rat following renin administration. A less spectacular, nevertheless, significant increase in the normal, spontaneous proteinuria of rats occurs following suitable doses of ACTH. If the animal is too large, or the administration of ACTH is not suitably timed, one may fail to observe any significant change from the normal in protein excretion. Though it might seem that the sharp fall in urinary volume in the hour following schedule C, renin administration was a manifestation of posterior pituitary contamination of the ACTH used, this possibility was discarded following assay of our ACTH preparations for anti-diuretic substances.¶ Further, we have recently demonstrated that a similar fall in post-renin urinary volume occurs in cortisone-treated rats. Other preliminary studies in our laboratory indicate that the fall in urine volume in ACTH and cortisone-treated animals given renin may be a result of actual renal tubular damage with plugging of the tubular lumina by protein casts. Along similar lines, Masson, Corcoran, and Page have reported renal damage in rats treated with saline, desoxycorticosterone acetate, and renin(7).

The mechanism by which ACTH augments proteinuria in the rat is not indicated by the data presented here.

Summary. 1. Normal rats respond to the intraperitoneal injection of renin with an intense, transient proteinuria. ACTH administration results in a 60 to 65% increase in proteinuria in the hour following renin administration over and above that shown by non-ACTH treated controls. 2. The normal, spontaneous proteinuria of the male rat is significantly increased by ACTH administration.

¶ The assays were performed by Dr. Irby Bunding of Armour and Co., Chicago, Ill.

7. Masson, G. M. C., Corcoran, A. C., and Page, I. H., *Fed. Proc.*, 1951, v10, 90.

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Effect of Intranasally Administered Immune Serum on Influenza Virus Present in the Mouse Lung.* (18908)

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Previous studies(1) have indicated that when mice are inoculated intranasally with small amounts of influenza virus, death occurs only in those instances in which the concentration of virus in the lungs reaches a certain level. However, when significantly larger amounts of virus are inoculated, death may occur within 2 to 4 days, but the maximum concentration of virus attained in the lungs is usually less than the critical concentration of smaller doses and, furthermore, may be less than frequently is found in the lungs of mice that have received sublethal doses. Hence, it was suggested that when the large amount of virus was inoculated the quantity introduced may, by itself, have been sufficient to kill the animal without subsequent multiplication of the agent. As part of a further investigation of this problem, a number of experiments were conducted comparing the effect of intranasally administered immune serum on the outcome of the infection, with the concentration of virus in the lungs of the mice at the time the serum was given. The purpose of this paper is to report the results of those experiments.

Materials and methods. A mouse-adapted line of the Cam strain of influenza A virus was used. The virus had been through 128 mouse passages at the beginning of the investigation and was maintained during the period of study by bi-weekly passage in mice. At each passage a number of 9- to 12-day-old

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1. Sugg, J. Y., *J. Bact.*, 1950, v60, 489.

embryonated eggs were inoculated intra-allantoically with a 10^{-3} dilution of the infected mouse lungs and were then incubated for approximately 48 hours at 35°C. After chilling, the allantoic fluids were harvested and were used as the source of virus; dilutions were prepared in 10% normal horse serum-distilled water. Between 0.08 and 0.1 ml of virus suspension was used as the infecting dose, and, the inoculations were made with the animals under light ether anesthesia. The mice were from a colony of albinos that had been maintained in this laboratory for over 14 years, and were approximately 4 weeks of age at the time of inoculation.

A single pool of serum, obtained from rabbits that had been immunized by 3 or 4 intraperitoneal injections of infected allantoic fluids, was used throughout. Each of the serum-treated mice received 0.06 ml of undiluted serum intranasally.

For each virus titration, 3 animals were sacrificed, the lungs pooled and ground in a mortar with alundum; sterile distilled water was added to make a 10% suspension. The suspensions were centrifuged at low speed for 5 to 10 minutes, and the supernatant fluids were removed and tested immediately. Tenfold dilutions of the fluids were prepared in infusion broth containing penicillin, and 0.1 ml of each dilution to be tested was inoculated into the allantoic sac of each of 4 to 7 (usually 5) 9- to 13-day-old fertile eggs. After 48 to 65 hours' incubation at 35°C, allantoic fluid was obtained from each egg and tested for the presence of hemagglutinins The egg-infectious titers are expressed as the reciprocal of the log of the 50% endpoint as calculated by the method of Reed and Muench(2).

Experimental. For each experiment a number of mice were inoculated intranasally with a selected virus preparation and were then divided into 2 groups. One group was held for observation as a control on the virulence of the inoculum (untreated mice), and the second group was treated with antiserum either 1 or 24 hours later in order to determine its effect upon the outcome of the infection. Comparison with the concentration of virus in the lungs of the mice was made by sacrificing 3 of the untreated animals and determining the virus titer as described under "Methods." When the serum was given 1 hour after infection, the animals to be used for the titrations were sacrificed 1 to 2 minutes following inoculation. This procedure was followed because it seemed important to allow adequate time for virus-cell combination to take place and, in addition, to base the comparison on the highest concentration of virus that had been present in the lungs; previous tests had indicated that the titer never increased but frequently decreased during the first 4 hours. When the comparisons were made 24 hours after infection, the mice were sacrificed for virus titration at the same time that the serum was given. In some experiments (Table II) additional virus titrations were made daily for a period of 3 days following the administration of serum in order to determine its effect upon the subsequent multiplication of the virus. All experiments were terminated on the 14th day.

Results. The data in Table I show that when the mice were inoculated with sufficient virus to produce immediately thereafter an egg-infectious titer of $10^{-5.6}$ or more in the lungs, the intranasal administration of immune serum 1 hour later had little or no effect in preventing the death of the mice. In contrast, when the mice were inoculated with relatively small amounts (10^{-5} dilutions) of virus and were not tested until 24 hours later, the same amount of the same serum protected either all, or the majority of the animals, even though those animals frequently had between 10 and 100 times as much virus in their lungs at the time of the administration of the serum as did the mice that had received the larger doses of virus and that were not protected. Thus, it is evident that the amount of virus required to overcome the protective effect of the immune serum was less when all of the virus present had been introduced directly into the lungs than it was when the virus present in that tissue had been produced by *in vivo* multiplication.

The data in Table II show that this dif-

2. Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, v27, 493.

TABLE I. Comparison of Protective Effect of Intranasally Administered Immune Serum with Concentration of Virus in Lungs of Mice 1 and 24 Hr after Infection with Different Doses of Virus.*

1 hr after infection			24 hr after infection		
Infecting dose	Egg-infectious titer of lungs†	Protection with immune serum	Infecting dose	Egg-infectious titer of lungs	Protection with immune serum
Undil.	6 ‡	0/7 §	10^{-5}	6.4‡	10/10§
,,	6	0/10	,,	6.6	6/7
,,	5.8	0/8	,,	6.7	7/7
,,	5.7	1/7	,,	6.8	5/8
,,	5.6	2/11	,,	7	7/8
10^{-1}	4.8	7/8	10^{-4}	7.8	0/7
10^{-2}	4.2	8/8	,,	8.4	0/7

* The virulence of the test dose was controlled by including in each experiment a number of infected mice that were not treated with serum. All of those animals died.

† The egg-infectious titers of the lungs were determined on animals sacrificed 1 to 2 minutes after infection; other tests indicate that the values shown are as high or higher than they would have been 1 hr later at the time the serum was given.

‡ Reciprocal of the log of 50% end point.

§ No. survived/No. inoculated.

TABLE II. Effect of Immune Serum Given Intranasally After Infection Upon the Multiplication of Virus in the Mouse Lung.

Infecting dose of virus	Hr before serum was given	Exp. No.	Treatment of infected mice	Egg-infectious titer of lungs hours after serum was given 0	24	48	72	Results of infection in mice
Undil.	1	1	none	5.5*	6.5	6.5	7.2	0/10†
			serum	—	6.7	6.5	6.7	1/10
		2	none	6	6.8	6.5	7.3	0/10
			serum	—	6.5	6.5	6.3	0/10
		3	none	7	8.1	7	7.2	0/12
			serum	—	7.4	7.3	6.3	0/12
10^{-5}	24	4	none	6.3	8.2	8.3	8.5	0/10
			serum	—	2.5	7	7.3	10/10
		5	none	6.5	9.5	8.6	9	0/7
			serum	—	5.2	7.7	8	5/7
		6	none	6.7	8.3	8.3	9.5	0/11
			serum	—	7.2	7.3	8	7/11

* Reciprocal of log of 50% end point.

† No. survived/No. inoculated.

ference in the effectiveness of the immune serum was not due to the amount of infectious virus that was subsequently produced in the lungs. Although the serum seemed to suppress virus multiplication to a greater extent when given 24 hours after inoculation of small amounts of virus than when given 1 hour after inoculation of the larger amounts, it will be noted that in the latter cases all but one of the animals died, even though the maximum titers observed in 2 of the 3 groups (Experiments 1 and 2) were less than the corresponding titers found in the serum-treated groups (Experiments 4, 5, and 6) which had been previously inoculated with the smaller virus doses and in which the majority of the animals were protected. These results would seem to indicate that when very large amounts of virus are used for infection death does not depend upon the subsequent multiplication of the virus.

However, when small doses were used for infection the protective effect of the immune serum apparently was related to the concentration of virus attained in the lungs. In those tests the maximum titer observed in one of the serum-treated groups (Experiment 4) was only $10^{-7.3}$ and all of the animals survived; but in the 2 other serum-treated groups (Experiments 5 and 6) the maximum titers found were $10^{-8.0}$, and in each of those groups some, but less than half, of the animals died.

These results are in agreement with those of the previous study(1) which indicated that if small amounts (less than 100 m.l.d.'s) of virus are inoculated intranasally, a titer of approximately $10^{-8.0}$ in the lungs represents a lethal concentration. It is suggested therefore that the immune serum protected those animals in which virus multiplication was suppressed sufficiently to keep the concentration in the lungs below that critical level.

Discussion. Immune serum introduced into the lungs of mice would not be expected to have any great effect on virus multiplication in previously infected cells, but it might be expected to interfere with the spread of the virus. Hence, the difference in the effectiveness of the immune serum in the two sets of experiments reported here might be due, at least in part, to a difference in the number of cells that were infected at the time the serum was given. It is probable that when large doses of virus were inoculated a majority of the lung cells were infected immediately, but that when small doses were used only a few cells were infected at the time of inoculation, and it is also possible that in the latter instances the virus produced during the first 24 hours was confined to a relatively small area of the lungs so that the serum was present before a critical number of cells became involved.

However, if the outcome of the infection was related to the number of lung cells that became infected, injury to those cells would not seem to have always depended upon virus multiplication. The results reported here, as well as those of experiments previously reported(1,3), strongly indicate that when very large amounts of virus are used for infection the extent of lung injury may be greater but the amount of infectious virus produced considerably less than when smaller doses are used. It is of course possible that when large doses are used there is produced, in addition to the infectious virus, a considerable amount of "immature" or "incomplete" virus similar to that which has been reported to be produced under certain conditions in embryonated eggs(4,5), and also in the brains of mice following intracerebral inoculation of non-neurotropic strains(6). But if particles of that sort occur in the mouse lung only when large doses are used for infection and not when smaller doses are used, it might be because the large amount of inoculated virus is toxic to the mouse lung and injures a majority of the cells to the extent that they are no longer able to support the reproduction of complete virus. And in that event, the injury produced at the time of inoculation might be sufficient to cause the death of the animal without any subsequent multiplication of the agent.

Summary. When mice were infected by the intranasal route with large doses of influenza virus and immune serum was introduced into the lungs 1 hour later, the animals died even though neither the amount of infectious virus in the lungs at the time the serum was given nor the amount subsequently produced was as great as the corresponding amounts found in the lungs of mice that had been infected with small doses of virus and that were protected when immune serum was given 24 hours later. The findings suggest that a large amount of influenza virus introduced directly into the mouse lung may, by itself, be sufficient to kill the animal without any subsequent multiplication of the agent.

3. Sugg, J. Y., *J. Bact.*, 1949, v57, 399.

4. Von Magnus, P., *Archiv for kemi, Mineralogi och geologi*, 1947, v24, 1.

5. Henle, W., and Henle, G., *J. Exp. Med.*, 1949, v90, 23.

6. Schlesinger, R. W., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 541.

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