treatment with progesterone and estrogen combinations(10,11,12). Thus, the deciduogenic effects observed in the uteri of rabbits treated with ethynodiol diacetate correspond closely to the pre- or pseudodecidual changes observed in the endometrium of women. The effect of other synthetic steroids known to be effective in the treatment of menstrual disorders and control of ovulation is being evaluated by this procedure.

Summary. Ethynodiol diacetate (E.D.) was administered to immature estrogen-primed rabbits in an attempt to stimulate formation of decidual tissue in the uterus. No uterine trauma was employed in these experiments. DCR were obtained after treatment with 1, 2, 4, or 10 mg of E.D. per day for 5 or 6 days. Combination of mestranol, in doses of 0.05 to 0.3 mg per day, with as little as 0.1 mg E.D. resulted in the formation of DCR. When E.D. was administered by itself, doses of 2 mg per day were required to stimulate deciduogenesis and to maintain the response over a 15-day period. The similarity of the responses in humans and rabbits is discussed.

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1. Elton, R. L., Calhoun, D. W., Nutting, E. F., 1965, submitted for publication.
2. Saunders, F. J., Edgren, R. A., Drill, V. A., Endocrinology, 1957, v60, 804.
3. Drill, V. A., Fed. Proc., 1959, v18, 1040.
4. Elton, R. L., Nutting, E. F., Proc. Soc. Exp. Biol. and Med., 1961, v107, 991.
5. Elton, R. L., Nutting, E. F., Saunders, F. J., Acta Endocrinol., 1962, v41, 381.
6. Elton, R. L., ibid., 1965, submitted for publication.
7. Mossman, H. W., Contr. Embryol. Carneg. Inst., 1937, v26, 129.
8. Pincus, G., Rock, J., Garcia, C.-R., Ann. N. Y. Acad. Sci., 1958, v71, 677.
9. Noyes, R. W., Hertig, A. T., Rock, J., Fertil. and Steril., 1950, v1, 3.
10. Eichner, E., Golar, G. G., Reed, J., Gordon, M. B., Am. J. Obstet. & Gynecol., 1951, v61, 253.
11. Long, R. C., Bradbury, J. T., J. Clin. Endocrin. & Metab., 1951, v11, 134.
12. Durham, W. C., Fertil. & Steril., 1961, v12, 45.

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Hydrocephalus in Mice Infected with Polyoma Virus. (31003)

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Previously this laboratory reported(1) that a strain of polyoma virus isolated from Swiss mice bearing sarcoma 180 produced tumors in hamsters by intracerebral inoculation. However, when the virus was inoculated intracerebrally (IC) into 1-day-old Swiss white mice, 30% developed hydrocephalus and the virus was recovered from the brain tissue of the hydrocephalic mice. No tumors were observed in the Swiss white mice after IC or subcutaneous inoculation of the virus.

Hydrocephalus in mice and small rodents caused by various viruses has been reported earlier. Levaditi *et al*(2) and Jones *et al*(3) produced hydrocephalus in mice and Findlay (4) demonstrated that pleuropneumonia-like organisms in association with a neurotropic virus caused hydrocephalus in the same animal. Shortly after our previous report(1), Vandeputte(5) produced hydrocephalus in rats with SE polyoma virus while Huebner *et al*(6) and Yabe *et al*(7) observed that the intracerebral inoculation of type 12 adenovirus produced hydrocephalus in hamsters.

In an attempt to clarify the nature of the hydrocephalus observed in this laboratory when mice were inoculated with our strain of polyoma virus, histologic sections prepared from the brains of 115 Swiss mice inoculated IC were examined. A number of (C_3 H + A K R) F_1 mice were also inoculated with the

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same virus to test its oncogenic capability. The results are reported here.

Materials and methods. Isolation of polyoma virus. The strain of polyoma virus used in the present study was isolated in 1958 during a study of serial IC passages of sarcoma 180 in Swiss mice and hamsters(8). A DBA mouse bearing a subcutaneous sarcoma 180 was obtained from the Laboratory of Chemical Pharmacology, National Cancer Institute. For passage the tumor was minced and a 20% suspension by weight was made in Eagle's medium with 2% calf serum. The suspension was allowed to settle for 5 minutes and the supernatant fluid was inoculated IC into an adult C_3H mouse. The same technique was used on the brain tissue of this mouse and for subsequent serial IC passages in 1-day-old Swiss mice or newborn hamsters. The hamsters and Swiss mice (randomly bred and inbred stock) were obtained from the Animal Production Unit, National Institutes of Health. The inoculated mice and hamsters showed swelling of the head; circumscribed tumors and scattered tumor cells were present in the brain tissue.

After 18 passages in mice and 10 passages in hamsters,‡ the hamster brain suspension

DBA ⟶ M_1 ⟶ M_2 - - - - → M_{16} ⟶ H_1 ⟶
(with sarcoma 180) (C_3H) (Swiss white) (Hamster)

⟶ M_{17} ⟶ M_{18} ⟶ H_2 - - - - → H_{10} ⟶ ME_1

was freed of bacteria and tissue cells by passage through a sintered glass filter and was inoculated into monolayer cultures of primary mouse embryo cells (ME) in Eagle's medium with 1% calf serum. A cytopathic effect like that produced by polyoma virus was observed, and filtered fluid from these cultures produced polyoma type tumors in the brains of hamsters but only hydrocephalus in Swiss mice. This cell culture fluid contained a virus which was subsequently identified as polyoma virus (1). The virus was serially passed in primary ME monolayers and the 5th, 9th and 10th passages (ME_5, ME_9, ME_{10}) were used in the present study. More mice bearing sarcoma 180 were again obtained from the same source and the tumors were dissected out as soon as the mice arrived in our laboratory. Polyoma virus was again isolated in primary ME cultures. A strain of cultured sarcoma cells of different origin was examined and polyoma virus could not be isolated by this technique.

Inoculation of Swiss mice for pathological studies. The technique used in the present experiments for inoculation of polyoma virus into Swiss mice was the same as that described previously(1) except for the following modifications and additions. The virus inoculum consisted of the supernatant fluid of the ME_9 or ME_{10} passages of the polyoma virus. The culture fluid, containing $10^{4.2}$ $TCID_{50}$ of virus in a volume of 0.01 ml, was inoculated periorbitally into the parietal or frontal areas of the cerebrum of 1-day-old mice. The same virus-containing culture fluid was heated at 100°C for 20 minutes and was then used as a control inoculum. In addition other control mice were injected with either unheated normal ME culture fluid or sterile saline solution. Control and infected mice were sacrificed for pathologic study at weekly intervals from 3 to 190 days after virus inoculation.

The sacrificed animals were decapitated and the heads fixed in formalin or formol-sublimate solution. The heads were then decalcified and embedded in paraffin. Sections from different parts of the brain of each animal were taken and strained with hematoxylin and eosin or von Gieson's stain.

Results. Hydrocephalus in Swiss mice. One hundred and fifteen Swiss mice were inoculated with viable polyoma virus. Hydrocephalus occurred in 34/76 (45%) of the group inoculated into the parietal area and in 11/39 (28%) of those inoculated in the frontal lobe of the cerebrum (Table I). None of the 45 control animals developed hydrocephalus. A midline longitudinal section of the head of hydrocephalic mice exposed the enlarged ventricles (Fig. 1). Lateral ventricles were the primary site of the distention but 26/45 (Table I) of these animals had, in addition, dilation of the third ventricle. The fourth ventricle was infrequently (2/45) enlarged. Examination of the microscopic sections revealed that the distinctive lesions in the hydrocephalic mice were an inflammatory response as evidenced by perivascular infiltra-

‡ Passage in mice and hamsters were carried out according to the following schedule:

TABLE I. Occurrence and Localization of Hydrocephalus in Mice.

Material inoculated	Site of inoculation in cerebrum	Total No. inoculated	No. with hydrocephalus	Localization of hydrocephalus No. with enlargement of			
				Lateral ventricle	3rd ventricle	Aqueduct of Sylvius	4th ventricle
Polyoma virus	Parietal	76	34				
	Frontal	39	11				
	Total	115	45	45	26	0	2
Uninfected control fluid	Parietal or frontal	45	0	—	—	—	—

tion, hemorrhage and connective tissue proliferation. As shown in Table II the frequency of these lesions was greater in the hydrocephalic animals. None of the brain sections, which included serial sections of the aqueduct of Sylvius, showed neoplastic changes. No tumors were observed in the viscera of the hydrocephalic mice.

A comparison of the frequency of microscopic lesions in hydrocephalic and non-hydrocephalic mice disclosed marked differences (Table II). Perivascular infiltration (Fig. 2) occurred in 30 (66%) of the animals with hydrocephalus. Similar lesions were observed in only 7 (10%) of the non-hydrocephalic mice inoculated with viable virus, and in 4 (10%) of the control mice (inoculated either with heated virus suspension or plain TC fluid). Evidence of brain hemorrhage was observed in 22 (50%) of the hydrocephalic mice, compared to 11 (10%) of the mice inoculated with viable virus but without hydrocephalus and in 4 (8%) of the control mice. Proliferation of connective tissue in the meninges (Fig. 3) was present in 28% of the hydrocephalic mice, in only 5% of the infected mice without hydrocephalus and in 3% of the control mice. In some of these mice there were hemosiderin and calcium deposits in this connective tissue.

Tumors in (C_3 H + A K R) F_1 mice. To determine whether the strain of polyoma virus used here was still oncogenic, a separate experiment was kindly performed by Dr. L. W. Law of the National Cancer Institute. Four litters of 1-day-old (C_3 H + A K R) F_1 mice were inoculated subcutaneously with polyoma virus, each receiving 0.05 ml of ME_5 culture fluid. The mice were observed for 12 months, and 28% developed tumors during that period. Many of the animals became "runty" but recovered. All injected mice had hemagglutination-inhibition titers of 7200 against polyoma virus at weaning. Usually when (C_3 H + A K R) F_1 mice were inoculated subcutaneously with SE polyoma virus, pleomorphic tumors of mucous glands such as parotid and submaxillary glands were most frequently observed. However, with the strain isolated in this laboratory the tumors were, for the most part, subcutaneous fibrosarcomas and kidney sarcomas. There was only microscopic involvement of the salivary glands.

Discussion. In view of previous findings and present data, it seems safe to conclude that polyoma virus can cause hydrocephalus

TABLE II. Comparative Histopathology of Experimental Hydrocephalus in Mice.

Condition of mice	Total No.	No. mice showing:		
		Perivascular infiltration	Hemorrhage	Proliferation of connective tissue in meninges
With hydrocephalus (inoc with viable virus)	45	30	22	13
Without hydrocephalus (inoc with viable virus)	70	7	8	4
Without hydrocephalus (inoc with heated virus, plain TC fluid or saline)	45	4	4	1

in mice. It is highly unlikely that the trauma from inoculation alone could have induced the hydrocephalus since hydrocephalus was absent in control animals given identical inoculations with heat-killed virus, or normal ME culture fluids. Moreover, as reported previously(1), antipolyoma serum prepared in rabbits neutralized the capacity of the virus for production of both hydrocephalus in mice and brain tumors in hamsters.

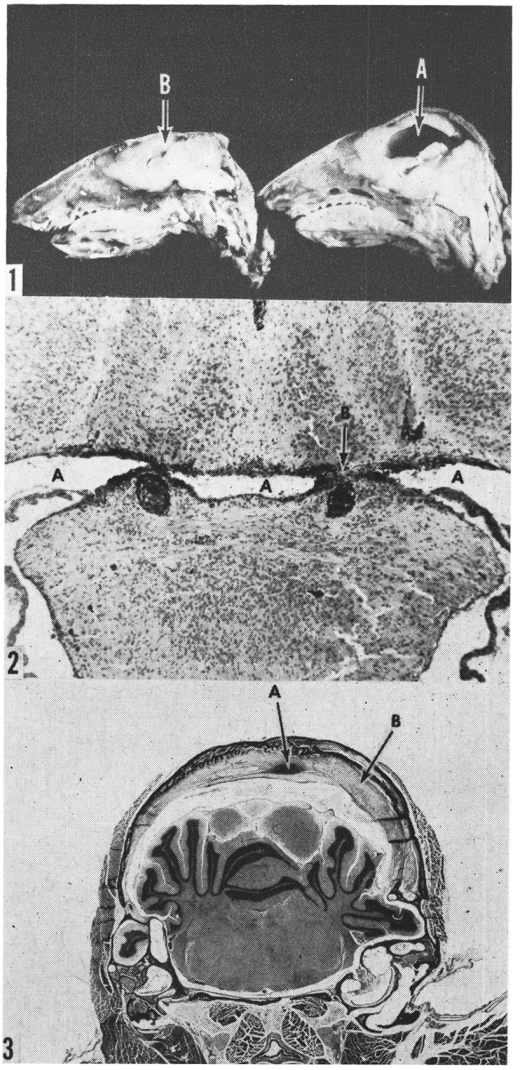

FIG 1. Mouse hydrocephalus (sagittal section following formalin fixation). Note enlarged lateral ventricle (A) in hydrocephalic mouse on the right, as compared to the same ventricle (B) in a normal mouse on the left.

FIG. 2. Microscopic section (× 33) of area of foramen of Monro (A) in 42-day-old mouse with hydrocephalus showing perivascular infiltration and compression of foramen (B).

FIG. 3. Microscopic section (× 4.62) through the head of a 61-day-old hydrocephalic mouse (2 months after virus inoculation). Arrow at A indicates calcium deposit in fibroblastic tissue (B).

Pathological examination disclosed evidence of an inflammatory response in the brain of the hydrocephalic mice. It is postulated that the inflammation produced subsequent obstruction to the flow of cerebrospinal fluid. The relative frequency of distention of lateral, third and fourth ventricles (Table I) suggests that the most likely area of obstruction was the aqueduct of Sylvius between the third and fourth ventricles, since 26/45 or 58% of the hydrocephalic animals had third ventricular enlargement. In the hydrocephalic animals, where only the lateral ventricles were enlarged, a functional block in the foramen of Monro (Fig. 3) between the lateral and third ventricles is suggestive but histologic sections did not show a clear-cut anatomic obstruction.

Some viruses have variants, one causing proliferation, the other necrosis and inflammation; and in still other cases the same virus can cause either neoplasms or non-neoplastic lesions depending on the host. Both of these phenomena have been described with the Rous sarcoma virus (RSV) by Duran-Reynals(9) and more recently by Groupe *et al*(10). Variants of polyoma virus with different oncogenic capacities have also been described(11,12, 13). The original strain of SE polyoma virus only rarely produced hydrocephalus in Swiss mice in our laboratory(1) and in another institution(14). It is therefore possible that the strain employed in the current experiments was a variant. The oncogenicity of the strain may have been modified during serial passages in animals or tissue cultures while the capability of inducing hydrocephalus was greatly increased. Modification of the virus is further suggested by the fact that our strain produced tumors in (C_3 H + A K R) F_1 mice different from those ordinarily produced by SE polyoma virus. An alternate hypothesis suggests that in the course of passage a non-oncogenic strain of polyoma virus was selected from a mixed population. Yabe *et al*(7) observed hydrocephalus in hamsters inoculated with adenovirus type 12 and suggested that a possible role of the virus in hydrocephalus

should be considered in view of the recovery of other types of adenoviruses from the cerebrospinal fluid of children suffering from certain neurological illnesses(15). This possibility is further strengthened by the observation by Russell(16) that in one form of hydrocephalus in young children there is gross dilation of the third and lateral ventricles, the fourth being normal in size, a phenomenon not unlike that found in the hydrocephalic mice as described above.

Summary. A strain of polyoma virus isolated during serial IC passages of sarcoma 180 in 1-day-old Swiss mice and newborn hamsters produced only hydrocephalus in white Swiss mice and neoplasms in (C_3 H + A K R) F_1 mice. It is postulated that the hydrocephalus was due to the inammatory response produced by this strain of polyoma virus.

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1. Li, C. P., Jahnes, W. G., Virology, 1959, v9, 489.

2. Levaditi, C., Schoen, R., C. R. Soc. de Biol., 1936, v122, 876.

3. Jones, H., Rake, G., Stearns, B., J. Inf. Dis., 1945, v76, 55.

4. Findlay, G. M., Klienberger, E., MacCallum, F. O., Mackenzie, R. D., Lancet, 1938, v235, 1511.

5. Vandeputte, M., Rev. Belg. Path. & Med. Exp. 1961, v28, 178.

6. Huebner, R. J., Rowe, W. P., Lane, W. T., Proc. Nat. Acad. Sci., 1962, v48, 2051.

7. Yabe, Y., Samper, L., Taylor, G., Trentin, J. J., Proc. Soc. Exp. Biol. and Med., 1963, v113, 221.

8. Li, C. P., Kirschstein, R. L., Jahnes, W. G., ibid., 1959, v101, 252.

9. Duran-Reynals, F., Yale J. Biol. & Med., 1940, v13, 99; ibid., 1950, v22, 555.

10. Groupe, V., Rauscher, F. J., Bryan, W. R., J. Nat. Cancer Inst., 1957, v19, 37.

11. Sachs, L., Medina, D., Nature (Lond.), 1960, v187, 715.

12. Gotlieb-Stematsky, T., Leventon, S., Brit. J. Exp. Path., 1960, v41, 507.

13. Law, L. W., Rabson, A. S., Dawe, C. J., Nature (Lond.), 1961, v190, 97.

14. Fox, J. R., Personal communication, 1959.

15. Faulkner, R., van Rooyen, C. E., Canad. Med. Assn. J., 1962, v87, 1123.

16. Russell, D., Medical Research Council, Special Rep. Series, 1949, No. 265.

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Electron Microscopy of Monkey Liver After Exposure of Animals To Pure Oxygen Atmosphere.* (31004)

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Electron microscopic studies of the livers of rats exposed to pure oxygen atmospheres at reduced pressures indicated that mitochondrial alterations occurred within a few days, persisted for a few weeks and then disappeared(1). Biochemical evidence for altered mitochondrial function was also obtained in the same group of animals(2,3). To determine whether observations in rats were applicable to other species, particularly primates, monkeys were exposed to pure oxygen atmospheres up to 3 weeks and their livers examined electron microscopically.

Material and methods. Male monkeys (Rhesus *Macaca mulatta*) were exposed in an altitude chamber to an atmosphere consisting of essentially 100% O_2 at a total pressure of 380 mm Hg. Surgical or needle biopsies were obtained on two monkeys on each of days 2, 3, 6, 8, 9, 15 and 22 of the oxygen exposure, and on days 10, 20 and 30 post

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