Leukemogenic Filterable Agent from Estrogen-Induced Thymic Lymphoma in RF Mice.* (30416)

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The problem whether chemicals induce neoplasms by activation of a latent virus has been extensively investigated with avian sarcomas without arriving at a definite conclusion(1). A similar induction mechanism was considered for radiation-induced leukemias and supported by observations of Gross, Liberman and Kaplan and others(2). There are no reports on isolation of a leukemic agent from estrogen-induced lymphomas observed by us in a study now to be described.

Induction of lymphoma with estrogen, first reported by Lacassagne, was thoroughly studied by Gardner(3). In this study, we have chosen to use mice of the Rf strain because, unlike most strains of mice, they are sensitive to development of myeloid leukemia following exposure to ionizing radiations(2), and subsequent infection with Gross virus(4). This strain probably contains a latent virus(5) and the question arose whether estrogen-treated Rf mice would also develop myeloid leukemias and yield a virus.

Materials and methods. Animals. Rf/Jax mice were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. This strain has a low incidence of spontaneous thymic lymphoma but develops both lymphomas and myeloid leukemias following exposure to ionizing radiations(2).

Estrogen-treatment. Thirty-five female Rf/Jax mice were given subcutaneously a pellet containing 1.0 mg diethylstilbestrol (DES) with 19.0 mg cholesterol at 8 to 9 weeks of age. The DES-pellet was renewed at 2-month intervals. The mice were fed Purina laboratory chow and tap water *ad libitum,* and kept until natural death, except 3 leukemic mice that were sacrificed in extremis and bioassayed for virus.

Donors of cell-free filtrates. Cell-free filtrates were made from 3 lymphoblastic thymic lymphomas with metastases. The donor of filtrate 70 was sacrificed at 160 days of age, 100 days after the beginning of DES-treatment, and that of filtrate 82, 130 days after the beginning of DES-treatment. The autopsy of these mice revealed a thymic tumor occupying about two-thirds of the thoracic cavity, moderately enlarged lymph nodes and slightly enlarged spleen and liver. Mouse 89 was sacrificed 150 days after the beginning of DES-treatment; it had a generalized lymphoma with a moderately enlarged thymus and a large mesenteric lymphoma weighing about 1.5 g.

Preparation of cell-free filtrates. The thymic tumor mixed with enlarged lymph nodes were homogenized and filtered, as described earlier(6), except that chilled tissue culture medium Mixture #199 with sodium bicarbonate was used as a diluent instead of chilled normal saline. The cell-free filtrate was divided into 1 ml aliquots in small ampules, sealed and stored at —70°C.

Test of cell-free filtrates. Whenever 2- to 4-day-old litters of Rf/Jax mice were available, frozen filtrate was rapidly thawed and inoculated intraperitoneally in amounts of 0.1 ml per mouse. Nursing mothers were given bread and milk daily. The mice were weaned at 3 to 4 weeks of age, and males and females were separated. The experiments were terminated about 9 months after the inoculation, when the surviving mice were sacrificed and autopsied. Histologic sections were made from hemopoietic organs of all grossly leukemic and several grossly non-leukemic mice.

Results. Thymic lymphoma-induction with DES. The age distribution of lymphomas in the DES-treated mice is shown in a histogram (Fig. 1). No simultaneous (untreated) controls were set up. In numerous experiments, the spontaneous incidence of lymphoma in Rf mice was uniformly low, as in the mice of the present series injected with

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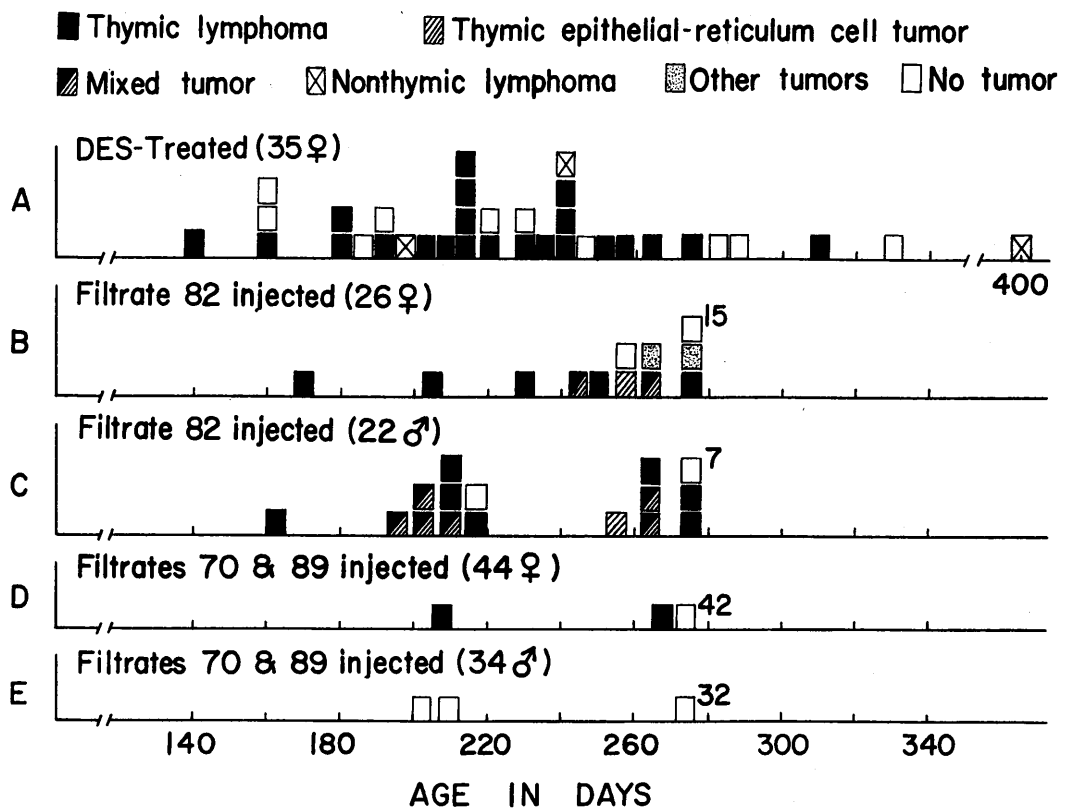


FIG. 1. Histograms of development of thymic lymphoma and other tumors: A, in DES-treated mice; B and C, in mice injected with cell-free filtrate 82; D and E, in mice injected with cell-free filtrates 70 and 89. Numbers in right upper corners indicate number of mice that were sacrificed at termination of experiments and found to be free from neoplasms. Each symbol represents one mouse.

filtrates 70 and 89 (Fig. 1). Thymic lymphoma occurred in 22 of 35 DES-treated mice (62.9%). The first death with thymic lymphoma occurred in a 140-day-old mouse, 80 days after the beginning of the DES-treatment. The mean age at death with thymic lymphoma was 220 days and the mean latency period after the beginning of the DES-treatment, 160 days. The mean age at death of 3 mice with non-thymic lymphomas (8.6%) was 300 days and the mean latency period, 240 days.

Thus, estrogen is capable of inducing thymic lymphoma in Rf mice. The incidence of non-thymic lymphoma occurring late in life is highly variable in this, as in other strains of mice. The number of non-thymic lymphomas in the present series was small and allowed no conclusion as to ability of estrogens to induce them.

Injection of filtrates prepared from primary DES-induced thymic lymphomas. The results of injections of cell-free filtrates from 3 lymphomas occurring in estrogen-treated mice are summarized in Table I and Fig. 1. Eight of 26 females (30.7%) and 14 of 22 males (63.6%), injected with filtrate 82, developed thymic tumors. The gross and microscopic appearance of the 12 lymphomas are illustrated in Fig. 2, 3. The 2 thymic epithelial-reticulum cell tumors were sharply circumscribed and encapsulated. They are illustrated in Fig. 4, 5. They are identical with thymomas induced by polyoma virus called either reticulum cell sarcoma or epithelioma(7). We listed them as epithelial-reticulum cell tumors since most investigators believe that the reticular cells of the thymus are entodermal in origin.

In the 8 mixed thymic tumors, the two

TABLE I. Results of Injection of Cell-Free Filtrates from 3 Thymic Lymphomas Occurring in DES-Treated Rf Mice.

Filtrate No.	No. & sex of mice inj	Mice with thymic tumors: Lymphoblastic No.	%	Epithelial-reticulum celled No.	%	Mixed No.	%	Total No.	%	Mice with other tumors No.	%
70	20 ♀	2	10.0	0	0	0	0	2	10.0	0	0
	23 ♂	0	0	0	0	0	0	0	0	0	0
82	26 ♀	5	19.2	1	3.8	2	7.7	8	30.7	4	15.4
	22 ♂	7	31.8	1	4.5	6	27.3	14	63.6	2	9.1
89	24 ♀	0	0	0	0	0	0	0	0	0	0
	11 ♂	0	0	0	0	0	0	0	0	0	0

types of lesions occurred either side by side in one "lobe" or involved separately the 2 "lobes." The other types of tumors seen in 4 female and 2 male mice that had been injected with filtrate 82 were an osteogenic sarcoma, an undifferentiated myosarcoma (in the same mouse), a renal sarcoma, a chondrosarcoma, and 3 parotid tumors. Such tumors are common in mice infected with polyoma virus and we believe that filtrate 82 contained this agent. This view is supported by the microscopic examinations of the kidneys of the mice treated with this filtrate that disclosed non-neoplastic lesions (Fig. 6, 7) characteristic of polyoma virus infection (8). Mice treated with filtrates 70 and 89 developed neither similar neoplasms nor similar kidney lesions.

Untreated controls were not set up simultaneously; however, mice treated with the inactive cell-free filtrates (Fig. 1, Table I) had as low an incidence of lymphomas as normal Rf mice, reported in several earlier experiments(4,9).

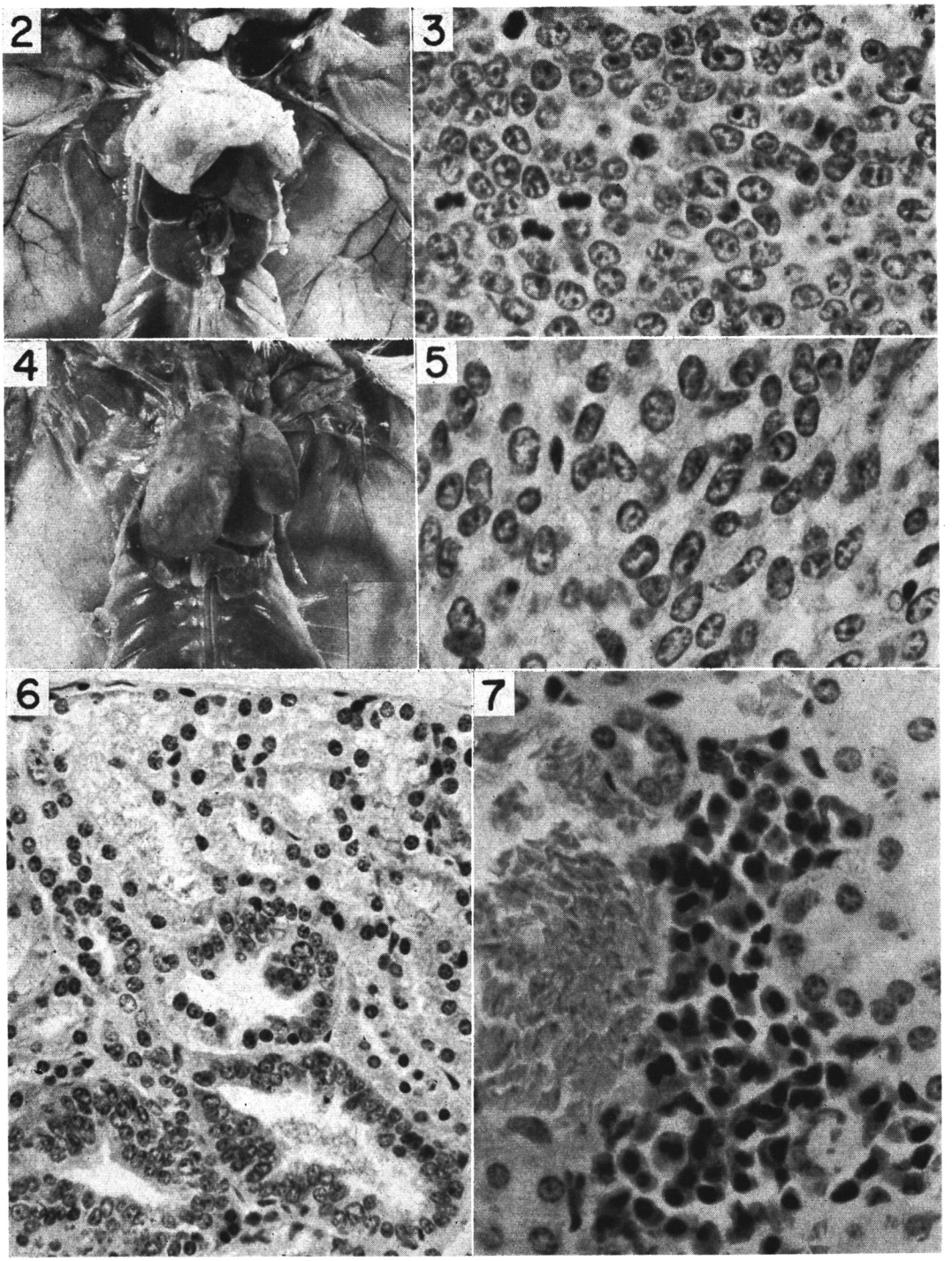
Two mice, which developed lymphomas following injection with filtrate 82, were sacrificed at 206 and 212 days of age. Filtrates prepared from their thymic tumor and lymph nodes were injected into 13 newborn Sprague-Dawley and 17 $W/_{Fu}$ rats, none of which developed leukemia within 6 months. Both of these rat strains are highly susceptible to Gross passage virus(6). In numerous recent experiments, newborn $W/_{Fu}$ rats, never failed to develop thymic lymphoma. Thus it seems that the agent in filtrate 82 is not identical with the passage virus of Gross.

Comments. The relation of virus to estrogen-induced lymphomas was studied by us in Rf mice. This strain has a low spontaneous incidence of leukemia. Irradiated Rf mice develop both myeloid and lymphoid leukemias(2) and injection of Gross virus in irradiated Rf mice enhances the development of both lymphoid and myeloid leukemia(4). Filtrates of radiation-induced leukemia in Rf mice yielded a virus(2).

Most lymphomas induced in mice by estrogens, carcinogenic hydrocarbons, ionizing radiations and viruses are thymic in origin. On the basis of the experiments using X-irradiation(2), it is now believed by many investigators that carcinogens other than ionizing radiations may also induce leukemia by "activating" a latent virus.

Failure to find virus in chemically-induced lymphomas in mice was reported by several investigators(10). Recently Toth(10) reported an isolation of a filterable agent from lymphoma induced in Swiss mice by DMBA. It should be considered, however, that spontaneous lymphomas are common in the Swiss strain and that leukemogenic viruses were isolated from non-leukemic tumors carried in this strain(11,12). Furthermore, characteristic virus particles have been demonstrated in germ-free mice of this strain(13).

Our findings, like those of others, can be interpreted by assuming that the presence of a latent virus played a major role in induction of lymphoma by estrogens. However, the following alternate possibility remains. Nonviral carcinogens can produce neoplastic transformation of lymphoid cells which, as many replicating cells, can "pick up" some viruses latent in the respective hosts. The



FIGS. 2, 3. Filtrate induced thymic lymphoma.
FIGS. 4, 5. Thymic epithelial-reticulum cell tumor.
FIGS. 6, 7. Non-neoplastic lesions in the kidney characteristics of polyomavirus infection: hyperchromatic and anaplastic tubules (Fig. 6) and perivascular plasma cell infiltration (Fig. 7).

isolation of virus from several transplanted neoplasms, capable of producing leukemia but not the tumor from which the virus originates (11), favors this possibility.

The present study was complicated by the presence of polyoma virus in the strain used. Since this virus is common in mice(14), it was probably present unrecognized in many similar earlier studies. Its presence does not invalidate the conclusion that estrogen-treatment greatly increases the incidence of thymic lymphomas and that cell-free filtrate of one of these is capable of producing thymic lymphomas in Rf mice. It is well known that polyoma virus does not produce lymphomas.

It is noteworthy that estrogens and the agent from an estrogen-induced lymphoma failed to induce myeloid leukemia in Rf mice. The pathogenesis of induction of thymic lymphoma by estrogens is still puzzling. Estrogens cause thymic atrophy but so do many other non-leukemogenic hormones. There is one property of estrogen not mentioned earlier which links it to the thymus. It produces hyperplasia and tumors of pituitary acidophilic cells, the hormones of which have growth promoting activities, including marked enlargement of the thymus(15). Hyperplasia inducing agents are in general promoters of carcinogenesis.

Summary. The role of a latent virus in induction of lymphoma by diethylstilbestrol in Rf mice was investigated. Thymic lymphoma developed in 62.9% and non-thymic lymphoma in 8.6% of DES-treated mice within 400 days of age. Unlike radiations, estrogen failed to induce myeloid leukemia in Rf mice. Cell-free filtrates of one thymic lymphoma (No. 82) was lymphomogenic; that of 2 other lymphomas was apparently inactive. Whereas filtrate 82 caused lymphomas in 41.7% of 48 mice within 9 months of age, the incidence of lymphoma in 78 mice injected with the other 2 filtrates was 2.6%; this is about the spontaneous lymphoma incidence in Rf mice within 9 months of age.

The development of 17 diverse tumors other than lymphomas in mice injected with filtrate 82 pointed to the presence of a polyoma virus in this filtrate contaminating the leukemia virus.

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