THE ENHANCING EFFECT OF URETHANE ON THE SEVERITY OF INFECTION WITH PNEUMONIA VIRUS OF MICE (PVM)*

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The factors which influence the severity of a virus infection, other than dosage and virulence of the virus, and specific immunity of the host, are at best but poorly understood. Evidence has been presented (1) that pneumonia virus of mice (PVM) or an antigenically similar agent may cause latent infection in a variety of animal species. Furthermore, it has been shown that the severity of this infection in mice is influenced by the strain of mouse employed (2), the age of the mouse (3), and the diet which it is fed before (4) and during (5) the period of infection.

In 1943 Nettleship, Henshaw, and Meyer (6) reported that urethane stimulated the development of pulmonary adenomas in susceptible strains of mice. This phenomenon was confirmed by Jaffé (7) and has been extensively studied in mice and rats by others (8–14). Because of this extraordinary effect on lung tissues, it seemed of interest to determine the influence of urethane on infection with the strictly pneumotropic virus PVM. The enhancing effect of urethane on the severity of this virus infection has been reported previously in abstract (15) and is more completely described below.

Materials and Methods

Mice.—Albino Swiss mice used in all the experiments with PVM were obtained from a commercial breeder, Mrs. Flora O'Grady. In some experiments concerning tumor development, strain A mice were employed. These were from our own breeding colony of mice originally obtained from the National Cancer Institute through the courtesy of Dr. C. D. Larsen. All mice were caged in glass jars on pine shavings, fed Purina dog chow, and given water to drink. Unless otherwise stated, the mice weighed approximately 10 gm. each at the time of infection with PVM.

Virus.—Pneumonia virus of mice, strain No. 15, was originally obtained from Dr. Frank L. Horsfall, Jr., and maintained by occasional intranasal passage in susceptible young mice. Infective extracts of the lungs were stored in a dry-ice chest.

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Virus Titrations.—The technique for measuring the infectivity titer of virus was similar to that employed by Horsfall and Curnen (16) and has been described in detail elsewhere (5). The methods for performing hemagglutination and hemagglutination inhibition tests were identical with those described by Curnen and Horsfall (17).

In some experiments the immunity of individual mice was determined by a microtechnique (18). Heparinized tail blood of the test mouse was mixed in a capillary tube with an equal volume of heated mouse lung extract containing four or more hemagglutinating units of PVM. The absence of erythrocyte agglutination indicated the presence of antibody in the test plasma.

Urethane.—Ethyl urethane (Merck and Co.) was employed throughout. Solutions were made in distilled water and stored in dark bottles at room temperature.

EXPERIMENTAL

Effect of Parenteral Urethane on PVM Infection.—The effect of a single parenteral injection of urethane the day before infection with PVM was tested in nine experiments.

Duplicate titrations of virus were made in two comparable groups of 20 mice, one of which received urethane while the other served as control, using a total of 300 mice. It was found that the intraperitoneal injection of 0.5 mg./gm. into 10 gm. mice resulted in slight and irregular stimulation of the infection. This is half the amount used by Nettleship *et al.* (6) in larger mice to stimulate tumor formation. With larger doses the effect on PVM was somewhat more marked. The 50 per cent maximum lesion score (M.S.50) in mice, each of which had received 2 mg./gm. the day before injection, was 0.97 log units higher than in controls. Uninfected mice given 3 mg./gm. or more of urethane died within a day or two of the injection and the lungs appeared hemorrhagic in the gross. However, uninfected mice, given 2 mg./gm. or less had lungs that were normal in the gross at the end of the 12 day experimental period used for testing PVM infection.

Effect of Intranasal Urethane on PVM Infection.—The effect of intranasal urethane was tested in 3 experiments.

0.05 cc. of urethane 10 per cent in water was inoculated into mice on the day after PVM was titered. The M.S.50 was slightly greater (average 0.48 log) in each instance in the mice which had received urethane.

Effect of Oral Urethane on PVM Infection.—Since urethane is excreted very rapidly, the majority of it in 24 hours (19), it was decided to test its effects when given in the drinking water throughout the entire 12 day experimental period. The virus titers observed in 27 experiments in mice receiving from 0.1 to 0.5 per cent urethane to drink are shown in Table I. It will be seen that the M.S.50 titers in the urethanized mice were consistently higher than in the controls. Moreover, as shown in Fig. 1, the increased titers observed vary directly with the concentration of urethane administered. It will be shown subsequently (Table IV) that the mice given 0.5 per cent urethane to drink actually ingested about twice as much of the drug as those given 0.1 per cent.

The Duration of Urethane Ingestion in Reference to PVM Infection.—The effect of urethane when drunk for varying periods before and after PVM inoculation was tested in a total of 23 experiments.

TABLE I

The Effect of Oral Urethane on PVM Infection

No. of experiment	Urethane		Control	Difference urethane from control
	Concentration in drinking water	M.S.50	M.S.50	
	per cent	*log*	*log*	*log*
1	0.1	2.00	1.11	+0.89
2	0.1	2.43	<2.00	>+0.43
3	0.1	4.13	3.19	+0.93
4	0.1	3.38	3.46	−0.08
5	0.1	3.22	2.46	+0.76
6	0.1	3.50	<2.00	>+1.50
7	0.1	3.39	3.29	+0.10
Average				>+0.52
8	0.2	3.34	3.29	+0.05
9	0.2	3.62	2.54	+1.08
10	0.2	5.00	3.81	+1.19
11	0.2	4.88	3.81	+1.07
12	0.2	3.30	2.73	+0.57
13	0.2	4.07	3.93	+0.14
14	0.2	>5.00	4.36	>+0.64
15	0.2	4.59	4.41	+0.18
16	0.2	4.51	4.24	+0.27
17	0.2	4.83	4.24	+0.59
18	0.2	>5.00	3.90	>+1.10
Average				>+0.63
19	0.3	4.05	3.59	+0.46
20	0.3	3.91	3.29	+0.62
21	0.3	>5.00	2.91	>+2.09
22	0.3	>4.00	3.11	>+0.89
23	0.3	>5.00	3.78	>+1.22
Average				>+1.06
24	0.4	4.29	2.73	+1.56
25	0.5	4.62	2.46	+2.16
26	0.5	5.80	<2.00	>+3.80
27	0.5	4.73	2.63	+2.10
Average				>+2.69

Duplicate titrations of virus in two similar groups of mice were done in the usual fashion. The control groups were given water to drink throughout the entire experimental period. The other groups were given urethane 0.2 per cent to drink starting from the 4th day before

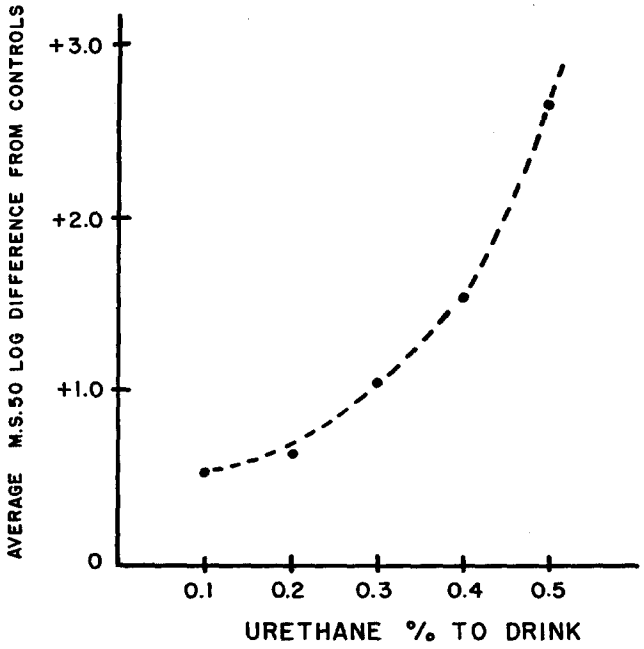

FIG. 1. Relation of urethane concentration in drinking water to enhancement of M.S.50 titers of PVM above the control titers. Averages of 27 experiments.

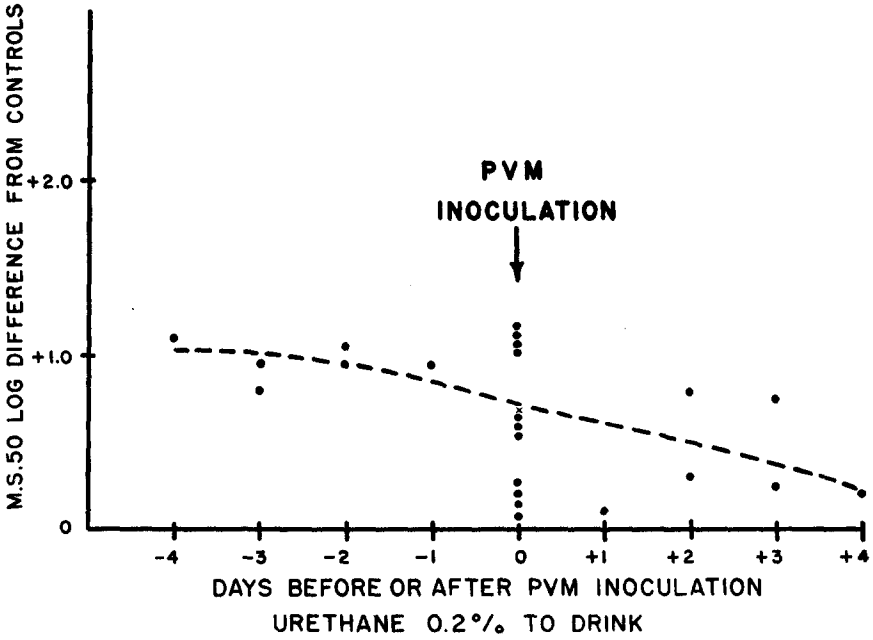

FIG. 2. Relation of the time and duration of urethane ingestion to its enhancing effect on PVM infection. Urethane 0.2 per cent was given the mice to drink on the day indicated and continued for the entire 12 day experimental period after PVM inoculation.

virus inoculation until the 4th day after. The differences between the M.S.50 titers in the urethanized mice from the control are plotted in Fig. 2.

It will be seen that, within the limits tested, the observed lesion scores varied directly with the duration of urethane ingestion. When urethane was given 4 days or more after virus inoculation, no effect was noted.

Effect of Urethane on the Lungs of Uninfected Mice.—Control mice which received the higher concentrations of urethane to drink (0.4 to 0.5 per cent), but which were not inoculated with virus, frequently died during the 12 day experimental period. At autopsy their lungs appeared edematous in the gross

TABLE II

Effect of Oral Urethane on the Titer of PVM (Experiment 27)

Urethane (0.5 per cent in drinking water)				Control (given water as such)				Dilution of PVM 0.05 cc. intranasally
D8*	D8	D8	D10	D8	D8	D9	S12	10^{-2}
++++	++++	++++	++++	++++	++++	++++	++	
D9	D10	D10	D10	S12	S12	S12	S12	10^{-3}
+++	++++	+++	+++	+	++	++	+	
D7	D11	D12	S12	S12	S12	S12	S12	10^{-4}
++++	++++	++++	+	+	0	0	0	
D12	S12	S12	S12	S12	S12	S12	S12	10^{-5}
++++	+++	+	0	0	0	0	0	
M.S.50 $10^{-4.73}$				$10^{-2.63}$				

D signifies death.

S signifies sacrifice.

* Number indicates the day following virus inoculation.

0 to ++++ indicates extent of consolidation.

with fluid running from the bronchi or cut surfaces. Moreover, the lungs often showed patches of plum colored consolidation which, on standing, resembled in the gross infection with PVM. In fresh specimens the lesions could be differentiated from PVM pneumonia by the greater prominence of the engorged superficial pulmonary vessels in the urethanized mice.

It is not believed that a simple summation of two indistinguishable lesions will account for the increased virus titers observed in urethanized mice since the drinking of lesser concentrations of urethane (0.1 to 0.3 per cent), which did not cause death or result in gross changes in the lungs of control mice, had enhancing effects on the lesion score. Moreover, the death of infected mice given 0.5 per cent urethane to drink obviously bore a time relationship

to the amount of virus inoculated, as is shown in Table II. Finally, as described below, more virus was present in the lungs of infected urethanized mice than in controls.

Nettleship *et al.* (6), Orr (10), and Cowen (11) have all remarked on the monocytic inflammatory lesions observed in the lungs of mice 8 or more weeks after the weekly injection of urethane, 1 mg./gm. These observations in addition to our own findings suggested that urethane administration might have resulted, in some unknown manner, in the activation of latent PVM. This possibility seems to be eliminated since lung extracts from uninfected mice, dying after the ingestion of 0.5 per cent urethane, did not produce pneumonia when inoculated intranasally into susceptible young mice. Moreover, heated extracts of the lungs from uninfected urethanized mice did not cause the agglutination of mouse erythrocytes. And, finally, the intranasal or intra-

TABLE III

Virus Titers in the Lungs of Mice Drinking Water Containing Urethane

Day after virus inoculation 10^{-5}	Experiment 1 Hemagglutinating titer (reciprocal)			Experiment 2 Infective titer log M.S.50		
	Urethane			Urethane		
	0	0.1 per cent	0.5 per cent	0	0.1 per cent	0.5 per cent
6	64	128	512	2.34	2.88	3.08
7	256	512	1024			
8	256	512	1024	3.18	3.50	4.47
9	32	128	Dead			Dead

peritoneal inoculation of immune anti-PVM rabbit serum, in amounts sufficient to completely protect the mice against PVM infection, did not protect them against death with pulmonary congestion and consolidation after the ingestion of 0.5 per cent urethane.

Effect of Urethane on PVM Titer in Lung.—All the observations described above, and summarized in Table I and II and Fig. 1, concern the lesion score in the lungs of mice infected with PVM. It seemed important to know whether urethane enhanced only the inflammatory reaction resulting from the virus infection, or whether virus growth was affected also.

To test this point 48 mice were inoculated intranasally with 0.05 cc. each of a 10^{-5} dilution of PVM. Sixteen of the mice were given water to drink, 16 urethane 0.1 per cent, and the remaining 16 urethane 0.5 per cent. On the 6th, 7th, 8th, and 9th days after virus inoculation, 4 mice from each group were sacrificed, their lungs pooled separately and extracted in the usual fashion. The amount of virus in these extracts was determined both by its infectivity titer and its hemagglutinating titer after heating. The results are recorded in Table III and Fig. 3. It will be seen that the highest virus titers in each group were present on the 7th and 8th days after inoculation and increased with the amount of urethane ingested. All mice

receiving 0.5 per cent urethane to drink had died before the 9th day so no determinations could be made on that day.

In a second similar experiment, also shown in Fig. 2, the hemagglutinating titers of heated lung extracts from urethanized and control mice were determined on days 2, 3, 4, 5, 6, 7, and 8 after virus infection. It was found in each group that virus, measurable by this technique, appeared on the 4th day after inoculation. The titer was always higher in the urethanized mice. As in the first experiment, all these mice died before the 9th day when the maximum virus titer was measured, whereas in the control group the titer had commenced to fall by that time.

It is concluded that urethane not only causes an increased lesion titer in PVM infection but also causes increased multiplication of the virus. Some

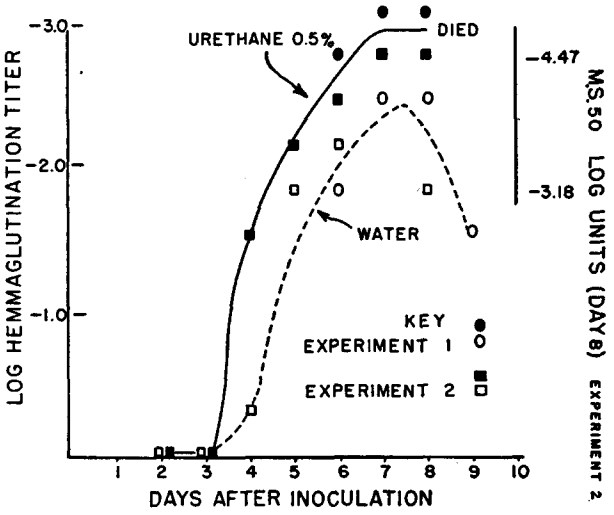

FIG. 3. Comparative virus titers in lungs of mice drinking water as such or containing 0.5 per cent urethane: a composite of 2 experiments.

experiments were planned in an attempt to elucidate the mechanism of these effects.

Effects of Urethane on Fluid Consumption and Weight Gain of Mice.—Urethane is a narcotic in doses of 1 mg./gm. and greater. Following the single intraperitoneal injection of half this dose into 10 gm. mice, they were somnolent for about ½ hour but then recovered and behaved normally, eating, drinking, and gaining weight like controls. When given increasing concentrations of urethane from 0.1 to 0.5 per cent to drink *ad libitum*, there was a progressive decrease in fluid consumption and in weight gain. In Table IV are recorded the measured fluid and urethane intake and weight changes in groups of mice observed over a 12 day period corresponding to the one usually used for studying PVM infection. It can be seen that control mice gained an average of 9

gm. over the 12 day period and drank on the average of 0.51 cc. of water per gm. per day. Those which received a single injection of urethane or drank 0.1 per cent urethane behaved much as the controls. Mice given 0.2 to 0.3 per cent urethane to drink gained only about 1 gm. in weight and drank about half as much fluid as the controls. Those given 0.5 per cent urethane actually lost 0.8 gm. in weight and drank only 0.17 cc. per gm. per day. Data concerning mice given a synthetic diet containing 4 per cent protein (5), such that their weight remained approximately constant, and other mice given water limited to the amount ingested by mice drinking 0.2 to 0.3 per cent urethane *ad libitum*, are also recorded in Table IV.

TABLE IV

Effects of Urethane on Growth and Water Consumption of Young Mice

No. of mice	Urethane	Average weight			Average water consumed		Average urethane
		Day 1	Day 12	Gain			
		gm.	gm.	gm.	cc./mouse/day	cc./gm./day	mg./gm./day
16	0 (Control)	9.6	18.6	9.0	7.2	0.51	
8	0.5 mg./gm. intraperitoneally on day 1	9.4	18.1	8.7	8.2	0.60	0.50
4	0.1 per cent in drinking water	10.0	18.9	8.9	6.2	0.43	0.43
4	0.2 per cent in drinking water	10.5	11.5	1.0	2.5	0.23	0.46
4	0.3 per cent in drinking water	10.0	11.2	1.2	2.9	0.27	0.81
4	0.5 per cent in drinking water	9.7	8.9	0.8	1.6	0.17	0.85
4	0*	10.4	10.9	0.5	11.0	1.00	
4	0‡	9.7	16.0	6.3	3.2	0.25	

* Synthetic diet containing 4 per cent protein.

‡ Water limited to 3.2 cc./mouse/day.

It seemed possible that simple limitation of growth or fluid intake might account for the changes in susceptibility to PVM noted in mice receiving urethane. It has been shown previously (5) that mice receiving the 4 per cent protein diet were equally susceptible to PVM as controls. It is possible, of course, that urethane intoxication may result in some other deficiency than limited protein. However, most acute deficiencies studied (4, 5) have, unlike the urethane effect, resulted in decreased susceptibility of the host to viral infections.

Sprunt (20) has reported that dehydration resulted in increased susceptibility of rabbits to infection with vaccinia virus. However, when duplicate titrations of PVM were done in mice receiving water limited to 2.5 to 3.0 cc./mouse/day, no differences in titers were found from those in control mice given unlimited water to drink.

Effects of Urethane on Lung Weights.—It was noted above that the lungs of

mice given the larger concentrations of urethane seemed congested and edematous. In 1922 Carrier (21) reported that the injection of urethane into human skin resulted in dilatation of both capillaries and arterioles. Löhr (22) found that this drug resulted in bronchodilatation and a marked vascular expansion in the lung. Winchester and Higgins (23) observed pulmonary edema in leukemic mice following treatment with urethane, and Rosin (14) observed similar changes in rats. To quantitate our observations the lungs of normal mice, of mice given various concentrations of urethane to drink, and of others fed a synthetic diet containing 4 per cent protein, were weighed at the end of 12 days. These weights and their relation to total body weight are recorded in Table V. It will be seen that the relationship of lung weight to body weight

TABLE V

*Effect of Urethane on the Weights of Lung, Spleen, and Thymus**

No. of mice	Urethane drunk	Average weight at 12 days						
		Total	Lung		Spleen		Thymus	
				Per cent of total		Per cent of total		Per cent of total
		gm.	*mg.*		*mg.*		*mg.*	
4	0 (Control)	21.8	211	0.97	101	0.46	50	0.23
4	0.1 per cent	18.9	201	1.06	60	0.32		
4	0.3 per cent	11.2	200	1.79	9	0.08		
4	0.5 per cent	8.9	207	2.33	—‡	—	8.4	0.01
8	0§	11.2	120	1.07	25	0.22		

* Mice each weighed 9.6 to 10 gm. at beginning of experiment and received urethane to drink for 12 days when they were sacrificed.

‡ Minute.

§ Diet contained 4 per cent protein.

was progressively greater in the mice given increasing amounts of urethane. These observations can be taken to support our impression that the lungs of urethanized mice became edematous and congested. The possible relation of these changes to the effects of urethane on PVM infection will be discussed subsequently.

Effects of Urethane on Lymphoid Tissues.—In addition to the gross changes in the lungs described above it was noted that the spleens and thymus glands of mice which had received urethane were smaller than in controls. Murphy and Sturm (24) had previously described the atrophy of lymphoid tissues in rats given urethane. In Table V the spleen and thymus weights of mice given various concentrations of urethane to drink for 12 days are recorded, together with the lung weights. The thymus glands of mice given 0.5 per cent urethane to drink were less than 20 per cent the size of controls. The spleens were pro-

gressively smaller in the mice given increasing amounts of urethane. This reduction in spleen size was considerably greater than could be accounted for by simple inanition.

Effect of Urethane on Antibody Production.—In view of the suppressing effect of urethane on lymphatic tissues, it seemed possible that this substance might exert its enhancing effect on PVM infection by interfering with the normal immune response.

To test the point 20 mice, approximately 6 weeks old, were inoculated intranasally with 0.05 cc. each of a sublethal dilution (10^{-4}) of PVM, and 2 days later they were reinoculated intraperitoneally with 0.1 cc. each of an undiluted virus suspension. Half of these mice were given 5 mg. each of urethane intraperitoneally on the 1st day of virus inoculation and 0.1 per cent urethane to drink for the remainder of the experimental period. The other half served as controls and received no urethane. Twenty-one days after the virus inoculation the mice were bled from the heart and the serum from each group pooled separately. The titer of each serum pool which would inhibit 4 hemagglutinating units of virus was tested by the standard method and the titers were found to be equal.

This experiment was repeated with two groups of uninfected mice and the native capacity of their sera to inhibit virus hemagglutination was again found to be equal. In another experiment virus was titered intranasally in two comparable groups of mice in dilutions from 10^{-5} to 10^{-10}. One group was given water to drink and the other urethane 0.5 per cent to drink for 6 days and then water. After 23 days antibody was measured in all surviving mice by the micro test described elsewhere (18). The majority of the urethanized mice receiving virus dilutions of 10^{-5} and 10^{-6} died from PVM infection before antibody was measured. Those receiving inocula of 10^{-7} and 10^{-8} developed a higher titer of antibody than controls. Moreover, the majority of the urethanized mice inoculated with virus dilutions of 10^{-9} and 10^{-10} developed antibody whereas none of the control mice receiving these higher dilutions of virus developed any antibody measurable by this technique.

It is concluded that urethanized mice can be infected with smaller amounts of virus than normal controls and that the enhancing effect of urethane on PVM infection is not to be explained by a suppression of the normal immune response to this virus.

Effect on PVM of Certain Substances Chemically or Pharmacologically Related to Urethane.—Larsen (8) tested the activity of a series of esters of carbamic acid, alkylated urethanes, and miscellaneous compounds for oncogenic activity and found them all inactive or not nearly as active as the parent substance, urethane. Cowen (25), using a small series of mice, reported that pentose nucleotides inhibited the carcinogenic properties of urethane. We have tested a few substances chemically related to urethane for activity against PVM. These included urea 0.5 per cent, methyl urethane 0.4 per cent, and uracil 0.37 per cent, all administered in the drinking water, and pentose nucleotide (Smith, Kline, and French Laboratories) intranasally or by repeated injection. None of these substances seemed to exert any clear cut effect on the virus infection.

Certain interesting pharmacological activities of urethane have been described by others. Its administration is said to result in lymphopenia (26, 27),

arrested mitosis (28), alkalosis (26), and antihistaminic action (29, 30). In view of these reports the effects of benzol, colchicine, sodium bicarbonate, and the antihistaminic drug dramamine (G. D. Searle and Co.) were all tested on PVM infection. Since no significant effect was noted in any case details will be omitted.

The Relation of PVM to Pulmonary Adenomas in Mice.—The discovery that urethane, which causes pulmonary adenomas to appear in mice, also enhances PVM infection immediately suggested that there might be some relation between the virus and the tumor.

To test the point two strains of mice were employed, the O'Grady strain used in this laboratory to study PVM infection and strain A mice which are most commonly employed in the study of adenomas. A variety of observations were made.

Both strains of mice proved equally susceptible to PVM infection. At the age of 6 months 31.3 per cent of 96 O'Grady mice and 15.1 per cent of 185 strain A mice had one or more spontaneous adenomas. In other mice of the same sort given 0.1 per cent urethane to drink for 7 weeks starting at 4 weeks of age, 93.8 and 100 per cent respectively had tumors when they were sacrificed at 6 months of age. PVM was recovered in each of two attempts on the 6th serial intranasal passage at 6 day intervals of lung extracts of O'Grady mice with or without urethane, but was not recovered from strain A mice in either of two tries after 12 serial passages. The intranasal inoculation of tumor extracts into each strain did not result in pneumonia resembling PVM infection nor in an increased incidence of adenomas at 6 months. Heated extracts of adenomatous lungs did not agglutinate mouse erythrocytes. Mice immunized intranasally and intraperitoneally against PVM developed adenomas almost as often as the controls, and immunization with extracts of the tumors did not protect mice against adenoma development or PVM infection. In individual mice there was no correlation between immunity to PVM, as measured by the micro test (18) at 6 months of age, and the presence of pulmonary adenomas. Finally, *Salmonella typhosa* extracts which we have found to suppress PVM infection (31), when inoculated three times at monthly intervals, had no suppressive action on the occurrence of adenomas at 6 months. It was concluded that the available evidence indicated no relationship between latent or overt PVM infection and the development of pulmonary adenomas.

DISCUSSION

The effects of urethane on bacteria have been widely studied *in vitro.* It has been shown to inhibit nitrate reduction by *Escherichia coli* (32), certain dehydrogenase systems of *Staphylococcus aureus* (33), and the respiratory systems of luminescent bacteria (34). Weinstein and McDonald (35) have described the bacteriostatic and bacteriocidal action of urethane on many species of bacteria. It was found (36) to inhibit a bacteriophage-resistant variant of *Staphylococcus aureus* in growth and

respiration to a greater extent than the parent strain. Little has been written on the effect of urethane on viruses. Bawden and Pirie (37) found that it inactivated the plant viruses of tobacco mosaic, tomato bushy stunt and potato "X" diseases. Fraser *et al.* (38) have shown that the thermal denaturation of tobacco mosaic virus is accentuated by urethane and Foster *et al.* (39) have found that urethane also accelerates the thermal inactivation of bacteriophage.

In the study of animal viruses the problem is greatly complicated by the effects of urethane on many systems of the host. Burney and Golub (40) found that urethane had no effect on psittacosis virus in tissue culture. Favilli (41) found that it inhibited infection of the rabbit's skin by vaccinia virus. Fiala, however, reported (42) that vaccinia virus, given intracerebrally or intravenously, resulted in both skin lesions and fatal encephalitis in rabbits receiving urethane subcutaneously, whereas control rabbits developed no apparent lesions or symptoms from the amounts of virus employed.

Our experiments show that urethane enhances the severity of the pulmonary infection of mice caused by PVM and leads to increased multiplication of the virus. A decrease of lymphoid tissues was observed in urethanized mice but the drug did not seem to impair the production of antibody. The mechanism of its action on PVM is unknown but may be related to the pulmonary edema it induces; for McClean (43), Olitsky and Schlesinger (44), and Sprunt (20) have shown that procedures which produce local edema may result in increased infection of the skin with vaccinia and herpes viruses. Or, the action of urethane may depend on increased cellular permeability. Anselmino and Hoenig (45) have reported that urethane caused increased permeability of the cell membrane of erythrocytes. Boyd and Perry (46) found that the bronchial secretions of urethanized cats contained 3 to 5 times as much potassium as those of decerebrate animals. This increase was accompanied by only a slight fall of serum potassium and might reflect an increased permeability of the membranes of cells lining the respiratory tract.

Finally, the possibility should be considered that urethane might mediate its effects on PVM indirectly by stimulating the adrenal glands. Murphy and Sturm (24) found the adrenal glands of rats given urethane to be 40 per cent larger than normal and we have observed, as described elsewhere (47), that the adrenal cortical steroids may enhance the severity of PVM infection in mice.

SUMMARY

Urethane, given parenterally or orally to mice, increased the severity of PVM infection. Not only were the lesions more extensive but mice could be infected with smaller inocula of virus and the multiplication of virus in the lung was enhanced. There was atrophy of lymphoid tissues but no suppression was noted of antibody formation. No relation could be found between PVM infection and the development of pulmonary adenomas in mice.

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BIBLIOGRAPHY

1. Horsfall, F. L., Jr., and Curnen, E. C., *J. Exp. Med.*, 1946, **83**, 43.
2. Horsfall, F. L., Jr., and Hahn, R. G., *J. Exp. Med.*, 1940, **71**, 391.
3. Unpublished data.
4. Mirick, G. S., and Leftwich, W. B., *J. Exp. Med.*, 1949, **89**, 175.
5. Leftwich, W. B., and Mirick, G. S., *J. Exp. Med.*, 1949, **89**, 155.
6. Nettleship, A., Henshaw, P. S., and Meyer, H. L., *J. Nat. Cancer Inst.*, 1943, **4**, 309.
7. Jaffé, W., *Rev. Policlin. Caracas*, 1944, **13**, 445.
8. Larsen, C. D., *J. Nat. Cancer Inst.*, 1946–47, **7**, 5; 1947–48, **8**, 63, 99; 1948–49, **9**, 35.
9. Haddow, A., and Sexton, W. A., *Nature*, 1946, **157**, 500.
10. Orr, J. W., *Brit. J. Cancer*, 1947, **1**, 311.
11. Cowen, P. N., *Brit. J. Cancer*, 1947, **1**, 401.
12. Smith, W. E., and Rous, P., *J. Exp. Med.*, 1948, **88**, 529.
13. Rogers, S., *J. Exp. Med.*, 1951, **93**, 427.
14. Rosin, A., *Cancer Research*, 1949, **9**, 583.
15. Leftwich, C. I., and Mirick, G. S., *Bull. Johns Hopkins Hosp.*, 1949, **85**, 171.
16. Horsfall, F. L., Jr., and Curnen, E. C., *J. Exp. Med.*, 1946, **83**, 25.
17. Curnen, E. C., and Horsfall, F. L., Jr., *J. Exp. Med.*, 1946, **83**, 105.
18. Smith, J. M., and Mirick, G. S., *J. Immunol.*, 1951, **67**, 539.
19. Bryan, C. E., Skipper, H. E., and White, L., Jr., *J. Biol. Chem.*, 1949, **177**, 941.
20. Sprunt, D. H., *J. Exp. Med.*, 1942, **75**, 297.
21. Carrier, E. B., *Am. J. Physiol.*, 1922, **61**, 528.
22. Löhr, H., *Z. ges. exp. Med.*, 1924, **39**, 67.
23. Winchester, W. W., and Higgins, G. M., *Science*, 1948, **107**, 568.
24. Murphy, J. B., and Sturm, E., *Cancer Research*, 1947, **7**, 417.
25. Cowen, P. N., *Brit. J. Cancer*, 1949, **3**, 94.
26. Hawkins, J. A., and Murphy, J. B., *J. Exp. Med.*, 1925, **42**, 609.
27. Paterson, E., Haddow, A., ApThomas, I., and Watkinson, J. M., *Lancet*, 1946, **250**, 677.
28. Dustin, P., Jr., *Brit. J. Cancer*, 1947, **1**, 48.
29. Katz, G., *Arch. exp. Path. u. Pharmakol.*, 1929, **141**, 366.
30. Farmer, L., *J. Lab. and Clin. Med.*, 1939, **24**, 453.
31. Smith, J. M., and Mirick, G. S., *Bact. Proc.*, 1950, **1**, 69.
32. Aubel, E., Schwazkoff, O., and Glaser, *Compt. rend Soc. biol.*, 1937, **125**, 223.
33. Bach, D., and Lambert, J., *Bull. Soc. chim. biol.*, 1938, **20**, 818.
34. Taylor, G. W., *J. Cell. and Comp. Physiol.*, 1936, **7**, 409.
35. Weinstein, L., and McDonald, A., *J. Immunol.*, 1946, **54**, 117.
36. Henry, J. E., and Henry, R. G., *J. Bact.*, 1946, **52**, 527.
37. Bawden, F. C., and Pirie, N. W., *Biochem. J.*, 1940, **34**, 1278.
38. Fraser, D., Johnson, F. H., Baker, R. S., *Arch. Biochem.*, 1949, **24**, 314.

39. Foster, A. C., Johnson, F. H., Miller, V. K., *J. Gen. Physiol.*, 1949, **33**, 1.
40. Burney, T. E., and Golub, O. J., *J. Immunol.*, 1948, **60**, 213.
41. Favilli, G., *Sperimentale*, 1933, **87**, 451.
42. Fiala, S., *Zentr. Bakt., 1 Abt., Orig.*, 1943, **150**, 260.
43. McClean, D., *J. Path. and Bact.*, 1930, **33**, 1045.
44. Olitsky, P. K., and Schlesinger, R. W., *Science*, 1941, **93**, 574.
45. Anselmino, K. J., and Hoenig, E., *Arch. ges. Physiol.* (Pflüger's), 1930, **225**, 56.
46. Boyd, E. M., and Perry, W. F., *Proc. Soc. Exp. Biol. and Med.*, 1944, **57**, 334.
47. Smith, J. M., Murphy, J. S., and Mirick, G. S., *Proc. Soc. Exp. Biol. and Med.*, 1951, **78**, 505.