plasmic constituents accessible to the action of lysozyme and trypsin.

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Influence of Thymectomy on Tumor Induction by Polyoma Virus in C57BL Mice. (29237)

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Polyoma virus will induce tumors in many strains of mice when inoculated in the neonatal period(1). Resistance to isogenic transplants of polyoma tumor cells is evident in adult mice which have been inoculated with virus at birth(2,3). It is also evident in mice injected with virus in adult life(2,3) or with cells of a histoincompatible polyoma tumor (4). Such a resistance is not dependent on the presence of viral antibodies since passive transfer of a heterologous immune serum will not give protection(2). Apparently resistance can be transferred to normal recipients by means of lymphoid cells from immunized donors(5). To explain these phenomena, it has been postulated that the polyoma virus transforms normal cells to tumor cells in both newborn and adult mice, that the transformed cells contain a distinct cellular antigen deficient or absent in normal host tissues, that the immunologically immature newborn tolerates this new antigen thus allowing tumor growth, and that the immunologically mature adult is capable of rejecting the antigenic tumor cells by a mechanism similar to classical transplantation immunity.

A strain resistance to polyoma virus has been observed in C57BL mice(6). Such intact mice are highly insusceptible to the oncogenic effect of all strains of polyoma virus tested, even when inoculated on the day of birth(7). However salivary gland epithelium of C57BL mice in organ culture, shows the same proliferative response as C3H tissues (8) and is readily transformed to a neoplastic state(9). It is not known whether the low susceptibility of C57BL mice to viral oncogenesis has an immunologic basis. Since mice thymectomized in the neonatal period are incapable of responding to foreign cellular antigens(10), the influence of thymectomy on the oncogenic activity of polyoma virus in C57BL mice was investigated.

Materials and methods. Mice. Inbred C57BL/KaLw mice of either sex were used. This strain was obtained from Dr. Henry Kaplan in 1951 and has been inbred here continuously since that time by brother to sister matings. Salivary gland tumors within the breeding colony have not been observed.

Virus. Dulbecco's large plaque polyoma virus strain(11) derived from LID-1, originally obtained from Dr. W. P. Rowe of Nat. Inst. of Health, was grown on C3H mouse embryo cells. The virus used for inoculation was a 40-fold diluted material, 0.05 ml being

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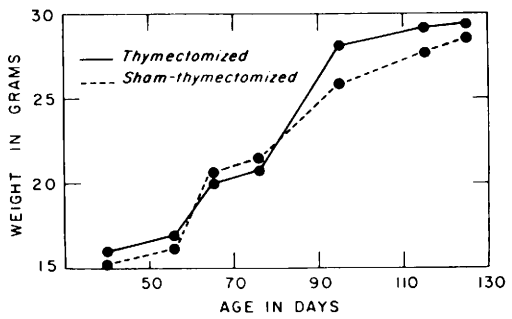

FIG. 1. Mean body weight curves of 13 thymectomized and 8 control C57BL male mice injected with polyoma virus. Only 2 mice, both thymectomized, showed weight curves outside the range of this whole group. These did not gain weight beyond 17 g and both developed parotid tumors at 68 and 70 days respectively.

injected subcutaneously into each mouse at 4 days of age. This represented an inoculum of 2×10^6 plaque forming units.

Thymectomy. Thymectomy or sham-operation was performed in 3-day-old mice by a method similar to that used for adult mice (12).

Antibody titration. The mice were bled at about 5 to 8 weeks of age and their sera were tested for polyoma hemagglutination-inhibiting (HI) antibodies. Heat-inactivated sera (56°C for 30 minutes) were diluted in phosphate buffered saline, mixed with 8 hemagglutinating units of polyoma virus, incubated at room temperature for 1 hour, then mixed with 0.5% sheep erythrocytes and held at 4°C for 12 hours. End points are expressed as the reciprocal of the dilution giving complete inhibition of hemagglutination.

Hematology. Absolute and differential white cell counts were made from tail vein blood of mice between 5 and 6 weeks of age. Blood smears were stained with May-Grunwald-Giemsa and differential counts were made on 400 leucocytes for each sample.

Histology. Tissues were fixed in Tellyesniczky's fluid and stained routinely with hematoxylin and eosin. Mice with tumors were sacrificed before the tumor could grow to a size that would cause discomfort. The lymphoid organs were evaluated at autopsy both grossly and microscopically. The absence of all mediastinal thymic tissue was verified by inspection and, whenever necessary, by microscopic sectioning.

Results. Thymectomy at 3 days of age did not in general impair the growth of the mice used in these experiments. Most of these mice gained weight at the same rate as sham-thymectomized controls (Fig. 1), few showing any of the features of the wasting syndrome which characteristically develops in C57BL mice thymectomized within 24 hours of birth(13).

The absolute number of lymphocytes in the peripheral blood of thymectomized and control mice is shown in Table I. Most of the sham-operated controls had counts at or above 5000 per cu mm. In contrast, most of the thymectomized mice had lymphocyte levels below 5000 per cu mm.

Table II shows that all the inoculated mice had developed HI antibodies between 5 and 8 weeks of age. There was no evidence of impairment of HI antibody production in mice thymectomized at 3 days of age. Many of these mice, however, produced somewhat lower titers than many of the controls. Thus only 2 of 16 thymectomized mice had titers at or above 1600 as compared to 9 of 15 controls. None of 8 non-inoculated mice had any detectable HI antibody titers.

The incidence of tumors occurring in thymectomized and control mice inoculated with polyoma virus is shown in Table III. Eighteen of 20 male and female mice thymectomized at 3 days of age and inoculated at 4 days

TABLE I. Peripheral Blood Lymphocyte Levels of 5- to 6-Week-Old C57BL Mice Thymectomized at 3 Days and Injected with Polyoma Virus at 4 Days.

Treatment	No. of mice in group	No. of mice showing following lymphocyte count per mm³: 3000	4000	5000	6000	7000
Thymectomized + virus	8	1	6	1		
Sham-thymectomized + virus	8		1	2	4	1

TABLE II. HI Antibody Titers in 5- to 8-Week-Old C57BL Mice Thymectomized at 3 Days and Injected with Polyoma Virus at 4 Days.

Treatment	No. of mice in group	No. of mice showing following HI antibody titers: <100	100	200	400	800	1600	3200
Thymectomized + virus	16		2	2	5	5		2
Sham-thymectomized + virus	15		1		3	2	4	5
Non-thymectomized and no virus	8	8						

TABLE III. Induction of Tumors in C57BL Mice Thymectomized at 3 Days and Injected with Polyoma Virus at 4 Days.

Group	Sex	Treatment	No. of mice in group	Mice developing tumors: No.	Mean latent period in days (range)	%
I	♂	Thymectomized + virus	13	11	65 (52–73)	85
		Sham-thymectomized + virus	8	0		0
II	♀	Thymectomized + virus	7	7	74 (64–78)	100
		Sham-thymectomized + virus	8	1	118	12
I + II	♂ + ♀	Thymectomized + virus	20	18	69 (52–78)	90
		Sham-thymectomized + virus	16	1	118	6
III	♂ & ♀	Thymectomized, no virus	15	0		0
		Sham-thymectomized, no virus	9	0		0

developed parotid gland tumors at an average age of 2 months. In contrast, only 1 of 16 inoculated sham-operated controls developed a very slowly growing unilateral parotid tumor at the age of 4 months. No tumors were seen in uninoculated sham-thymectomized or thymectomized mice. In the thymectomized inoculated group 9 mice had grossly evident bilateral parotid tumors and a further 2 had gross involvement of both parotid and of one submandibular gland; 5 mice had grossly evident unilateral parotid tumors and involvement of the other gland was seen only at autopsy or after microscopic examination and 2 mice had only unilateral parotid tumors with no involvement of other sites. A few mice had small tumors of the hair follicles but other primary tumors or lesions were absent. The histologic appearance of the neoplasms encountered was very similar to that of parotid tumors seen in other strains of mice receiving polyoma virus on the day of birth. Most of the tumors were pleomorphic with poorly defined tubular structures and numerous round and spindle cells presenting a sarcoma-like appearance (Fig. 2 and 3).

All the thymectomized mice developing tumors had no evidence of any thymus remnants in the mediastinum. In all but 2 animals which were runted, the gross appearance of the spleen, lymph nodes and Peyer's patches was normal. Some depletion of lymphocytes was evident microscopically in these tissues but it was not as obvious as that seen in mice thymectomized within 24 hours of birth(13). The lymphoid tissues in the 2 runted animals were very markedly reduced in size and depleted of lymphocytes.

No neoplasms or lesions of any type and no abnormality of the lymphoid tissues were found in 5 sham-thymectomized virus-inoculated mice sacrificed at 4 months of age. The other sham-thymectomized mice in that group are being kept for long term observations.

Discussion. The data presented above clearly show that C57BL mice thymectomized at 3 days of age are highly susceptible to the oncogenic activity of polyoma virus. Ninety percent of such mice developed tumors of one or both parotid glands within 2 months. In contrast, only one of 16 sham-thymectomized inoculated controls developed a slowly growing unilateral parotid tumor at the age of 4 months. It is unlikely that the high sus-

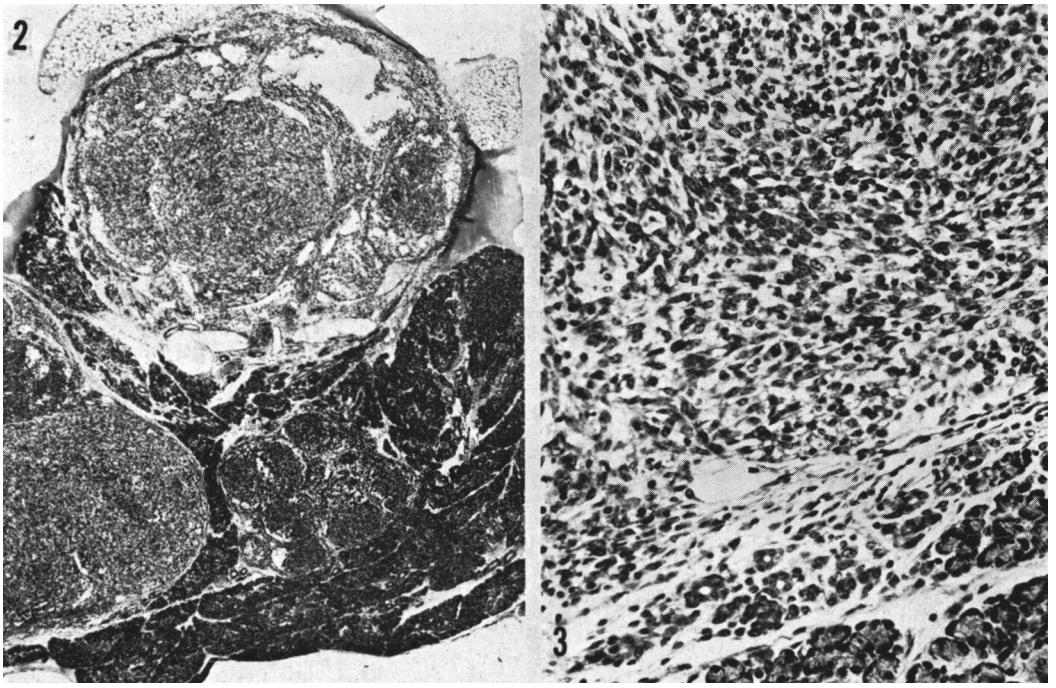

FIG. 2. Parotid gland tumor nodules in C57BL mouse thymectomized at 3 days and inoculated with polyoma virus at 4 days (× 64).

FIG. 3. High power view of cells of tumor shown in Fig. 2. Note normal gland at bottom and tumor tissue at the top showing tubular-like structures and numerous round and spindle cells (× 224).

ceptibility of the thymectomized mice is due to non-specific factors affecting the general health and operating in the absence of the thymus: most of the mice which developed tumors were in good condition and showed no evidence of marasmus nor any of the trophic disturbances which are characteristically seen in C57BL mice thymectomized on the day of birth(13). It is also unlikely that the susceptibility after thymectomy is the result of an inadequate antibody response to the virus: all mice produced detectable HI antibody titers, some as high as the sham-thymectomized controls. Further, some sham-thymectomized mice which did not develop tumors produced rather low levels of antibody.

The thymectomized mice in the present experiment showed a depletion of lymphocytes in their peripheral blood and lymphoid tissues comparable in degree to that found in mice thymectomized in adult life(14). Thymectomy at or after 2 months of age is not associated with any significant impairment of the immune response. However, mice thymectomized as late as 14 days after birth do show an impaired capacity to reject homografts, though only when antigenic differences between donor and host are slight. It is possible, therefore, that the high susceptibility of the C57BL mice thymectomized at 3 days of age to the oncogenic activity of polyoma virus is dependent on their impaired capacity to react against the foreign cellular antigen of the induced tumor. Normal newborn C57BL mice may be capable of dealing with such foreign antigens in the same manner as normal adult mice of other strains. If the immune capacity of the inoculated adult is temporarily reduced by total body irradiation, then tumors develop(15). It is thus possible that when the maturation of immunological faculty is impaired by thymectomy, the animal will not have the means to prevent the growth of antigenically "foreign" neoplastic clones.

The results presented here are in harmony with those obtained in other experiments

which showed that the neonatally thymectomized animal is more susceptible than the normal animal to the oncogenic activity of some chemical(16) or viral(17,18) carcinogens. They show, in addition, that genetically determined strain resistance to a carcinogen can be abolished by neonatal thymectomy. Interference with the cellular immune mechanism may thus be necessary, in some cases, to allow the full expression of a carcinogenic process. It is felt that the experimental system used here is a very convenient model with which to evaluate the role of host factors in the natural resistance to tumor growth.

Summary. Mice of the C57BL strain are normally highly resistant to the oncogenic activity of polyoma virus. Such mice were thymectomized at 3 days and injected with the virus at 4 days. Controls were sham-operated and injected with virus. Most of the thymectomized mice gained weight at the same rate as the controls and produced hemagglutination inhibiting antibodies. Pleomorphic tumors of the salivary glands developed within 2 months in 18 of 20 thymectomized mice and in only 1 of 16 controls at 4 months. It is suggested that the resistance of the C57BL strain to the oncogenic action of polyoma virus has an immunologic basis. Impairment of the maturation of immunologic faculty in mice of this strain allows the progression of antigenically distinct neoplastic clones induced by the virus.

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Osmotic and Respiratory Behavior of Surviving Rat Liver Cells.* (29238)

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An extensive literature is available on the osmotic behavior of mammalian erythrocytes [Ponder(1,2)] while the osmotic behavior of nucleated mammalian cells has been studied less extensively. Past studies have dealt with osmotic behavior of isolated cells and have been largely concerned with changes in total cell volume as a function of extracellular osmotic pressure. The present experiments were designed to study changes in nuclear and total cell volume as a function of changing effective extracellular osmotic pressure on surviving rat liver slices. In addition, changes in liver cell O_2 uptake were studied in relation to cell water content.

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Materials and methods. Male Sprague-Dawley rats weighing 150-200 g were main-