That one observes no glial reaction prior to 10 days of age suggests that the absence of reactive gliosis in the immature animal may be secondary to the absence of specific inducing agents rather than simple glial immaturity. In adult rats reactive gliosis occurs early in Wallerian degeneration, a situation in which components of the axone and myelin sheath alone are liberated. Reactive gliosis is also seen in human multiple sclerotic plaques and following cerebral-spinal fluid exchange in the cat(9), two lesions in which the myelin sheath alone is destroyed. Thus it is suggested that some component contained in, but not limited to, the myelin sheath may be implicated as the inducing factor responsible for reactive gliosis.

Conclusions. Studies of experimental central nervous system lesions in the immature rat indicate that the morphologic and enzymic alterations of reactive gliosis are not observed prior to 10 days of age. The absence of reactive gliosis in the newborn animal may be due to immaturity of glial cells, absence of specific inducing agents, or some combination of the two factors. It is suggested that absence of certain inducing substances found in, but not limited to, the myelin sheath may be the determining factor.

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Immunologically Privileged Sites in Studies of Polyoma Tumor Antigens. (28303)

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The production of tumors by cells inoculated into appropriate animals is the only universally accepted criterion of oncogenic transformation. Yet, in the case of studies on *in vitro* transformation by tumor viruses, a serious limiting factor in showing the oncogenic properties of the transformed cells is their immunological rejection by the inoculated host as the animal reacts to the "foreign" cell antigen contained in the cells(1,2). To overcome this resistance, usually large numbers of cells must be inoculated and some procedure used, such as whole body X-irradiation or cortisone therapy, to reduce the host's immunological competence. The same limiting factors also reduce the sensitivity of biological tests for antigens in these virus-induced tumors where animals immunized with viral or tumor materials are tested for resistance to tumor challenge, since large numbers of cells frequently must be used to produce tumors in the control animals.

It has been well established that certain organs represent "immunologically privileged" sites in the sense that homografts and even heterografts are either not rejected at all or are rejected less efficiently when the "foreign" cells are inoculated into these areas. The brain, anterior chamber of the eye, the testes, and, in the case of the hamster, the cheek pouch have been shown to be such immunologically privileged sites. The purpose of the experiments reported here was to find whether the use of these areas could increase

the sensitivity of the biological tests for oncogenic properties of virus-transformed cells and for immunological studies of tumor antigens in virus-induced tumors.

Materials and methods. Animals. Inbred strains of C57BL/6 Jn and C_3H/Hen mice were obtained from the Nat. Inst. of Health animal colony. All mice except those used for intratesticular inoculation were females between 4 and 8 weeks of age. Non-inbred, female Syrian hamsters from the same source were used between 6 and 12 weeks of age. *Virus.* The standard polyoma virus pool had been produced in primary mouse embryo tissue cultures made from embryos of random-bred Swiss mice and the pool contained 1.5 x 10^7 pfu of virus per ml. Animals were made "virus-immune" by a single intraperitoneal inoculation of 0.1 ml of the standard virus pool 3 to 6 weeks before tumor challenge. *Tumors.* The 695 and 1923 transplantable C57BL mouse polyoma tumors have been previously described(1) and were free of demonstrable polyoma virus. ME 52 P is an established tissue culture of C57BL embryo cells transformed *in vitro* by polyoma virus(2) and still maintaining high titers of virus. The MC tumor is a transplantable sarcoma produced by 3-methylcholanthrene in C57BL mice. Tumors were trypsinized into single cell suspensions and counts made of viable cells. All animals receiving tumor cell inoculations were observed for a period of 2 months.

Results. Titration of transplantable polyoma tumors by different routes of inoculation. A suspension of single cells from the 34th transplant passage of the 695 tumor was made and serially diluted so that 0.03 ml contained 1.6 times the number of cells indicated in Table I. Subcutaneous (SC) inoculation was into the interscapular area; SC-burn inoculation was under the crust formed over a third degree burn inflicted in the lumbosacral area 4 days previously, and here there was frequent leakage of part of the inoculum from under the edge of the crust; SC-incision inoculation was subcutaneous under the center of healing multiple-crossing linear incisions made with sharp scissors in the lumbosacral area 4 days previously. The multiple incisions were repeated over the same area at weekly intervals for 4 weeks after tumor challenge. Intradermal (ID) inoculation was into the clipped skin of the lumbosacral area producing a superficial bleb; intramuscular injection (IM) was into the left gastrocnemius muscle; intracerebral (IC) inoculation was into the right temporal area of the brain; intraocular (IO) injections were made into the anterior chamber of the eye by introducing the needle through the area near the periphery of the cornea; intratesticular inoculation (IT) was made directly into the left testicle and usually the testicle could be felt to rupture as the result of the injection. All inoculations were of 0.03 ml through a 27-gauge short-bevel needle from a tuberculin syringe. From Table I it can be seen that the SC and ID routes were the least efficient with IM being perhaps slightly better. When SC inoculation was done in the presence of trauma by burn or incision there was a 100-fold increase in the efficiency of tumor takes. IC, IO, and IT were all at least 1000-fold more effective than SC. Those mice getting tumors after IC inoculation developed evidence of involvement of the central nervous system such as convulsions and paralysis, and at death a day or two later showed gross bulging of the cranium. IO tumor growth was apparent by bulging of the eyeball and IT tumor filled the scrotum with a mass extending up into the peritoneal cavity.

Table II shows the comparison of the titrations of the 37th transplant passage of 1923 polyoma tumor SC and IC in isologous C57BL

TABLE I. Titration of Transplantable Isologous Polyoma Tumor in C57BL Mice by Different Routes of Inoculation.

Route of inoculation*	No. of 695 tumor cells inoculated			
	10^2	10^3	10^4	10^5
Subcutaneous	0/5†	0/5	1/5	5/5
" (burn)	0/5	3/5	4/4	4/5
" (incision)	1/5	2/5	5/5	6/6
Intradermal	0/5	0/5	2/5	5/5
Intramuscular	0/5	0/5	4/5	5/5
Intracerebral	4/5	5/5	5/5	5/5
Intraocular	2/5	4/4	5/5	5/5
Intratesticular	4/5	5/5	5/5	5/5

* See text for details of inoculation.
† In this and subsequent tables, fraction is No. of mice developing tumors over No. inoculated.

TABLE II. Titration of Isologous Mouse Polyoma Tumor Intracerebrally and Subcutaneously.

Route of inoculation	No. of 1923 tumor cells inoculated				
	10^1	10^2	10^3	10^4	10^5
Intracerebral	0/5	1/5	3/5	5/5	5/5
Subcutaneous	—	0/5	0/5	2/5	5/5

mice. The efficiency of tumor takes is about 100-fold greater by the IC route although it is less efficient by this route than was 695 tumor in the previous experiment.

Ability of in vitro transformed cells to produce tumors in X-irradiated and normal mice on SC and IC inoculation. The ME 52 P culture cells were used at the time of the 64th subculture 22 months from their original inoculation with polyoma virus. Cells were trypsinized from Blake bottles, suspended and counted. The numbers of viable cells indicated in Table III were inoculated either IC or SC into adult C57BL mice. One set of mice were normal, the other had received 400 r whole body X-irradiation a few hours before challenge with tumor. Although no endpoint was reached in the IC titer in normal mice, it is obvious that there could have been no great increase in efficiency of tumor takes between IC and SC inoculation in the un-X-rayed animals. X-irradiation itself led to a definite increased efficiency whether the SC or IC route was used. There is some evidence that the combination of IC inoculation along with irradiation was the most efficient procedure.

Increased sensitivity of tests for resistance against IC challenge with polyoma tumors. Previous experiments(1) had shown that polyoma virus immune animals can resist SC challenge with 10 to 100 times the minimal number of polyoma tumor cells required to produce tumors in control animals. On the other hand, when animals are immunized with virus-free tumor cells the degree of resistance to SC challenge is minimal and usually is demonstrable only as a retardation of tumor growth rather than complete suppression. After finding the greatly increased sensitivity of normal mice to IC and IT challenge with the 695 polyoma tumor, the ability of virus-immune mice to resist challenge by these routes was tested. In the experiments summarized in Table IV, polyoma immune and normal adult

TABLE IV. Resistance of Polyoma Virus-immune Mice to Challenge with Transplantable Isologous Polyoma Tumor Inoculated by Different Routes.

Route of challenge	Mice	No. of 695 tumor cells inoculated					
		10^1	10^2	10^3	10^4	10^5	10^6
Subcutaneous	N	—	—	1/5	1/5	4/5	—
	I	—	—	—	0/5	0/5	4/5
"	N	—	—	2/3	4/4	—	—
(incision)	I	—	—	1/5	2/4	3/6	—
Intradermal	N	—	—	0/5	0/5	5/5	—
	I	—	—	—	0/5	0/5	3/5
Intra-	N	4/5	5/5	5/5	5/5	—	—
cerebral	I	—	0/4	0/5	0/5	1/5	—
Intrates-	N	5/5	5/5	5/5	5/5	—	—
ticular	I	—	0/5	0/5*	0/4*	—	—

* One in each of these groups developed tumors after 3 months.
N = normal adult mice.
I = Polyoma virus-immune adult mice.

C57BL mice were inoculated by the methods described. As in previous experiments the difference in endpoint between immune and normal mice challenged by the SC route was one log as was also true of those with SC inoculation followed by repeated trauma and with ID inoculation. However, in the IC challenge group the difference was at least 5 logs and in the IT group at least 4 logs.

That this resistance was on a specific immunological basis is suggested from the results shown in Table V. Here polyoma immune and normal mice were challenged SC, IC and IT

TABLE III. Effect of Route of Inoculation and X-irradiation on Ability of Mice to Support Tumor Growth from Cells Transformed *in vitro* by Polyoma Virus.

Mice	Route of inoculation	No. of ME 52 P cells inoculated					
		10^1	10^2	10^3	10^4	10^5	10^6
Normal	Subcutaneous	—	—	0/5	0/4	1/5	2/5
Irradiated*	"	0/5	0/5	1/4	5/5	—	—
Normal	Intracerebral	0/5	0/5	0/4	0/5	—	—
Irradiated*	"	0/5	1/5	2/5	5/5	—	—

* 400 r whole body X-irradiation before challenge.

TABLE V. Lack of Resistance of Polyoma Virus-Immune Mice to Intracerebral and Intratesticular Challenge With Non-Polyoma Tumor.

Route of inoculation	Mice	No. of MC tumor cells inoculated 10^2	10^3	10^4	10^5	10^6	10^7
Subcutaneous	N	—	—	4/5	4/5	5/5	5/5
	I	—	—	3/5	2/5	5/5	5/5
Intra-cerebral	N	0/5	1/5	3/5	5/5	5/5	5/5
	I	0/5	0/5	5/5	5/5	5/5	5/5
Intrates-ticular	N	0/5	0/5	5/5	5/5	5/5	5/5
	I	0/5	0/5	4/5	5/5	5/5	5/5

N = Normal adult mice.
I = Polyoma immune adult mice.

with a non-polyoma methylcholanthrene induced tumor. There was no difference in the endpoints between the immune and normal groups by any route of inoculation. It is of interest, however, that this tumor's ability to develop was not enhanced by inoculation into the brain or testes.

Evidence summarized in Table VI suggested that inoculation with immunizing cellular antigen into the same privileged site in which subsequent challenge was to be made might have increased the sensitivity of tests for immunizing antigens. Groups of mice received a single immunizing IC or SC dose of 8 x 10^5 cells of 1923 polyoma tumor after the cells had received 15,000 r X-irradiation to prevent their multiplication. Two weeks later they were quantitatively challenged IC with a different isologous polyoma tumor (695). With this small antigenic stimulus there was no evidence of resistance in those immunized by the SC route, but a 10-fold degree of resistance in the IC immunized group.

Attempts to produce tumors by inoculation of polyoma virus into immunologically privileged sites. Groups of 10 adult C_3H mice were inoculated with undiluted polyoma virus by the following routes: SC, SC with burn, SC with repeated incision, ID, IC, IO, and IT. After one year's observation only one polyoma tumor had developed and that was a parotid tumor in the SC burned group. Many of the mice in all groups developed mammary tumors which were shown to be non-polyoma in origin by a lack of resistance to their transplantation in polyoma-immune mice. They were probably caused by the mammary tumor agent which is carried in this strain of mice. Nine adult hamsters were also inoculated into the cheek pouch with 0.1 ml of undiluted virus and observed for one year with no tumors induced.

TABLE VI. Immunization With X-irradiated Tumor Cells by Different Routes Followed by Intracerebral Challenge.

Route of immunization*	No. of 695 tumor cells inoc intracerebrally 10^1	10^2	10^3	10^4	10^5
Control	0/5	5/5	5/5	5/5	5/5
Intracerebral	—	0/5	3/4	5/5	5/5
Subcutaneous	—	4/5	5/5	5/5	5/5

* Single dose of 8 × 10^5 1923 tumor cells X-irradiated with 15,000 r given 2 weeks before intracerebral challenge with 695 tumor cells.

Discussion. The basis for the enhanced ability of homologous or in our experiments, isologous tumors to transplant in the so-called immunologically privileged sites is not definitely established. The available evidence suggests a lowered efficiency of adsorption of antigen rather than a decreased ability of antibody or immune cells to get to the site. Our results with virus-immune animals are consistent with this explanation. It is obvious from our results that enhanced transplantability by the IC route is not demonstrable for all tumors. The increased efficiency with our 1923 tumor was not as great as with 695, and in the case of the MC tumor there was no difference between the SC and IC titers. Time factors obviously must be important and the slower growing transplants have more opportunity eventually to be rejected as the immunological reaction to foreign antigens slowly develops. Furthermore, there might well be differences as to the effect on the tumor cells of local environmental conditions at the different sites of inoculation.

For those tumors like our 695 for which only a few cells inoculated IC into normal mice suffice for a successful transplant, the sensitivity of any test for resistance as a result of some immunization procedure is greatly enhanced. An immunological relationship between immunizing antigen and tumor may thus be demonstrated which would not be apparent by the SC or IM routes of challenge. The type of procedure used in the experiment

presented in Table VI offers another possible way of increasing the sensitivity of tests for relationship of antigens to tumors—both immunization and challenge being inoculated IC.

In those situations where the ability of a given cell suspension to produce tumors is being tested, such as in the *in vitro* transformations by viruses in tissue culture systems, the inoculation into immunologically privileged sites in X-irradiated animals might give positive transplants where conventional routes of inoculation would be negative. The negative results of the attempt to obtain oncogenesis by polyoma virus in adult animals by inoculation into privileged sites are probably explained by the fact that virus particles so inoculated can readily disseminate to other areas, calling forth the immediate usual immunological response(3).

Summary. Two polyoma tumors in mice have been found to have a 1,000-fold increased transplantability if the IC or IT routes of inoculation are used instead of SC. When polyoma virus-immune mice were challenged by the IC or IT routes, the degree of demonstrable resistance was 10^5 and 10^4 minimal transplantable numbers of cells as compared to 10^1 by the SC route. Mice immunized IC or SC with a single dose of X-irradiated tumor cells and later challenged IC showed protection in the IC but not the SC immunized group. The ability of *in vitro* polyoma transformed tissue culture cells to produce tumors on inoculation of isologous adult mice was most markedly enhanced by whole body X-irradiation, but best results were obtained if IC inoculation was made into irradiated animals.

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Characteristics of Rumen Pigment in Lambs Fed Pelleted Rations.* (28304)

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The development of a condition in lambs fed pelleted rations called parakeratosis was described by Jensen *et al.*(1). The affected papillae were dark in color, and, microscopically, the epithelium was thickened. There also were excessive layers of keratinized nucleated cells on the surface. Vidacs *et al.*(2) suggested that the changes in the epithelium were due to chemical factors rather than to the physical characteristics of the ration. The pigment associated with the condition in lambs has not been characterized. The objectives of this work were to study the histological changes in rumen epithelium associated with the feeding of pelleted rations and to characterize the pigment associated with these changes.

Materials and methods. Trial 1. Three lambs were randomly selected from 7 lambs on each of the following treatments for collection of rumen tissue specimens at time of slaughter. The lambs had been on the experimental ration for a 60-day period. The treatments were (1) conventional ration composed of shelled corn, long alfalfa hay and supplement; (2) ration 1, ground and mixed (molasses added); (3) ration 2, pelleted; (4) ration 2 plus minerals and vitamins, pelleted; and (5) ration 2 plus bicarbonate, pelleted. As quickly as possible after slaughter, speci-

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