carcinogen was to give rise to a small number of local tumors which were not observed in the untreated controls. In comparing this experiment with that of Engelbreth-Holm and Lefèvre(8) who studied the effect of carcinogen in adult AKR mice it can be observed that the accelerating effect on the lymphomas was

† Eastman Organic Chemicals, N. Y.

the same as that observed in the present study but that the number of local tumors induced was considerably higher. It is difficult to compare this investigation with the current study in detail since there were differences in dosages and other aspects of technic. Nevertheless, it appears to be consistently the case that newborn mice differ from adults in having an enhanced response of the lymphoid tissues and a diminished response to local sarcoma formation by chemical carcinogens.

The mechanism of enhancement of these lymphomas having a viral component by a chemical carcinogen is obviously of basic interest but is beyond the scope of the present investigation.

Summary. Subcutaneous injection of 100 μg of 7,12-dimethylbenz(a)anthracene in 0.05 ml tri-n-caprylin in newborn AKR mice considerably shortened the latent period of malignant lymphomas. All malignant lymphomas observed in the treated group before the time of appearance of the first lymphoma in the controls were of the stem-cell type.

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Growth of Eaton PPLO in Broth and Preparation of Complement Fixing Antigen. (27681)

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Although the Eaton agent was first recognized in 1944, its natural history and relative importance in human lower respiratory tract disease are not fully understood(1) primarily because of technical difficulties involving propagation and identification of the organism and quantitation of antibody. Since 1957 the standard system for antibody assay has been the indirect fluorescent antibody test in which frozen sections of infected chick embryo lung serve as the source of antigen(2). The preparation of such frozen sections is slow and cumbersome, hence many laboratories have been reluctant to initiate diagnostic or epidemiologic studies of Eaton infection.

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