indicate that vitamin E is not concerned in either of the above phenomena and that as much as one gm. of royal jelly of the honey bee is insufficient to cure the sterility of E-deficiency in the female rat.

9018 C

Antigenic Differences in Strains of Human Influenza Virus.

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On the basis of earlier studies with mouse and ferret passage virus,[1] it was concluded that the Puerto Rico (PR8) and the Philadelphia (Phila) strains of human influenza virus were immunologically identical, while the swine influenza virus was serologically distinct. The 2 strains of human virus were also indistinguishable from the WS strain of the English workers.[1, 2] After repeated inoculation of ferrets with human influenza virus, however, it was noted that the serum of an animal so treated developed the capacity of neutralizing the swine virus as well.[3] Moreover, the serum of rabbits (a non-susceptible animal species) vaccinated with ferret-passage human influenza virus developed antibodies against both the human and swine viruses, whereas rabbits vaccinated with swine influenza virus produced antibodies which neutralized only the swine virus.[3] Identical results were obtained with horse sera prepared by Laidlaw, Smith, Andrewes and Dunkin.[3, 4] It was suggested, therefore, that the human and swine viruses, while immunologically distinct contained common antigens and that the swine antigenic components were present in the human virus as secondary antigens.[3, 5]

Since it seemed likely that the antibody response of an insusceptible animal might reflect the secondary antigens of the virus more completely than that of the susceptible animal in which antibodies to the primary antigen rather than to the secondary antigen

1 Francis, T., Jr., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1172.

2 Andrewes, C. H., Laidlaw, P. P., and Smith, W., *Brit. J. Exp. Path.*, 1935, **16**, 566.

3 Francis, T., Jr., and Shope, R. E., *J. Exp. Med.*, 1936, **63**, 645.

4 Laidlaw, P. P., Smith, W., Andrewes, C. H., and Dunkin, G. W., *Brit. J. Exp. Path.*, 1935, **16**, 275.

5 Francis, T., Jr., and Magill, T. P., *J. Exp. Med.*, 1936, **63**, 655.

would probably constitute the initial response, further studies were carried out in rabbits using the tissue culture virus as the source of the various strains. Rabbits were inoculated intraperitoneally with 2 cc. of culture fluid containing the PR8, Phila, and the Swine-2 strains, respectively. Blood was taken from the marginal ear vein 9 to 15 days later. Neutralization tests were then performed with the serum and tissue culture virus, employing mice as the test animal. The 3 sera were tested simultaneously against the homologous and 2 heterologous virus strains.

TABLE I.
Neutralizing Capacity of Serum against Homologous and Heterologous Strains of Influenza Virus.

Strain of Culture Virus	Serum of Rabbit Vaccinated with			
	PR8	Phila	Swine	Normal
PR8	0	++	+	d4 ++++
	0	++	++	d4 ++++
	+	+	+	d4 ++++
	0	++	+	d5 ++++
	0	++	+	d5 ++++
Phila	d4 ++++	0	d5 ++++	d3 ++++
	d4 ++++	0	d6 ++++	d3 ++++
	d6 ++++	0	d6 ++++	d4 ++++
	d6 ++++	0	d7 ++++	d4 ++++
	d7 ++++	0	d7 ++++	d8 ++++
Swine	d4 ++++	d5 ++++	0	d5 ++++
	d4 ++++	d6 ++++	0	d6 ++++
	d5 ++++	d7 ++++	0	d6 ++++
	d5 ++++	+++	0	d7 ++++
	d8 ++++	++++	0	d7 ++++

Survivors were sacrificed on 8th day after infection.
0 = No pulmonary lesions.
+ to ++++ = degree of pulmonary involvement.
d4 = Died on 4th day.

It was found that a comparatively high degree of specificity was exhibited by each serum. Table I represents the results of such a test. In each instance a serum completely neutralizes the homologous virus strain; the Phila serum in addition partially neutralizes the PR8 strain but not the swine virus; the anti-swine serum partially neutralizes the PR8 strain of human influenza virus but not the Phila strain. These results were not due to differences in the virulence of the respective strains for mice, as shown by the fact that, measured by lung lesions in inoculated mice, the titer of all 3 strains at the time of the test was 1:1,000.

In Table II the results of another test with the serum of different rabbits bled 9 days after a single intraperitoneal injection of 2 cc. of tissue culture virus are shown. At this time the end-point of

TABLE II.
Titration of Serum against Homologous and Heterologous Strains of Influenza Virus.

Serum of Rabbit		Dilution of Serum				
Vaccinated with virus strain	Tested against virus strain	Undil.	1:2	1:5	1:10	1:20
PR8	PR8—160th transfer	S	S	S	S	S
		S	S	S	S	S
		S	S	S	S	S
		S	S	S	S	S
	Phila—160th transfer	d6	d5	d5	d4	
		d7	d6	d5	d4	
		d7	d6	d5	d4	
		d7	d6	d6	d4	
	Swine—149th transfer	d4	d4	d4	d4	
		d4	d4	d4	d5	
		d4	d4	d4	d5	
		d5	d5	d4	d5	
Phila	PR8	d7	d6	d5	d4	
		d8	d6	d5	d6	
		d8	d6	d7	d6	
		S	d8	d7	d7	
	Phila	S	S	S	d8	d3
		S	S	S	d8	d7
		S	S	S	d9	d8
		S	S	S	S	d8
	Swine	d5	d5	d4	d5	
		d5	d5	d4	d5	
		d6	d5	d5	d6	
		d6	d5	d5	d6	
Swine	PR8	S	S	d5	d4	
		S	S	d5	d4	
		S	S	d7	d4	
		S	S	S	d5	
	Phila	d5	d4	d4	d4	
		d6	d4	d4	d4	
		d6	d6	d4	d4	
		d7	d6	d4	d5	
	Swine	S	S	d7	d6	d3
		S	S	d8	d7	d4
		S	S	d8	d7	d4
		S	S	d8	d8	d5

S = Survived (Experiment terminated on 10th day).
d4 = Died on 4th day.

the virus titration in each case was 1:10,000. Titrations of the respective sera were made against the homologous and heterologous strains.

Using survival and death of the mice over a period of 10 days as the criterion of protection, it is seen that the PR8 and Phila sera protected only against their homologous strain, while the anti-swine serum protected equally against the swine and the PR8 strains.

These results, which closely parallel those of Alexander in studies

on the neurotropic virus of horsesickness,[6] indicate a definite serological difference in the PR8 and Phila strains of human influenza virus and are in direct opposition to those previously reported when it was concluded that the 2 strains were serologically identical. The present results apply, however, only under the conditions outlined. When serum is obtained from the same rabbit after a longer interval, with or without an additional injection of virus in the meantime, it is found that cross-protection by the sera against PR8 and Phila strains is clearly demonstrable. The anti-swine virus serum may then also exert a protective action against both human strains although in most instances this effect is most pronounced against PR8. In contrast, however, under the present conditions no instance has been encountered even with late bleedings in which serum derived against human strains of virus has neutralized the swine virus. This would suggest, contrary to previously expressed opinion[5] that the swine virus comprises a more complex antigen than the human strains and that the common antigen which elicits the cross-neutralizing antibodies is more effectively present in the swine virus.

Several possibilities must be considered in interpreting the discrepancies between the present results and those previously reported. The strains of virus have been grown in tissue culture medium for a considerable period and some degradation of the virus may have occurred during the interval of cultivation outside the animal body, although this was not previously thought to have occurred.[7] The serum employed was derived very early in the period of serological response from animals not susceptible to the infectious agent, and such serum may not be comparable to that of animals of a susceptible species following recovery from infection. The question of heterophile reactions between the tissues of the different species employed in the experiments does not seem to apply to the results.

The conclusion appears warranted that the first and probably most specific serological response of rabbits to intraperitoneal injections of tissue culture strains of human or swine influenza virus yields a serum which is essentially specific for the homologous strain, and that these differences in the immune response reflect differences in the antigenic structures of the PR8 and Phila strains of human influenza virus.

[6] Alexander, R. A., *Onderstepoort J. Vet. Sci. and Animal Ind.*, 1935, **4**, 349.

[7] Magill, T. P., and Francis, T., Jr., *J. Exp. Med.*, 1936, **63**, 803.