3. Mowry, R. W., *Lab. Invest.*, 1958, v7, 566.
4. Love, R., Liles, R. H., *J. Histochem. Cytochem.*, 1959, v7, 164.
5. Spicer, S. S., *ibid.*, 1960, v8, 18.
6. Pearse, A. G. E., *Histochemistry*, Little, Brown & Co., Boston, 1960, 917.
7. Warren, L., Spicer, S. S., *J. Histochem. Cytochem.*, 1961, v9, 400.
8. Gasic, G., Baydak, T., in *Biological Interactions in Normal and Neoplastic Growth*. International Symposium, Detroit, May 18-20, 1961, Little, Brown & Co., Boston, 1962, 709.
9. Gasic, G., Gasic, T., *Nature*, 1962, v196, 170.
10. Gasic, G., Loebel, F., Badinez, O., in *VIII International Cancer Congress* (Abst.), Moscow, July 22-28, Medgiz Publishing House, Moscow, 1962, 149.
11. Spicer, S. S., Wollmann, S. H., Warren, L., *Am. J. Path.*, 1960, v37, 599.

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Development of Malignant Lymphomas by Cell-Free Filtrates Prepared from a Chemically Induced Mouse Lymphoma.* (28195)

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It is known that Ak mouse malignant lymphomas can be transmitted by injection of cell-free filtrates into newborn C3H mice(1). Malignant lymphomas have also been induced following inoculation into newborn hosts of cell-free filtrates, prepared from x-ray-induced malignant lymphomas of C57Bl/Ka and C3H mice(2,3). Recently, several unsuccessful attempts have been made to transmit chemically-induced mouse lymphomas by cell-free filtrates(4,5,6).

In the present investigation a further effort is made to demonstrate a viral component by cell-free filtration in malignant lymphomas induced with a single subcutaneous injection of 100 μg of 7,12-dimethylbenz(a)anthracene (DMBA) in Swiss mice.

Materials and methods. Malignant lymphomas have been induced in our laboratory by subcutaneous injection of 100 μg DMBA in Swiss mice at birth with an incidence of 50.0 % in the females and 63.6% in the males. The clear distinction between induced and spontaneous malignant lymphomas both on the basis of histological type and time of origin has been described(7). At the age of 16 weeks one male mouse from the above mentioned group was sacrificed with ether and showed a marked enlargement of the thymus. Small sections from the thymus and other organs of this mouse were fixed in 10% buffered formalin and stained with hematoxylin eosin. The cytologic diagnosis revealed a malignant lymphoma, stem-cell type.

A cell-free extract from the thymus was prepared by Gross technique(1), using a 20% cell suspension in physiological saline, centrifuged at 0°C, first at 3000 rpm (1400 $\times$ *g*), for 15 minutes, then at 9500 rpm (7000 $\times$ *g*) for 7 minutes; the supernate was passed through a Selas, porosity 02, porcelain filter candle. The filtrate was used for injection as follows: 0.05 ml was injected subcutaneously to each of 8 newborn (less than 24 hours old) Swiss mice and 0.1 ml was injected intraperitoneally into each of 10 eight-week-old Swiss mice. As a control, 29 female and 28 male Swiss mice were kept untreated.

The mice used were all Swiss albino of the colony bred randomly in this laboratory. They were housed in plastic cages with wood shavings, separated according to sex at weaning, fed Rockland diet in pellets and tap water *ad libitum*. The animals were carefully checked and weighed at weekly intervals and the changes recorded. They were allowed to die spontaneously or were killed when found in poor condition. Complete necropsies were performed on all animals. All organs were fixed in 10% buffered formalin and sections from these tissues were stained with hematoxylin-eosin.

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TABLE I. Distribution of Tumors in Treated and Control Groups.

Groups	No. of animals inj	No. of survivors at weaning	Filtrate dilution/vol of inj (ml)	Animals with malignant lymphoma			Animals with other tumors
				No.	%	Cytological type* and time of death (wk)	
1. Cell-free filtrate inj into newborn mice	8	7 (2♀, 5♂)	1:5/(.05)	6	85.7	l (32); l (38); l (44); l (59); l (70); h (94)	1[a]
2. Cell-free filtrate inj into 8-wk-old mice	10♂	10♂	1:5/(.1)	—	—	—	1[b]
3. Untreated controls	—	29♀	—	5	17.4	l (48); h (65); h (82); h (94); h (95)	20[c]
	—	28♂	—	1	3.5	l (43)	8[d]

* l, lymphocytic type; h, histiocytic type.

[a] lung adenomas [b] lung adenomas [c] 7 adenocarcinomas of breast, 10 lung adenomas, 1 subcutaneous fibroma [d] 1 granulocytic leukemia, 1 anal hemangioma, 1 papilloma of forestomach, 3 lung adenomas, 1 sebaceous gland adenoma of skin, 1 squamous cell papilloma of skin.

Results. The number and types of neoplasms of treated and control groups are recorded in Table I.

In Group 1, injected at birth with the cell-free filtrate of the DMBA induced malignant lymphoma, 7 out of 8 mice survived after weaning. Of these 7 mice, 5 developed malignant lymphoma of the lymphocytic type, one a malignant lymphoma of the histiocytic type, and one died at the age of 38 weeks with enlarged spleen, liver and lymph nodes showing a histological pattern consistent with a lymphoid tumor, although advanced autolytic changes made the cytological diagnosis impossible. Thus the incidence of malignant lymphoma is 85.7%, excluding the latter case.

In Group 2, injected at the age of 8 weeks with the same cell-free extract, none of the 10 mice developed such tumors.

In Group 3, consisting of 29 females and 28 males kept untreated, the incidence of malignant lymphoma was 17.4% in females and 3.5% in males. These incidences are in accord with our previously reported findings in controls(8).

Macroscopically the malignant lymphomas were either thymic or generalized in the filtrate injected and untreated control groups.

In addition, a few other tumors appeared in all groups, as recorded in Table I.

Discussion. The transmission by cell-free filtrates of fowl sarcomas, induced with chemical carcinogens, has been reported only in 2 instances(9,10).

All attempts to transfer mouse lymphomas induced by chemical compounds using filtration methods have failed so far(4,5,6).

The preliminary results obtained in these experiments indicate that malignant lymphoma induced with DMBA in Swiss mice can be transmitted into newborn hosts of the same strain by cell-free filtration.

It should be pointed out that the possibility that the malignant lymphoma used for this experiment was spontaneous rather than induced is remote. In the first instance the malignant lymphoma used arose a matter of only 16 weeks after carcinogen treatment. No lymphoma has been observed in untreated mice of this stock for many years before the age of 35 weeks. Secondly, the malignant lymphoma used was of the stem cell type; this histological type is rarely seen among the spontaneous lymphomas most of which are lymphocytic or histiocytic types and occur beyond the age of 35 weeks.

It appears very unlikely that the filtrate could still contain some DMBA remaining from the initial treatment and the presence of such carcinogen could be responsible for induction of tumors. Even if we assume for the sake of argument, that the whole amount of 100 μg DMBA remained in the animal during 16 weeks and that it concentrated entirely in the thymus and then passed through the filter, we could still have had only approximately 5 μg DMBA in each dose of filtrate, since the total filtrate was divided into 18 sin-

gle doses. Even this hypothetical amount of 5 μg DMBA is insufficient to cause malignant lymphoma, as was shown in our previous experiments(7).

Consideration should be given to the fact, that in the present instance a malignant lymphoma of the stem cell type was filtered and injection of this filtrate resulted in more differentiated types of lymphoma, either of the lymphocytic or the histiocytic types. Whether or not this phenomenon represents a biologically different course is not clear. However, in our experience virtually all malignant lymphomas occurring in carcinogen treated or untreated Swiss mice above the age of 35 weeks were of the lymphocytic or histiocytic types(7). Except for one instance the animals injected with cell-free filtrate were above this age when they died or were sacrificed (Table I).

It is tempting to speculate about the interpretation of the present finding, as to whether DMBA activated the latent lymphoma virus, if it exists at all in our untreated mouse colony, or a carcinogen-altered nucleic acid is responsible for transmission of this neoplasm. Further experiments are in progress.

Summary. A cell-free filtrate was prepared by the Gross technique from a thymic tumor induced by a single subcutaneous injection of 100 μg DMBA in a Swiss mouse and diagnosed cytologically as a malignant lymphoma, stem cell type. Out of 7 survivors injected with the filtrate at birth, 6 developed malignant lymphomas, while no such tumor occurred in 10 mice treated similarly at the age of 8 weeks. Cytologically they represented the more differentiated malignant lymphomas, namely the lymphocytic type in 5 cases and in one instance the histiocytic type.

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1. Gross, L., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 27.
2. ———, *ibid.*, 1959, v100, 102.
3. Lieberman, M., Kaplan, H. S., *Science*, 1959, v130, 387.
4. Salaman, M. H., *Brit. J. Cancer*, 1959, v13, 76.
5. Latarjet, R., *Cancer Res.*, 1960, v20, 807.
6. Miller, J. F. A. P., *Advances in Cancer Research*, 1961, v6, 352.
7. Toth, B., Rappaport, H., Shubik, P., *J. Nat. Cancer Inst.*, in press.
8. Toth, B., Della Porta, G., Shubik, P., *Brit. J. Cancer*, 1961, v15, 322.
9. McIntosh, J., Selbie, F. R., *Brit. J. Exp. Path.*, 1939, v20, 49.
10. Oberling, C., Guerin, M., *Bull. Cancer*, 1950, v37, 5.

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Effect of Early Postnatal Steroid Treatment on Ovarian Function in Prepuberal Rats.* (28196)

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Pfeiffer(1) implanted testis into very young female rats and observed that at puberty their vaginal smears consisted of predominantly cornified cells. Other investigators by injecting androgens(2,5) or estrogens(3,4) were able to induce a similar phenomenon. Wilson(3), on the basis of impaired mating behavior, suggested these compounds had an effect upon the central nervous system (CNS) and Barraclough(5) presented more direct evidence that the steroids modified the CNS area(s) concerned with ovulation. Everett and coworkers(12,13) concluded that the pituitary and CNS were involved in the ovulatory process in adult cycling female rats.

Because of interest in this induction phenomena and the consequences of such treatment on the differentiating reproductive system a more adaptable system of analysis was desired. The prepuberal period was there-

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