

their effects in castrates by operating through the pituitary. One would have to assume, under these circumstances, that estrogens and androgens affect this organ in opposite directions and this is contrary to general evidence. It is possible that the sex hormones influence the blood picture through their well known actions on metabolism and general somatic growth. Estrogens, for example, depress the thyroid which has been shown to be concerned in erythropoiesis.¹⁶ If, however, the thyroid is the only organ concerned, to explain the results, it would have to be assumed that androgens stimulate the production of thyroid secretion, for which there is no direct evidence.

Summary. 1. The RBC and hemoglobin content of normal rats show higher values than those of normal females. 2. Following castration, the RBC and hemoglobin contents of the males drop, while those in the females rise. 3. Injections of estradiol into castrated females, and testosterone into castrated males produce, respectively, a drop and rise in RBC and hemoglobin. 4. Bone marrow studies indicate that androgens stimulate and estrogens inhibit erythropoiesis. 5. Additional support is thus given to the sex hormone explanation of the normal sex difference in RBC reported for many species of animals.

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A New Strain of Virus of Influenza B Isolated During an Epidemic in California.*

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Isolation of strains of influenza virus not antigenically related to the virus of influenza A¹ has been reported by Francis² and by Magill.³ Francis designated his strain influenza B. The virus iso-

¹⁶ Meyer, O. O., Thewlis, E. W., and Rusch, H. P., *Endoc.*, 1940, **27**, 932.

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¹ Horsfall, F. L., Jr., Lennette, E. H., Rickard, E. R., Andrewes, C. H., Smith, W., and Stuart-Harris, C. H., *Lancet*, 1940, **2**, 413.

² Francis, T., Jr., *Science*, 1940, **92**, 405.

³ Magill, T. P., *Proc. Soc. Exp. Biol. and Med.*, 1940, **45**, 162.

lated by Magill was obtained during the same outbreak of influenza in New York, but no data on the antigenic relationship of the 2 viruses have yet been published. The purpose of this paper is to report the isolation of another strain of the virus of influenza B which is antigenically related to, but not identical with, the strain found by Francis.

During January and February, 1940, an outbreak of influenza occurred in California. This epidemic was more or less concurrent with the appearance of influenza in other parts of the United States and the incidence of acute upper respiratory disease of relatively mild character appears to have been quite high. About 8,000 cases were reported from the entire state. Blood specimens were obtained during and after illness from 121 cases of influenza and throat washings were taken on the 2nd to 4th day of illness from 101 of these.

Ferrets were inoculated intranasally with throat washings from 12 cases. One throat washing obtained in the early part of January, 1940, gave an indefinite temperature response in the first ferret and a more marked reaction in the second ferret passage. In 9 subsequent ferret passages a slight rise in temperature was observed in 6 animals on the second or third day after inoculation. Nasal symptoms and loss of appetite occurred in less than half of the ferrets inoculated, but at autopsy the turbinates were usually found to be congested. No definite pulmonary consolidation was seen. At this time four separate attempts to adapt the agent to mice were unsuccessful. Throat washings from many other cases were also inoculated intranasally into mice and onto the chorioallantoic membrane of chick embryos with negative results.

Several months after these observations had been made, Francis announced the isolation of the Lee strain of influenza B. With this strain, which was very kindly sent to us by Dr. T. Francis, Jr., neutralization tests were done on the stored serum specimens from human cases. Fifty out of 76 of the cases of influenza tested showed an increase in antibodies to the Lee strain. In no case was a definite increase in antibodies to influenza A detected. Ferrets inoculated with lung and turbinate material from the 3rd, 6th, 7th, 9th, and 11th ferret passages in the series described in the preceding paragraph were found to have developed antibodies to the Lee strain of influenza B, but had no antibodies to the virus of influenza A (strains PR8, W.S., and 188). It was concluded, therefore, that the ferrets had been infected with a virus antigenically related to the Lee strain. Ferrets inoculated with 11 other throat washings

collected during the same epidemic developed no antibodies to influenza A or B.

After the ferret passage virus (referred to as Montgomery strain) had been tentatively identified as influenza B, two subsequent attempts were made to adapt the agent to mice. One of these starting from the 11th ferret passage, was successful and lung lesions appeared in the 11th mouse passage. Some of the mice of the fourth passage although having no lesions were saved for subsequent immunity tests. These were found to be resistant to intranasal inoculation with virus of the 20th mouse passage which killed normal mice in 5 or 6 days with characteristic plum-colored pulmonary consolidation.

In the course of attempts to infect Syrian hamsters (*Cricetus auratus*) and the California kangaroo rats (*Dipodomys deserti deserti* and *Dipodomys merriami simiolus*) with the two strains of influenza B it was noted that these animals developed neutralizing antibodies to rather high titers after intranasal inoculation with mouse passage material.⁴ The immune sera from kangaroo rats, hamsters, rabbits, guinea pigs, and ferrets were used in cross-neutralization tests with the Lee and Montgomery strains. In these tests the same suspensions of infected mouse lung kept at constant titer by storage at minus 70°C were used throughout. The titer based on the 50% mortality endpoint of the suspension of the Lee strain was 0.05 cc of a dilution of 10^{-3.05} and of the Montgomery strain 0.05 cc of a dilution of 10^{-1.99}. In the neutralization test a dilution of 10⁻² or 11 M.L.D. of Lee strain and 10^{-1.30} or 5 M.L.D. of the Montgomery strain were used with 3 or 4 different dilutions of serum. With the amount of virus used in the tests practically all of the control mice died. In reading the results of the test complete, partial, or no neutralization was recorded by comparing the degree of pulmonary consolidation in the test mice with that in controls according to score method as previously applied.⁵

The results of the cross-neutralization tests are presented in Table I. The titers of sera from animals immune to the Lee strain were approximately 2 to 16 times as high with the homologous strain (Lee) as with the heterologous strain (Montgomery) except with one rabbit serum in which there was no difference. Conversely, the apparent neutralizing capacities of sera from animals immune to the Montgomery strain were 4 to 64 times as high against the

⁴ Eaton, M. D., Martin, W. P., and Talbot, J. C., *Proc. Soc. Exp. Biol. and Med.*, 1941, **48**, 181.

⁵ Eaton, M. D., and Pearson, H. E., *J. Exp. Med.*, 1940, **72**, 635.

TABLE I.
Cross-Neutralization with Two Strains of the Virus of Influenza B.

Animal serum No.	Anti-strain	Neutralization of strain									
		Montgomery with serum dilutions					Lee with serum dilutions				
		1:1	1:4	1:16	1:64	1:256	1:1	1:4	1:16	1:64	1:256
Guinea Pig 10-7	Lee	—	P	O	O	O	—	C	P	O	O
Guinea Pig 1-10	Lee	—	O	O	O	O	—	P	O	O	O
Rabbit 3-5	Lee	—	C*	C*	P*	O*	—	C*	C*	C*	O*
Rabbit 3-8	Lee	—	P*	O*	O*	O*	—	P*	O*	O*	O*
Dipodomys 2-9	Lee	—	C	P	P	O	—	C	C	C	P
Dipodomys 6-3	Montgomery	—	C	C	C	—	—	C	P	O	—
Dipodomys 6-7	Montgomery	—	C	C	P	—	—	P	O	O	—
Dipodomys 3-5	Montgomery	C	C	P	O	—	P	P	O	O	—
Hamster 3-54	Montgomery	—	C	C	P	—	—	C	P	P	—
Hamster 3-89	Montgomery	C	P	P	P	—	P	O	O	O	—
Ferret 6-7	Montgomery	—	C	C	C	P	—	C	P	P	O
Ferret 6-8	Montgomery	—	C	C	P	P	—	C	P	P	O
Ferret 2-0	Montgomery	C	C	P	O	—	P	P	O	O	—
Ferret 4-4	Montgomery	C	P	P	P	—	P	O	O	O	—

C Complete neutralization, percentage scores zero (no definite lung lesions).

P Partial neutralization, percentage scores less than half those of controls.

O No definite neutralization, percentage scores more than half those of controls.

— Test not done with serum-dilution indicated.

* Neutralization with 1 M.L.D.

homologous as against the heterologous strain. On the basis of the work of Horsfall and Lennette,⁶ these differences in antibody titer could not be accounted for by the small difference in the amounts of the 2 viruses used in the test.

Mice actively immune to the Montgomery strain showed no signs of infection after inoculation with the Lee strain, but were not immune to the virus of influenza A (strain PR8) or to a virus of mouse pneumonia isolated in this laboratory.⁷

Summary. A new strain of the virus of influenza B was isolated from the throat washing of a case of influenza during an epidemic in California in January, 1940. The difficulties of isolating this virus by the usual methods of passage in ferrets and mice are noted. The new strain was not identical in antigenic composition with the Lee strain isolated by Francis, but was closely related to it.

⁶ Horsfall, F. L., Jr., and Lennette, E. H., *J. Exp. Med.*, 1941, **73**, 327.

⁷ Pearson, H. E., and Eaton, M. D., *Proc. Soc. Exp. Biol. and Med.*, 1940, **45**, 677.