THE SUSCEPTIBILITY OF SWINE TO THE VIRUS OF HUMAN INFLUENZA*

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Plates 45 to 48

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The similarity in the pathogenic properties of human and swine influenza virus for ferrets led Elkeles (1) to attempt the transmission of the human agent to swine. He found that very young pigs (2 to 6 weeks old), developed a mild illness when the virus from man was given intranasally under light ether narcosis. At autopsy these animals sometimes showed scattered dark red bronchopneumonic areas of consolidation in the upper lobes of the lung. When cultures of either swine or human influenza bacilli were added to the virus at the time of its administration, the swine developed a more severe illness. The clinical picture was characterized by a low grade fever, apathy, loss of appetite, and sometimes cough. At autopsy varying degrees of bronchopneumonia were encountered. Virus, pathogenic for ferrets, could be recovered from the pneumonic lungs. It thus appeared that Elkeles had produced a disease somewhat resembling swine influenza by the administration to young pigs of human influenza virus mixed with influenza bacilli of either human or swine origin. This observation made more credible the theory that swine influenza may have arisen as the result of the infection of swine in 1918 from human sources (2–4).

The question of the pathogenicity of human influenza virus for swine was of such importance that it seemed to warrant further investigation. The present paper reports our experience in the transmission of human influenza virus to swine.

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EXPERIMENTAL

The Infection of Swine with Human Influenza Virus Alone

The PR 8 (Francis (5)) and WS (Smith, Andrewes, and Laidlaw (6)) strains of the human influenza virus were used in our experiments. No difficulty was encountered in establishing either strain in swine 6 to 14 weeks of age. Older or younger animals were not tried. All animals were inoculated intranasally using the same methods employed in earlier studies of swine influenza (7, 8). The instillations were made with a Luer syringe without needle by pressing the tip of the syringe into the external nares. It was not necessary to anesthetize swine in order to induce infection with human influenza virus alone, although animals infected under ether narcosis exhibited somewhat more extensive pulmonary lesions at autopsy than those inoculated without anesthetic.

The WS virus was transferred 2, and the PR 8 virus 5, serial passages in swine before the experiments were discontinued. 10 per cent suspensions of infected mouse lung (5, 9) in physiological saline served as the source of virus for the first swine inoculation in each of the passage series and, after the first swine passage, similar suspensions of infected swine lung were used to inoculate swine of subsequent passages. 6.5 cc. of virus suspension was administered to each animal.

The disease produced in swine by human influenza virus was clinically very mild and, apart from a transient and indefinite malaise, no constant symptoms were observed. It was sometimes difficult to be certain on antemortem examination that the animals had actually been infected. In most cases there was no significant elevation in temperature though a few had fever on the afternoon of the day following inoculation. 4 animals were kept under observation for 3 weeks or longer and made uneventful recoveries. 8 others were killed either by stunning and bleeding or by chloroforming on the 3rd or 4th day after inoculation and these exhibited the following picture at autopsy. There was a scattered, patchy, lobular atelectasis of one or more of the anterior lobes of the lung (Figs. 1 and 2). On cut section the involvement about the bronchi was seen to be more extensive than was apparent from the uncut surface. The involved lung was a reddish purple in color, beefy in appearance, felt rubbery, and was moist. A rather scant glassy tenacious mucous exudate could sometimes be pressed from the cut bronchi.

Histologically in involved areas of lung the alveolar walls were folded, greatly thickened, and infiltrated with large numbers of mononuclear cells (Figs. 3 and 4). Capillaries in the alveolar walls were dilated and packed with red blood cells. The partially collapsed alveoli themselves were either free of cells or contained at the most an occasional desquamated epithelial cell or a few lymphocytes. Polymorphonuclear leucocytes were conspicuously absent. There was a pronounced peribronchial round cell infiltration. The bronchial epithelium was, in places, fragmented and partially desquamated, and the cilia were either denuded or badly matted together. Some bronchi contained a scant, mixed lymphocytic and polymorphonuclear leucocytic exudate. Lymph channels in the interlobular

septa were sometimes dilated and the septa themselves thickened and loosely infiltrated with mononuclear cells.

The trachea, bronchi, and lungs of swine infected with human influenza virus were in most instances bacteriologically sterile. The few animals from whose respiratory tracts organisms could be cultivated showed no evidence that their disease had been modified by the bacterial contaminant.

Influenza virus was demonstrated in the lung lesions of all passage swine by the inoculation of mice (5, 9). No evidence was obtained that serial transmission of the virus in swine modified its pathogenicity for mice. Also, the pathogenicity of the virus for swine appeared to remain constant through the number of serial passages to which it was submitted. It was of interest that the areas of swine lung that appeared free of lesions were also free of virus.

The symptoms and pathology observed in swine infected with human influenza virus were indistinguishable from those of the filtrate disease (10) produced in swine by the intranasal administration of swine influenza virus alone.

The Infection of Swine with Human Influenza Virus and H. influenzae suis

Sixteen swine have been inoculated intranasally with mixtures of human influenza virus and the bacterium, *H. influenzae suis* (11). These animals were included in two serial passage experiments. One series, started with virus from infected ferret lung (6), was continued for 5 passages, and the other, initiated with virus from infected mouse lung, was carried through 3 passages in swine before being discontinued. 6.5 cc. of 10 per cent virus suspension to which had been added 0.5 cc. of a 24 hour horse blood culture (11)[1] of *H. influenzae suis*, strain 18, was given intranasally to each animal. It was known from other experiments conducted at the same time as those under discussion that this culture of *H. influenzae suis*, in combination with swine influenza virus, was capable of producing characteristic influenza in swine (10).

In 11 of the 16 swine inoculated with mixtures of human influenza virus and *H. influenzae suis* the resulting illness was definitely more severe than that caused by the virus alone. This enhancement was evident both clinically and at postmortem examination. The temperature reactions were similar to those seen in swine influenza although usually the fever was less persistent. Seldom was it maintained for longer than 3 days, whereas 5 to 6 day fevers are not uncommon in swine influenza. Fever, while it lasted, was high, exceeding 41°C. on 1 or

[1] 0.5 to 1 cc. of sterile defibrinated horse blood added to a plain agar slant. In this medium *H. influenzae suis* grows largely in the blood at the base of the slant with only scant colony formation on the agar surface.

more days in most cases. It tended to be diphasic, frequently being lower on the 2nd than on either the 1st or 3rd day. The animals were depressed, their appetites diminished, and they lay listlessly in their pens. The extreme prostration seen often in swine influenza was not observed in any of the swine infected with human virus and *H. influenzae suis*. Symptoms referable to pulmonary involvement were present but less marked than in swine influenza; respiration was accelerated and the animals exhibited the peculiar type of diaphragmatic breathing best described as "thumping." None, however, appeared to be in particular respiratory distress. 2 animals kept under observation for 2 weeks made uneventful recoveries.

The remaining 9 of the 11 swine which had shown an illness greater than that caused by the virus alone were killed either by stunning and bleeding or by chloroforming on the 3rd or 4th day after infection. Influenza virus was demonstrated in the lungs of all by mouse inoculation and *H. influenzae suis* was isolated, usually in pure culture, from either the pneumonic lung or bronchial exudate of each animal. The respiratory tract lesions encountered at autopsy were similar in character to but less extensive than those seen in swine influenza. The trachea contained a scant to moderately abundant, thick, tenacious, glassy mucous exudate. A similar but more copious exudate was present in the bronchi and, in the smaller bronchi and bronchioles of pneumonic lobes of the lung, it completely filled the lumen. The pneumonia was of the same character as that seen in swine influenza but much less extensive. Seldom were more than two lobes involved, whereas in swine influenza an involvement of five lobes is the rule, and not uncommonly portions of all seven lobes may be pneumonic. The right cardiac lobe was most frequently affected, next the azygos lobe, and after that either the right apical or upper portion of the right diaphragmatic lobe. Lobes on the left side were seldom observed to be involved. The affected areas of lung were slightly depressed when compared with uninvolved lung and the line of demarcation between normal and pathological lung was sharp. The scattered areas of lobular atelectasis which gave a checker-board appearance to the lungs of animals infected with virus alone were replaced by a confluent pneumonia in which large numbers of adjacent lobules participated. Frequently the entire right cardiac lobe, or most of the azygos lobe, or the whole base of the right apical lobe was consolidated. The involved lung was a purplish red in color, felt firm and leathery, did not crepitate, and, when grasped with forceps, was friable in contrast to its usual rubber-like consistency. The cut surface was moist and the small bronchi which protruded exuded a thick glassy white mucous exudate. A cloudy serous fluid was yielded by the cut lung surface itself. Photographs of the lung of a pig infected with human influenza virus and *H. influenzae suis* are shown in Figs. 5 and 6.

Histologically sections of pneumonic lung cut in such a way as to include small bronchi and terminal bronchioles exhibited the following features: The cilia lining the smaller bronchi were either entirely gone or badly matted together. The bronchial epithelium was fragmented, in places partially desquamated, and,

scattered between the cells, were leucocytes singly or in clumps. The lumina of the bronchi were filled with a polymorphonuclear leucocytic exudate and the bronchial walls outside the epithelium were densely infiltrated with mononuclear cells (Fig. 7). The alveolar walls were thickened and infiltrated with mononuclear cells as were also the interlobular septa. The alveoli themselves contained red blood cells, leucocytes, and coagulated plasma (Fig. 8). Leucocytes were most abundant in the alveoli opening directly into the terminal bronchioles. Dilated capillaries in the alveolar walls were packed with red blood cells and widened lymph channels in the interlobular septa were filled with coagulated lymph and small numbers of cells

From the above descriptions it is apparent that the pathological process caused in the hog lung by infection with a mixture of human influenza virus and *H. influenzae suis* differs qualitatively as well as quantitatively from that produced by infection with either the swine or the human influenza virus alone, while it differs only quantitatively from that seen in swine influenza (infection with a mixture of swine influenza virus and *H. influenzae suis* (10)).

As stated earlier, only 11 of the 16 swine to which human influenza virus and *H. influenzae suis* were administered developed an illness more severe than that caused by the virus alone. The remaining 5 pigs exhibited symptoms indistinguishable from those encountered in swine infected with the virus alone. At autopsy the lungs of these animals showed lesions characteristic of those caused by the virus alone and cultures of the trachea, bronchi, and lungs failed to reveal the presence of *H. influenzae suis*. Influenza virus was, however, demonstrated in the lung lesions by mouse inoculation. Thus in these 5 swine, inoculated in the usual fashion with human influenza virus and *H. influenzae suis*, the virus but not the bacterium had become established in the respiratory tract. Instances of this nature have never been encountered in swine inoculated with swine influenza virus and *H. influenza suis*.

Attempted Transmission of Human Influenza Virus by Contact in Swine

One of the characteristic features of swine influenza, as seen in the field, is its extreme contagiousness. This high degree of communicability may also be demonstrated in animals experimentally infected with swine influenza virus and *H. influenzae suis*. In a series of experiments that is now large, the swine influenza virus has rarely failed to transfer from sick to normal swine by pen contact, although under certain conditions (12) the accompanying bacterium may fail to do so.

In four experiments to test the communicability of human influenza virus, normal swine were placed in the same pens with pigs inoculated intranasally with either human influenza virus alone or human virus mixed with *H. influenzae suis*. None of the 4 exposed animals developed clinical evidence of illness. The respiratory tract of one, killed on the 4th day following exposure, appeared normal at autopsy and its turbinates and lung, tested by mouse inoculation, were found to be free of virus. The remaining 3 pigs were kept under observation for 3 weeks. Their sera were then tested for ability to neutralize human influenza virus in mice. The serum of one animal neutralized the virus completely while that of the other 2 contained no demonstrable antibodies for human influenza virus. Thus, virus had been transferred by pen contact to only 1 of the 4 animals exposed. In this single case the disease was too mild to be recognized clinically although it did result in the establishment of specific virus-neutralizing antibodies. A comparison of this small group of human virus experiments with similar contact experiments in which swine virus was employed leads to the conclusion that human influenza virus is much less communicable in swine than swine influenza virus.

Cross-Immunity in Swine Convalescent from Infection with Swine or Human Influenza Virus

In cross-immunity experiments thus far conducted it has been found that swine recovered from swine influenza are immune to infection with a mixture of human influenza virus and *H. influenzae suis*. Experiments to test the immunity to swine influenza conferred by a preliminary infection with human influenza virus have indicated that the development of cross-immunity in this direction may, to some extent at least, be influenced by whether the initial infection was with human virus alone or with this virus and *H. influenzae suis*. The results of these experiments, which are still in progress, will be reported later. Sera of swine recovered from infection with swine influenza neutralize the swine influenza virus but exert little or no neutralizing effect on the human virus, while sera of swine recovered from infection with human influenza virus neutralize only the human agent (13).

Identification of Human Influenza Virus after Serial Passage in Swine

It was important in the present experiments to establish that the influenza virus transferred in series through swine was actually a human type agent and not a swine influenza virus accidentally introduced during passage. All possible precautions as to isolation of animals were practiced throughout the period of investigation and

experiments with swine influenza virus were discontinued so far as possible while the human virus was under study. The possibility of cross-infection, though remote, was nevertheless recognized and for this reason at the termination of the present study with the human virus in swine the following two experiments were conducted. First, known anti-swine and anti-human influenza virus immune sera were tested for their ability to neutralize the virus recovered from the 5th and last serial passage swine. The virus was neutralized by anti-human but not by anti-swine virus sera. Second, convalescent sera from three 5th passage swine were tested for their ability to neutralize human and swine influenza virus; they neutralized only human virus. It is clear from these results that the virus, recovered after serial passage in swine, was the human influenza virus entirely unaltered immunologically from that with which the studies were begun.

DISCUSSION

The present experiments confirm Elkeles' observation that swine are susceptible to human influenza virus. Contrary to Elkeles' observations, however, it was not necessary to use baby pigs or to anesthetize the animals in order to induce infections. The lack of agreement may possibly be due to differences in the natural susceptibility of the swine used in our experiments and the Dutch pigs employed by Elkeles. In this respect, it is of interest that *Ferkelgrippe*, an enzootic pneumonia of baby pigs prevalent in Germany, is believed by Köbe (14) and Waldmann (15) to have a complex etiology similar to that of swine influenza and is transmissible only to young pigs. It is possible, from consideration of Elkeles' work, that, as in the case of *Ferkelgrippe*, only the very young of European breeds of swine may react to infection with human influenza virus.

When the disease produced in swine by the combined action of human influenza virus and *H. influenzae suis* is compared with swine influenza, it is apparent that the two are similar qualitatively but different quantitatively. The increased severity of the pneumonia produced by the swine virus and bacterium in comparison with that produced by the human virus and bacterium seems to constitute a significant difference between the strains of human and swine influenza viruses studied. An explanation of this difference in the two viruses

is not evident but it may be that human virus possesses less power than swine virus to prepare an extensive area of lung for the invasion of *H. influenzae suis*. A second possible explanation may be that the present human influenza virus is inherently less capable of acting synergistically with a second agent than is swine influenza virus. The failure of *H. influenzae suis* to establish itself in the respiratory tracts of 5 of 16 swine to which it was given in admixture with human virus, as contrasted with its invariable establishment in swine when administered in combination with swine influenza virus, would support this second possibility.

Elkeles' experiments, and those presented here, have shown that a virus from cases of influenza in man is capable of infecting swine when administered intranasally, that the pathogenic properties of this virus are usually enhanced by the presence of *H. influenzae suis*, and that the resulting pneumonia is qualitatively similar to that seen in swine influenza. However, it seems unlikely, in view of its low communicability, that this recent strain of the human influenza virus could establish itself in swine and progress as the cause of any widespread or serious epizootic disease. In this respect, it is of interest that two strains of swine influenza virus (Iowa, 1934, and Ohio, 1935), recovered from hogs since the present human strain was known to be prevalent, are serologically the same as swine influenza virus strain 15 (Iowa, 1930) and thus serologically different (2, 13, 16, 17) from human influenza virus of the WS or PR 8 type.

As mentioned earlier, it has been suggested that swine were originally infected with influenza from man in 1918 and that the swine virus is the surviving prototype of the virus prevalent at that time in the human population (2–4). If it could be assumed, for the sake of the present discussion, that swine influenza etiologically is a replica of the human pandemic disease and that man and swine react alike to infection with virus and bacterium, then the differences, discussed above, in the pathogenicity for swine of the viruses of swine and recent human influenza might reflect differences between severe pandemic influenza as it occurred in 1918 and the recent milder interpandemic form from which both the PR 8 and WS strains of virus were obtained. The swine influenza virus, highly communicable and capable of causing an extensive pneumonia when acting synergistically with a second

organism, would be expected to result in a disease that varied both epidemiologically and clinically from that caused by the recent human influenza virus, an agent less capable of acting synergistically with a second organism and less communicable.

SUMMARY

Swine inoculated intranasally with human influenza virus alone develop an ill defined, mild, and usually afebrile illness of short duration. At postmortem the anterior lobes of the lungs of such animals contain scant, scattered areas of lobular atelectasis. Transmission of the virus for 5 serial passages through two groups of swine failed noticeably to enhance its pathogenicity for this species. The disease produced in swine by infection with human influenza virus alone is indistinguishable clinically and pathologically from that caused by infection with swine influenza virus alone. Transmission of human influenza virus from swine to swine by contact succeeded in only one of four attempts.

Swine inoculated intranasally with a mixture of human influenza virus and *H. influenzae suis* usually develop a febrile, depressing illness similar to mild swine influenza. The pneumonia encountered in such animals at autopsy is similar to but less extensive than that seen in swine influenza. In some animals *H. influenzae suis* fails to become established and the disease then seen is identical with that caused by human influenza virus alone.

The human influenza virus recovered after 5 serial transfers in swine was immunologically the same as that with which the experiments were begun.

BIBLIOGRAPHY

1. Elkeles, G., Mededeelingen uit het Instituut voor Praeventieve Geneeskunde, 1934, 60.
2. Laidlaw, P. P., *Lancet*, 1935, **1**, 1118.
3. Andrewes, C. H., Laidlaw, P. P., and Smith, W., *Brit. J. Exp. Path.*, 1935, **16**, 566.
4. Shope, R. E., *J. Exp. Med.*, 1936, **63**, 669.
5. Francis, T., Jr., *Science*, 1934, **80**, 457.
6. Smith, W., Andrewes, C. H., and Laidlaw, P. P., *Lancet*, 1933, **2**, 66.
7. McBryde, C. N., Niles, W. B., and Moskey, H. E., *J. Am. Vet. Med. Assn.*, 1928, **73**, 331.

8. Shope, R. E., *J. Exp. Med.*, 1931, **54**, 349.
9. Andrewes, C. H., Laidlaw, P. P., and Smith, W., *Lancet*, 1934, **2**, 859.
10. Shope, R. E., *J. Exp. Med.*, 1931, **54**, 373.
11. Lewis, P. A., and Shope, R. E., *J. Exp. Med.*, 1931, **54**, 361.
12. Shope, R. E., *J. Exp. Med.*, 1934, **59**, 201.
13. Francis, T., Jr., and Shope, R. E., *J. Exp. Med.*, 1936, **63**, 645.
14. Köbe, K., *Centr. Bakt., 1. Abt., Orig.*, 1933, **129**, 161.
15. Waldmann, O., *Berl. tierärztl. Woch.*, 1933, **49**, 693.
16. Smith, W., Andrewes, C. H., and Laidlaw, P. P., *Brit. J. Exp. Path.*, 1935, **16**, 291.
17. Francis, T., Jr., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32**, 1172.

EXPLANATION OF PLATES

PLATE 45

FIG. 1. Dorsal aspect of lung of swine infected with PR 8 strain human influenza virus alone. There is a scattered, patchy, lobular atelectasis of the right cardiac lobe and the upper portion of the right diaphragmatic lobe. Animal chloroformed on 3rd day after infection.

FIG. 2. Ventral aspect of same lung.

PLATE 46

FIG. 3. Section of lung of a swine infected with PR 8 strain human influenza virus alone showing folded, thickened alveolar walls infiltrated with mononuclear cells. The small bronchi are cuffed by accumulations of round cells. Animal chloroformed on 3rd day after infection. Phloxine-methylene blue. × 68.

FIG. 4. Higher power of above section to show round cell infiltration of the alveolar walls in an area of atelectasis. × 262.

PLATE 47

FIG. 5. Dorsal aspect of lung of swine infected with mixture of PR 8 strain human influenza virus and *H. influenzae suis*. There is an atelectatic pneumonia of the right cardiac lobe. Animal chloroformed on 3rd day after infection.

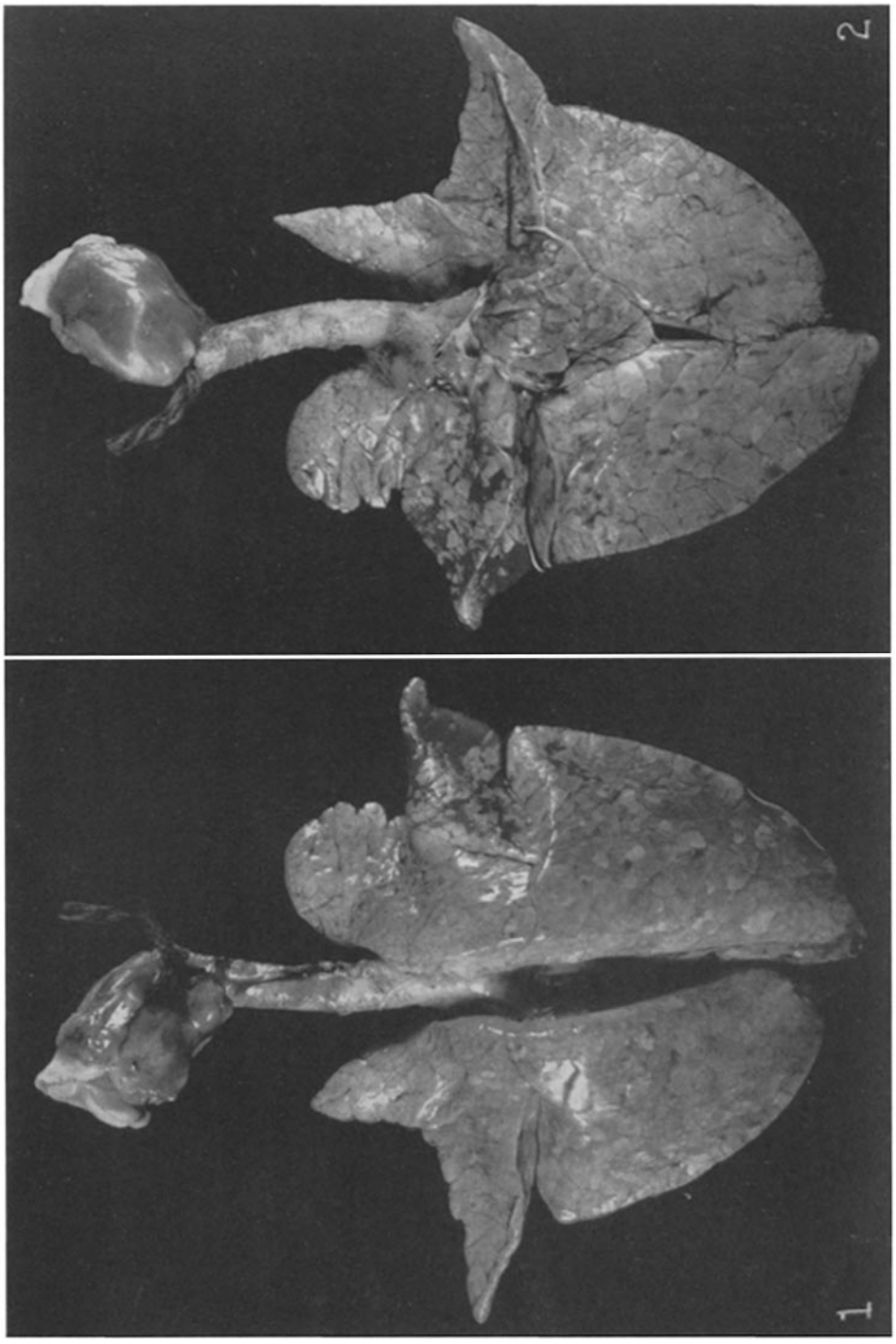
FIG. 6. Ventral aspect of same lung. The pneumonia involves all of the right cardiac lobe and lobular areas of the azygos and upper portion of the right diaphragmatic lobes.

PLATE 48

FIG. 7. Section of a small bronchus in lung of a swine infected with mixture of PR 8 strain human influenza virus and *H. influenzae suis* showing leucocytic bronchial exudate, fragmented and vacuolated bronchial epithelium denuded of cilia, and round cell infiltration of the submucosa. Leucocytes have invaded

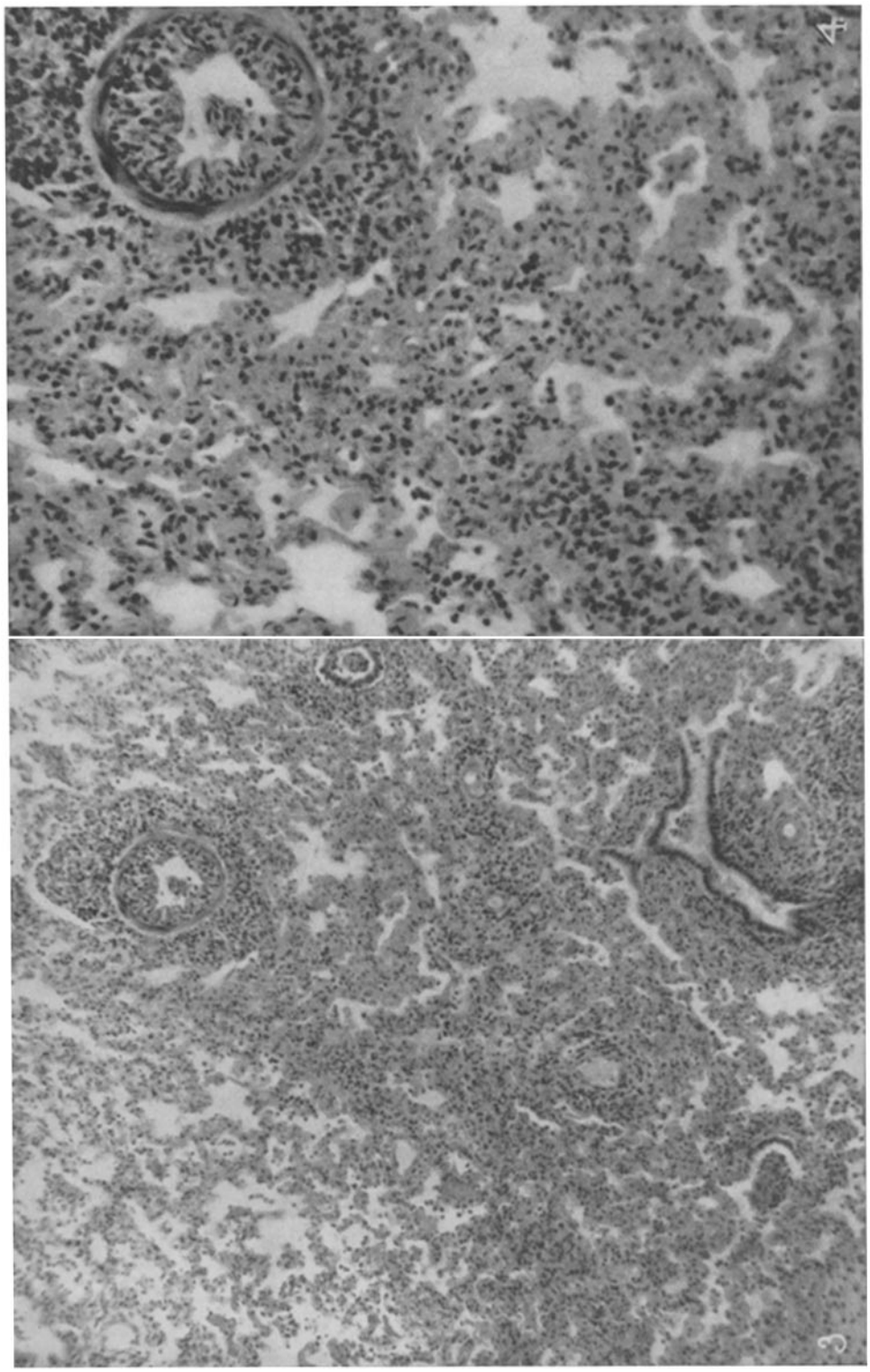
the mucosa. Animal chloroformed on 3rd day after infection. Phloxine-methylene blue. × 245.

FIG. 8. Section of lung of a swine infected with mixture of PR 8 strain human influenza virus and *H. influenzae suis*. The small bronchi contain a dense polymorphonuclear leucocytic exudate and are cuffed by round cells. The alveolar walls are thickened and infiltrated with round cells and some of the alveoli contain accumulations of polymorphonuclear leucocytes. Animal chloroformed on 3rd day after infection. Phloxine-methylene blue. × 56.



Photographed by J. A. Carlile

(Shope and Francis: Susceptibility of swine to human influenza)



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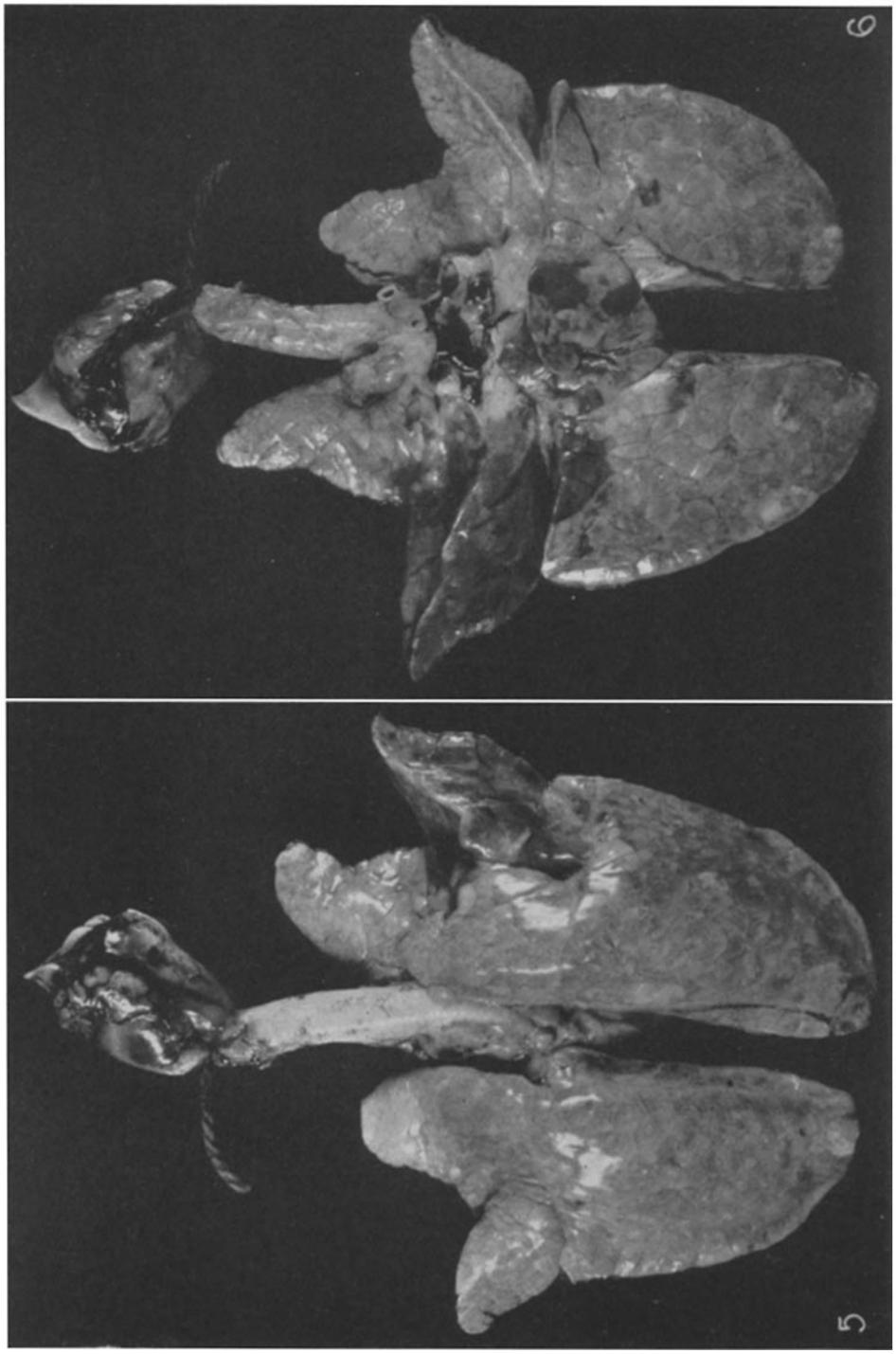

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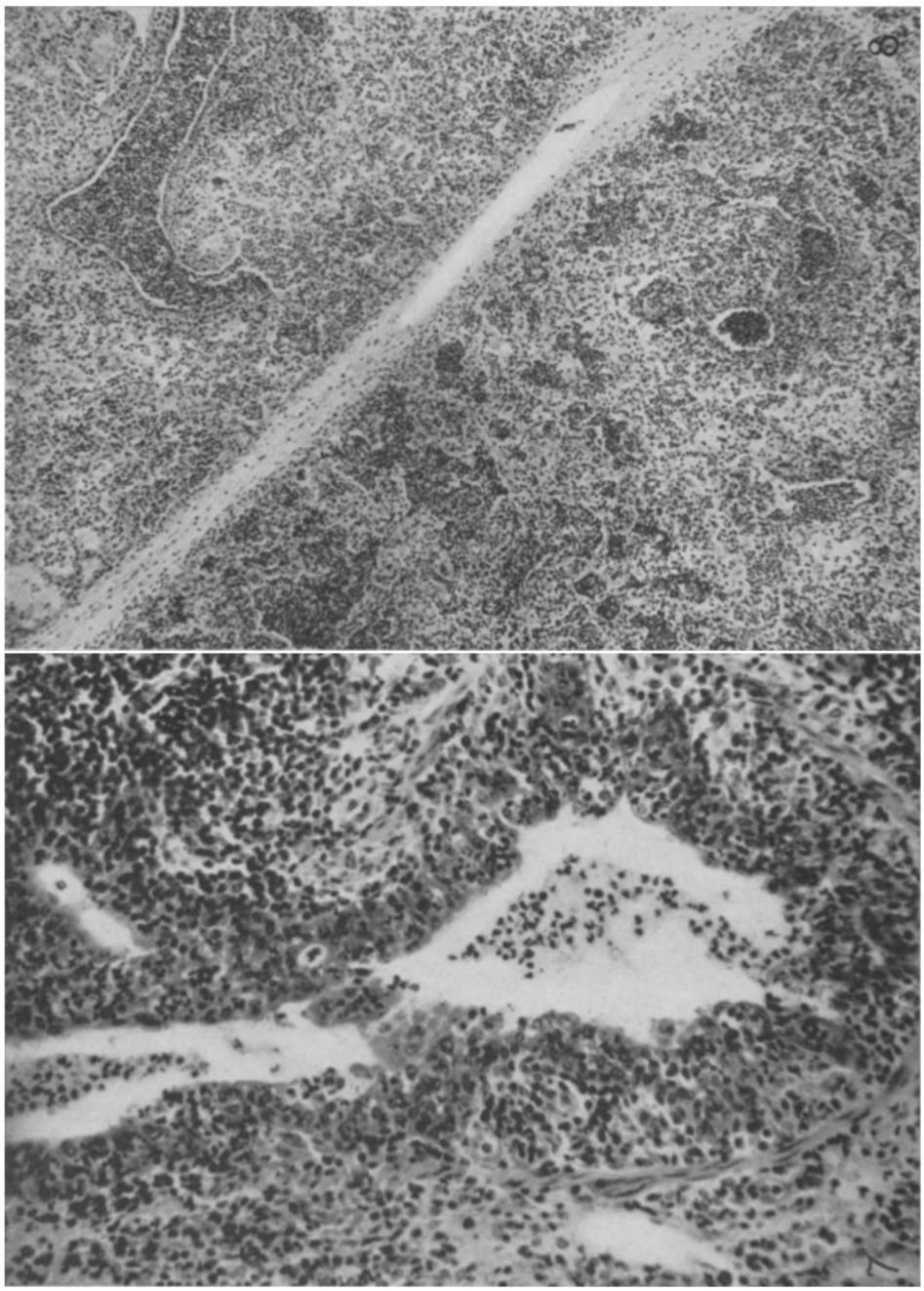

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