resent well defined disease categories, but rather patterns of abnormal behavior grouped together by clinical designations of practical usefulness, but not of well defined etiological and theoretical significance. It is possible that the differences in serum proteins demonstrated in this study correlate with as yet unknown behavioral and metabolic differences rather than with specific diagnostic categories.

It will be of considerable interest to determine in future studies whether or not significant variations in serum proteins could be demonstrated in those individuals whose behavior disorders are manifested by violence and criminality.

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Oncogenic Activity of Adenovirus 12 in Thymectomized BALB/c and C3H/HeN Mice. (29535)

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Human adenovirus 12 is oncogenic in Syrian hamsters(1,2) rats(3), C3Hf/Gs and C3H/Bi mice(4,5) and *Mastomys*(5). Yabe and his associates(4) also inoculated DBAf and Af mice with adenovirus 12; however, no tumors were produced. The present report describes the results of inoculation of adenovirus 12 in newborn BALB/c, C3H/HeN, dba and AL/N mice. Tumors were produced in the BALB/c and C3H/HeN mice, but this occurred only in animals thymectomized on the day of birth.

Materials and methods. Animals: Pregnant C3H/HeN, BALB/c, AL/N and dba mice were obtained from the Animal Production Unit, Nat. Inst. of Health. *Virus*: Human adenovirus 12 was obtained from American Type Culture Collection. It was passaged and titrated in primary cultures of human embryo kidney (HEK) using methods described previously(5). *Methods of inoculation*: Newborn animals (less than 24 hours old) were inoculated subcutaneously with 0.05 ml of virus preparation. *Thymectomies*: Some litters of newborn BALB/c and C3H/HeN mice were thymectomized within a few hours of birth. With aseptic technique, the skin and sternum of the chest were incised and the thymus removed by forceps and, in some animals, by means of suction. The sternum and skin were then brought together and closed with collodion. Sham thymectomies were performed by incising and then closing the skin and sternums without removal of any tissue. *Necropsies*: Complete necropsies were performed on all animals killed or found dead. Tissues for histopathologic examination were obtained from all viscera and from the brain. They were fixed in formol-sublimate and stained with hematoxylin and eosin.

Results. In the first experiments, 4 litters each of newborn C3H/HeN, BALB/c, dba and AL/N mice were inoculated subcutaneously with $10^{7.15}$ tissue culture infectious doses ($TCID_{50}$) of adenovirus 12. 25 C3H/HeN, 28 BALB/c, 25 dba and 22 AL/N

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TABLE I. Inoculation of BALB/c Mice and C3H/HeN Mice with Adenovirus 12.

Operation	Animals	Inoculum (subcut)	No. of liters	No. of animals surviving inoculation	No. with tumors	Tumor latent period (mo)
None	BALB/c mice	$10^{7.15}$ $TCID_{50}$*	4	28	0†	—
Thymectomy	*Idem.*	$10^{7.15}$ $TCID_{50}$	4	30	5	2–4
Sham	"	$10^{7.15}$ $TCID_{50}$	2	17	0‡	—
None	C3H/HeN mice	$10^{7.15}$ $TCID_{50}$	4	25	0†	—
Thymectomy	*Idem.*	$10^{7.15}$ $TCID_{50}$	4	18	3	3–4
Sham	"	$10^{7.15}$ $TCID_{50}$	2	10	0‡	—

* Tissue culture infectious doses.
† After 13 mo observation.
‡ After 6 mo observation.

mice were inoculated. These animals have now been observed for over a year and no evidence of tumors has been noted.

After these animals had been observed for 4 months without evidence of tumor formation, it was decided to attempt to decrease the resistance of the animals by neonatal thymectomy. Accordingly, 4 litters each of BALB/c and C3H/HeN mice were thymectomized within 8 hours of birth. Two hours after thymectomy, these mice were inoculated subcutaneously with $10^{7.15}$ $TCID_{50}$ of the same pool of adenovirus 12 that had been used in the first experiment. In addition 2 litters each of BALB/c and C3H/HeN mice were subjected to sham-thymectomy within 8 hours of birth, followed by inoculation of $10^{7.15}$ $TCID_{50}$ of adenovirus 12. The results are shown in Table I.

None of the non-thymectomized or sham-thymectomized mice developed tumors. Of the thymectomized BALB/c mice 5 of 30 developed tumors between 2 and 4 months after inoculation. Three of the 18 thymectomized C3H/HeN mice developed tumors from 3 to 4 months after inoculation. At necropsy, these 8 animals had encapsulated, soft white tumors with central areas of hemorrhagic necrosis in the subcutaneous tissue of the back. No metastases were found. Histologically, these tumors were composed of sheets and cords of cuboidal cells with pleomorphic nuclei. They resembled the tumors previously described in hamsters, *Mastomys* and mice(1,2,4,5) and were considered to be "undifferentiated malignant tumors."

None of the thymectomized mice have shown any evidence of runting or "Wasting disease" and indeed the weights of thymectomized and non-thymectomized animals were the same. Since this suggested the possibility of incomplete thymectomy, the tracheal, and mediastinal areas were carefully studied but no evidence of residual or ectopic thymic tissue was seen.

Discussion. These results indicate that the susceptibility of 2 inbred strains of mice to adenovirus 12 may be increased by neonatal thymectomy. These results correlate well with reports that the oncogenic effect of polyoma virus is potentiated by neonatal thymectomy(6,7).

The role of the thymus in development of immune mechanisms and the effects of neonatal thymectomy in mice have been discussed by Miller(8). Since Habel(9) has shown that immune mechanisms are important in the ability of animals to reject transplanted polyoma-induced tumor cells, it seems likely that the development of tumors in our thymectomized mice is related to impairment of immunity with loss of ability to reject neoplastic cells induced by adenovirus 12.

None of the thymectomized mice in our experiments showed any evidence of "Wasting disease"(10). Histologic examination of the mediastinum and tissue around the thyroid gland failed to reveal any remaining or ectopic thymic tissue; however serial sections of the thyroid region were not prepared. Law, Dunn and their associates(11) have examined serial sections of the thyroid region in

neonatally thymectomized BALB/c mice and found ectopic thymic tissue in 7 of 13 animals. Although the presence of such ectopic thymus in our BALB/c mice might have accounted for the absence of "Wasting disease," the removal of all thymic tissue from the mediastinum significantly increased the susceptibility of the mice to the oncogenic activity of adenovirus 12. Another explanation for the absence of "Wasting disease" in our animals might be the absence of bacterial infection in the thymectomy wounds because of careful aseptic technique. Azar has recently presented evidence that the wasting in neonatally thymectomized Sprague-Dawley rats may be related to such infection(12).

Summary. Newborn BALB/c and C3H/HeN mice inoculated subcutaneously with human adenovirus 12 developed tumors only when thymectomized at birth. Tumors developed in 5 of 30 BALB/c and 3 of 18 C3H/HeN thymectomized mice. These results suggest that neonatally thymectomized animals may be useful in evaluation of the oncogenic potential of viruses which have not produced tumors when injected into non-thymectomized animals.

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Interaction Between the Soluble Antigen of PR8 and NWS Virus.* (29536)

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Work on multiplicity reactivation(1,2), recombination between ultraviolet irradiated virus and live virus(3), recombination between heat inactivated virus and live virus (4,5,6,7), and recombination between incomplete and complete virus(8) has shown that non-infective forms of a virus can enter a cell and contribute to the formation of new virus particles. These works indicated that inactivation of the infectious property of a virus need not mean that whatever information these virus particles contain is lost or is inaccessible to the cell.

The present investigation is an attempt to ascertain whether the non-infective nucleoprotein (soluble antigen) of influenza A (PR8) virus can transmit information to a susceptible cell and upon subsequent infection of this cell by a heterologous virus contribute to the formation of either genetically or phenotypically mixed particles.

Materials and methods. Viruses. Two egg-adapted strains, PR8 and NWS, of influenza type A were used. They were prepared by suballantoic inoculation of 10- to 11-day-old embryonated eggs with 0.05 ml of a 10^{-4} dilution of virus in beef-heart infusion broth. After 40-48 hours' incubation at 35.5°C, the infected allantoic fluids were harvested and stored in ampoules in a —70°C electrical deep freeze.

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