does, it is suggested that pitocin is effective in the presence of pitocinase.

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A Comparison of Some Properties of Polyoma Virus and Pneumonia Virus of Mice.* (27024)

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Polyoma virus (PY) and pneumonia virus of mice (PVM) are not related to each other serologically(1), but because of their similarity in size and their semi-ubiquitous presence in a latent form in mice, the possibility was considered that they belong to the same or related groups of viruses. Since sensitivity to ether serves to distinguish groups of viruses(2, 3), and since it is known that PY is ether resistant(4), the reaction of PVM towards ether was investigated. Ability of the 2 viruses to grow in tissue culture, and their *in vivo* effects on mouse lung were also compared.

Materials and Methods. Three strains of PY were used in these experiments. Strain LID-1 (5) was kindly provided by Dr. R. J. Huebner. Strain Lp or large plaque virus was derived by Dr. R. Dulbecco by single plaque isolation from a stock originally provided by Dr. W.P. Rowe(6,7). Sp or small plaque virus was isolated from transformed hamster embryo cells after exposure to Lp virus(7). Both Lp and Sp viruses were kindly provided by Dr. R. Dulbecco. The stocks of the various strains of polyoma assayed on secondary mouse embryo layers, according to the procedure of Dulbecco and Freeman(8), contained approximately the same number of plaque forming units, about $1x10^8$ PFU/ml.

A single stock of PVM was used. This had been prepared by Dr. F.L. Horsfall, Jr. in 1953 and had been stored at $-60°C$ since that time.

Standard procedures were used for intranasal infection of mice(9) and for preparation and infection of tissue cultures. Ether treatment was done according to the procedure of Andrewes and Horstmann(2).

Lung specimens were fixed in 10% formalin and stained with hematoxylin-eosin.

Results. Results of experiments on ether sensitivity of PVM are recorded in Table 1.

TABLE I. Ether Inactivation of Pneumonia Virus of Mice.

	Mouse ID50 per ml.	
Exp. No.	Control	Ether treated‡
1	7.5×10^4	No lesions at 10^{-2} dilution
2	5.8×10^4	idem. 10^{-1}
3	6.5×10^5	<7.0

‡ Virus was exposed to 20% ethyl ether for 18 hrs at 4°C.

Virus infectivity was measured by the ability to produce gross lesions in the lungs of weanling mice(9). The per cent of mice with lesions was plotted vs. dilution on probit paper and ID_{50}/ml was estimated graphically. In all experiments the virus appeared to be highly sen-

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sitive to ether. In Exp. 3 large numbers of mice were inoculated with undiluted ether-treated virus. Lesions were produced in only a few of these mice, indicating that inactivation was almost complete.

The ether sensitivity of PVM suffices alone to eliminate this virus from the group containing PY. Nevertheless, several other properties of PVM were compared with those of PY. Various cell types were exposed to undiluted PVM and then observed for 6-14 days to determine whether PVM causes cytopathic changes in cells which are susceptible to PY. Three cell types were employed,—cell line L-929(10,11), primary mouse embryo cell cultures, and primary mouse kidney cell cultures. After monolayers had formed, the cells were washed twice and exposed for 60 minutes to 0.4 ml of a virus suspension containing approximately $1x10^5$ mouse infectious units of PVM. After 2 further washings, nutrient medium was added to one set of plates and nutrient agar to the other set. Cells with nutrient agar were maintained up to 14 days and those with liquid medium up to 6 days. In no case was there evidence of a cytopathic effect.

Since PVM produces typical lesions in the lungs of weanling mice, the *in vivo* reaction of weanling mice to PY was tested. In a preliminary experiment a stock of LID-1 having a HA titer of 256 was used at a 1:5 dilution in phosphate buffer. Weanling mice were infected intranasally with 0.05 ml. After 9 days one set of 10 mice was sacrificed and their lungs inspected. Two mice had normal lungs, 6 had lungs of abnormal color, and 2 had extensive gross lesions. One month after infection the remaining mice were killed, serum prepared from the pooled blood, and the lungs removed and inspected. In this case 2 had normal lungs, 8 had extensive lesions, and 11 had smaller lesions and abnormal color. Representative lungs from both sets of mice were examined histologically and compared with mice exposed to PVM, to phosphate buffer alone, and to Pf broth containing 5% heated horse serum, the diluent for PVM. Lungs of mice exposed to phosphate buffer or to Pf broth were completely normal by both macroscopic and microscopic criteria.

Microscopically, the changes in the lungs of mice infected with PVM are typical of a viral pneumonitis with many scattered areas of edema of the bronchial, bronchiolar, and surrounding alveolar walls. An exudate filling the lumina of these structures consisted mostly of mononuclear cells, but also of a few polymorphonuclear cells and necrotic epithelial cells. In some areas there was an eosinophilic exudate with few cells, lining the alveolar walls. No hemorrhages were seen.

TABLE II. Effect of Dilution on Production of Lung Lesions by PY.

LID-1 was diluted in phosphate buffer. Weanling mice were infected intranasally with 0.05 ml per mouse. After 1½ months, mice were sacrificed and lungs were examined for lesions.

Dilution of virus	No. of PFU per .05 ml	Proportion of mice with lung lesions
1:5	1.8×10^6	14/27
1:50	1.8×10^5	5/8
1:500	1.8×10^4	0/8
1:5000	1.8×10^3	2/7
1:50,000	180	0/8
1:100,000	90	0/10

The lesions seen in the lungs of mice infected with PY were characterized by some congestion of the alveolar septa and large confluent areas of hemorrhage into bronchioles and surrounding alveoli. At times hemorrhages extended to the pleural surface. There was no evidence of collapse of alveoli nor was there evidence of an inflammatory response.

Hemorrhagic lesions were produced by LID-1 and Lp viruses, but Sp virus did not produce lesions, macroscopic or microscopic, even when used undiluted. The effect of dilution on number of lesions produced by LID-1 is shown in Table 2. Although it is clear that a moderately high dilution of virus results in formation of lesions, there are insufficient data to determine how many plaque forming units are needed to form a visible lesion.

The serum prepared from mice exposed to LID-1 did not have antibodies to PY. The HA inhibition titer of the serum was 1:64, which is not significant. Some lungs from mice exposed to 1:5 dilution of LID-1 were also harvested sterilely and homogenized as a 10% suspension in phosphate buffer containing 5% fetal calf serum. No infectious PY virus could be detected by plaque assay on monolayers of mouse embryo cells.

TABLE III. Comparison of Some Properties of Polyoma Virus and Pneumonia Virus of Mice.

1. Physical-chemical properties	PY	Reference	PVM	Reference
Size	45 mμ	12, 14	40 mμ	15
Relative heat sensitivity				
Stable at 60°C	Stable (30′)	13		
" " 56°C	"	13	Inactivated	16
Sensitivity to UV	Very insensitive	13	—	—
" " trypsin	Stable	13	Inactivated	1, 16
" " ether	"	4	"	*
2. Biological properties				
Immunological	Not related to PVM	1	Not related to PY	1
Hemagglutination	+	5	+	16
Lesions in mouse lung	Hemorrhagic	*	Pneumonitic	*
Cytopathic effect in mouse embryo cell cultures	CPE in 12-14 days	17	No CPE after 2 wks	*
Cytopathic effect in mouse kidney cell cultures	CPE in 4-5 days	18	No CPE after 2 wks	*

* Refers to this paper.

Discussion. A summary of comparative data on PY and PVM is presented in Table 3. All evidence clearly indicates that these viruses are unrelated to each other. The meaning of the hemorrhagic lesions produced by PY in mouse lung is not clear. The fact that mouse lung did not react to Sp virus may be in keeping with the generally lowered virulence observed with viruses of this type(19).

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