15. Appelbaum, E., Shtokalko, S., Ann. Intern. Med., 1957, v47, 346.

16. Newcomer, V. D., Sternberg, T. H., Wright, E. T., Reisner, R. M., J. Chronic Dis., 1959, v9, 354.

17. Kravetz, H. M., Andriole, V. T., Huber, M. A., Utz, J. P., New Eng. J. Med., 1961, v265, 183.

18. Andriole, V. T., Kravetz, H. M., J. Am. Med. Assn., 1962, v180, 269.

19. Thirumalachar, M. J., Radhakrishman, C. V., Padhye, A. A., Manrai, M., Gopalkrishman, K. S., Rahalkar, P. W., Hindustan Antibiotics Bull., 1964, v7, 74.

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Ependymomas Produced in Syrian Hamsters by Adenovirus 7, Strain E46 ("Hybrid" of Adenovirus 7 and SV40). (30568)

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Several human adenoviruses have been shown to grow poorly in monkey kidney cell cultures in the absence of simian virus 40 (SV40), and marked enhancement of adenovirus growth has occurred when SV40 was added to the cultures(1). After the discovery of SV40(2), it was found that the "monkey kidney cell adapted" strains of human adenoviruses used for vaccine production were mixtures of adenovirus and SV40. To obtain a strain of adenovirus 7 free of SV40, vaccine strain LL was passaged 3 times in African green monkey kidney (AGMK) cell cultures in the presence of high titered SV40 antiserum. After the 3 passages, infectious SV40 could no longer be detected in the preparations although the virus grew well in AGMK cell cultures. When this strain (designated E46, substrain of strain LL) was inoculated subcutaneously in newborn hamsters, it produced subcutaneous tumors which contained the neoantigen of SV40(3). Evidence has been presented by Huebner and his associates(3), Rowe and Baum(4), and Rapp and his associates(5) that the portion of the SV40 genome which codes for production of the SV40 neoantigen is incorporated in some way within the capsid of the adenovirus 7 virion.

The subcutaneous tumors produced by E46 were described as resembling SV40 tumors grossly and microscopically although some tumors "also showed islands of epithelioid cells similar to those regarded as characteristic for adenovirus 12 tumors"(3). Since both SV40 and adenovirus 7 produce subcutaneous tumors after subcutaneous inoculation in newborn hamsters(6,7) and since these tumors do have certain histologic similarities as well as differences, it is difficult to be certain of the role played by the portion of the SV40 genome in the E46 strain of adenovirus 7 in tumor induction. When SV40 is inoculated intracerebrally in newborn hamsters, however, it produces well differentiated papillary ependymomas(8) which are readily distinguished from other tumors which have been produced by any known strains of oncogenic adenoviruses(7). This suggested to us that the inoculation of E46 intracerebrally into newborn hamsters might determine whether the portion of the SV40 genome believed to be within the adenovirus capsid was capable of inducing the characteristic SV40 ependymoma.

Materials and methods. Animals: Pregnant Syrian hamsters (*Cricetus auratus*) were obtained from the Animal Production Section of Nat. Inst. of Health.

Viruses: The Gomen strain of adenovirus 7, obtained from the American Type Culture Collection, was passed in Hep-2 cell cultures. Strain E46 was obtained from Dr. Wallace P. Rowe. Two pools of E46 were prepared in our laboratory. One, designated E46H1, was the first passage of the original material in human embryonic kidney (HEK) cell cul-

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TABLE I. Titers of Adenovirus 7 Preparations Used for Hamster Inoculations.

Virus strain	Titer ($TCID_{50}$/ml) in AGMK	HEK
E46M5	$10^{6.9}$	$10^{8.2}$
E46H1	$10^{6.4}$	$10^{8.9}$
Gomen	$10^{8.2}$	$10^{9.4}$

tures. The other, E46M5, was the 5th passage of the original material in AGMK cell cultures. The virus pools were titrated in both AGMK and HEK cell cultures by methods previously described(9) and titers are shown in Table I.

Tests for infectious SV40: The E46M5 pool was tested for infectious SV40 in AGMK cultures. Equal volumes of the pool and adenovirus 7 antiserum (prepared in Balb/c mice immunized with Gomen strain grown in Hep-2 cell cultures) were incubated for 30 minutes at room temperature and inoculated into roller tube cultures of AGMK cells. The tubes were observed for 21 days.

Tumor tissue was tested for SV40 by placing 5 to 10 explants 1 mm in diameter on monolayers of AGMK cells in roller tubes. The tubes were observed for 21 days.

Methods of inoculation: Newborn animals, less than 24 hours old, were inoculated either intracerebrally or both intracerebrally and subcutaneously. The subcutaneous inoculation consisted of 0.05 ml of virus preparation and the intracerebral inoculation consisted of 0.01 ml of the same preparation.

Necropsies: Complete necropsies were performed on animals showing neurologic dysfunction or having large subcutaneous tumors. Tissues for histopathologic examination were obtained from all viscera and from the brain. They were fixed in formal-sublimate, sectioned and stained with hematoxylin and eosin.

Electron microscopy methods used have been described(9).

Results. Results of animal inoculations are summarized in Table II. Three litters of newborn hamsters were inoculated intracerebrally with the Gomen strain of adenovirus 7. Thirty animals survived inoculation and none have developed tumors or any evidence of neurologic dysfunction after 140 days of observation. Three litters were inoculated intracerebrally with E46H1 and 34 animals survived inoculation. Three of these animals became lethargic 102 days after inoculation and were killed with ether. At necropsy, grossly each had tumor nodules in the lateral ventricle. The remaining 31 animals do not show any abnormalities 180 days after inoculation. Four litters were inoculated both intracerebrally and subcutaneously with E46-M5. Forty animals survived inoculation and 12 developed subcutaneous tumors 30 to 45 days after inoculation. These animals were not killed and 4 subsequently became lethargic 94, 100, 101 and 102 days after inoculation. These 4 were killed and found to have tumor nodules in the lateral ventricle similar to those found in the group inoculated with E46H1. The subcutaneous tumors were soft, friable and white with hemorrhagic areas.

Histologically, 2 of the brain tumors produced after inoculation with E46H1 and one produced after inoculation of E46M5 were well differentiated papillary ependymomas (Fig. 1). One of the brain tumors produced after inoculation with E46H1 and 3 produced

TABLE II. Inoculation of Newborn Hamsters with E46 and Gomen Strains of Adenovirus 7.

Strain of virus	Inoculum	Site of inoculation	No. of animals inoculated*	No. of animals with ependymomas	No. of animals with subcutaneous tumors	Ependymoma latent period before CNS symptoms (days)	Subcutaneous tumor latent period before grossly observable (days)
E46H1	$10^{6.9}$ $TCID_{50}$†	I.C.	34	3‡	—	102	—
E46M5	$10^{6.2}$ " † $10^{6.9}$ " †	I.C. S.C.	40	4‡	12‡	94–102	30–45
Gomen	$10^{7.4}$ " †	I.C.	30	0§	0§	—	—

* Surviving 48 hr after inoculation. † Titrated in HEK cell cultures. ‡ After 180 days of observation. § After 140 days of observation.

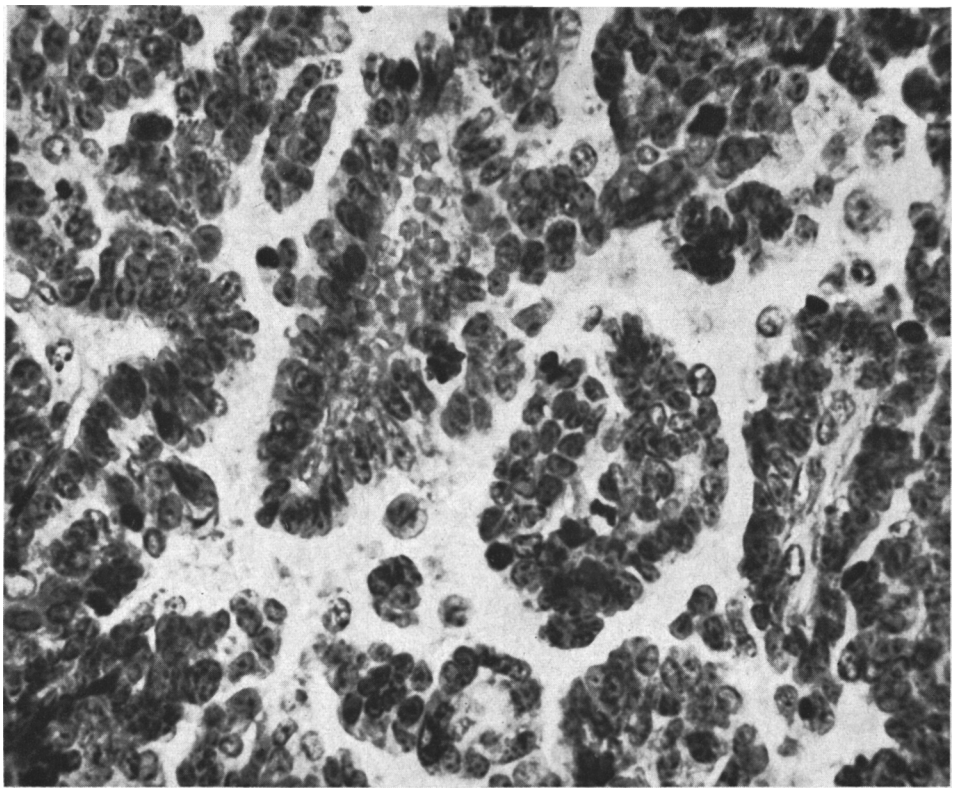

FIG. 1. Section of an ependymoma in lateral ventricle of brain of a hamster inoculated with strain E46 of adenovirus 7. Papillary architecture is clearly seen. Hematoxylin and eosin, 520 ×.

after inoculation of E46M5 were more poorly differentiated ependymomas. Electron microscopic study of one of the more poorly differentiated brain tumors showed the features of ependymoma cells such as cilia and blepharoplasts as described previously(10).

Microscopically, the subcutaneous tumors had the appearance of the undifferentiated malignant tumors previously described in hamsters, mice and mastomys inoculated with adenovirus 12(11,12).

Although Huebner and his associates(3) have presented evidence that E46 is free of infectious SV40 and it would be extremely unlikely that our E46H1 could have become contaminated with SV40, we thought that it would be desirable to rule out the presence of infectious SV40 in our E46M5 virus preparation. We were unable to detect SV40 by inoculation of AGMK cultures with E46M5 after neutralization of the adenovirus 7 by antiserum prepared against the Gomen strain. We were also unable to find SV40 particles electron microscopically in AGMK cultures inoculated with E46M5 and incubated for 72 hours. We could not isolate SV40 from one of the ependymomas induced by E46M5 even when explants of tumor were grown in contact with monolayers of AGMK cells.

Discussion. Huebner and his associates have shown that the E46 strain of adenovirus 7 contains the portion of the SV40 genome responsible for production of SV40 neoantigen(3). Since E46 grows well in monkey cells it also apparently contains the portion of the SV40 genome which produces enhancement of adenovirus growth in monkey cells(1). The present studies indicate that it also contains the portion of the SV40 genome responsible for induction of ependymomas in Syrian hamsters. It will of course be of great importance to determine whether

the neoantigen, the "enhancer" and the oncogenic factor which produces ependymomas are identical or different.

Although there is no evidence that the Gomen strain of adenovirus 7 can produce ependymomas, we have observed the animals for only 120 days and observation is continuing. We have not produced ependymomas in newborn hamsters inoculated with large doses of adenovirus 12 and observed for 2 years. The remote possibility remains that the E46 strain could produce ependymomas without the portion of the SV40 genome within its capsid. To eliminate this possibility, strains of E46 without the hybridized portion of SV40 must be shown to be incapable of inducing ependymomas. Such experiments are now in progress.

Summary. Strain E46 of adenovirus 7 produced ependymomas when inoculated intracerebrally in newborn hamsters. These tumors were morphologically identical to those produced by intracerebral inoculation of SV40 and unlike any tumors produced in hamsters by oncogenic adenoviruses. It has been previously shown that strain E46 is a "hybrid" virus containing the portion of the SV40 genome responsible for production of SV40 neoantigen within an adenovirus 7 capsid. The present results indicate that the "hybrid" contains that portion of the SV40 genome responsible for induction of ependymomas.

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1. Rabson, A. S., O'Conor, G. T., Berezesky, I. K., Paul, F. J., Proc. Soc. Exp. Biol. and Med., 1964, v114, 187.

2. Sweet, B. H., Hilleman, M. R., ibid., 1960, v105, 420.

3. Huebner, R. J., Chanock, R. M., Rubin, B. A., Casey, M. J., Proc. Nat. Acad. Sci., 1964, v52, 1333.

4. Rowe, W. P., Baum, S. G., ibid., 1964, v52, 1340.

5. Rapp, F., Melnick, J. L., Butel, J. S., Kitahara, ibid., 1964, v52, 1348.

6. Eddy, B. E., Borman, G. S., Grubbs, G. E., Young, R. D., Virology, 1962, v17, 65.

7. Girardi, A. J., Hilleman, M. R., Zwickey, R. E., Proc. Soc. Exp. Biol. and Med., 1964, v115, 1141.

8. Kirschstein, R. L., Gerber, P., Nature, 1962, v195, 299.

9. O'Conor, G. T., Rabson, A. S., Berezesky, I. K., Paul, F. J., J. Nat. Cancer Inst., 1963, v31, 903.

10. Rabson, A. S., O'Conor, G. T., Kirschstein, R. L., Branigan, W. J., ibid., 1962, v29, 765.

11. Trentin, J. J., Yabe, Y., Taylor, G., Science, 1962, v137, 835.

12. Rabson, A. S., Kirschstein, R. L., Paul, F. J., J. Nat. Cancer Inst., 1964, v32, 77.

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An Evaluation of the Variability in Measurement of Some Body Composition Parameters.* (30569)

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The measurement of body composition in gross biochemical terms has become increasingly important as an aid to clinical diagnosis and treatment(1). As better and simpler methods based on non-destructive dilution techniques or physical measurements have become available, these methods have been applied as routine procedures for the measurement of such parameters as total body water, extracellular water, blood volume, total circulating proteins, total exchangeable potassium and sodium and total body fat(2).

Survey of the literature has revealed very little information concerning the accuracy and reproducibility of the methods used. In studies of populations of well and sick subjects, the variations among individuals and

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