color intensity was determined for the German silver cell by making a long series of readings over a number of days on two standard intensities of color. The colored solutions were composed of pure carotene dissolved in chloroform and diluted with alcohol; the readings were made with a blue filter. With the more dilute solution light transmission was about 70%; concentrations calculated at this level showed a standard error of ±0.65%. With the more concentrated solution light transmission was approximately 25%; concentrations computed from these data disclosed a standard error of ±0.24%.

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11862 P

Differentiation of Influenza A and Influenza B by the Complement-Fixation Reaction.*

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A recent report[1] has described the isolation of a new serological type (B) of virus from epidemic influenza. By means of the neutralization test with sera from convalescent animals and human individuals, it was shown that this virus was readily differentiated from virus of the type (A) previously identified. In the process of adaptation of the new virus to mice, attempts were made to ascertain whether serological differentiation could also be obtained with the complement-fixation reaction. While the earlier results were indefinite, these efforts have been renewed since the virus has increased in virulence for mice.

Antigens were prepared from lungs of mice infected with the PR8 strain of the virus of Influenza A[2] and also from mice infected with the Lee strain of Influenza B virus. Two percent suspensions of the infected lungs in physiological salt solution, after clarification by

* This study was conducted under a grant from the International Health Division of the Rockefeller Foundation.

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

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

centrifugation, were used in the tests. Two units of complement were employed. Serial two-fold dilutions of serum were made and 0.1 cc of each dilution was mixed with 0.1 cc volumes of complement, antigen and saline. After incubation for 60 minutes at 37°C., 0.2 cc of sensitized cells were added. The tubes were again incubated in the waterbath for 45 minutes when readings were made. A complete set of controls was included in each test.

Acute and convalescent sera obtained from patients infected with Type A virus in 1938-39 and from others shown to have been infected in 1940 with Type B virus were tested for their capacity to fix complement in the presence of each antigen. Representative results are presented in Table I, where the titer of neutralizing antibodies in each serum against 100 M.L.D. of the 2 types of virus is also recorded.

It is seen that the convalescent sera of patients who had suffered from epidemic influenza in 1938-39 showed an increase in complement-fixing antibodies to the PR8 strain of Influenza A virus but not to the Lee strain of Influenza B virus. On the other hand, the convalescent sera from 4 of the patients suffering from epidemic

TABLE I.
Complement-Fixation with Sera of Patients and Influenza Virus, Types A and B.

Type of Infection	Pt.	Serum	Type A (PR8) antigen, Serum dilution 1: 2	4	8	16	32	C	Type B (Lee) antigen, Serum dilution 1: 2	4	8	16	32	C	Neutralization titer Type A	Type B
Influenza A 1938-1939	V	A*	4	2	0	0	0	0	4	1	0			0	6	0
		C	4	4	4	4	1	0	4	1	0			0	240+	0
	J	A	1	0	0	0		0	3	0	0			0	4	6
		C	4	3	1	0		0	1	0	0			0	140	6
	A	A	4	0	0	0	0	0	3	0	0			0	30	0
		C	4	4	4	2	0	0	1	0	0			0	240	6
	L	A	0	0	0			0	0	0	0			0	120	6
		C	4	3	0			0	0	0	0			0	480	6
	Ac	A	0	0	0			0	0	0	0			0	30	8
		C	4	2	0			0	0	0	0			0	120	15
Influenza B 1940	M	A	0	0	0			0	0	0	0	0	0	0	17	0
		C	0	0	0			0	4	4	3	2	0	0	17	60
	T	A	0	0	0			0	0	0	0	0	0	0	10	12
		C	0	0	0			0	4	4	4	3	0	0	16	240
	C	A	2	1	0			0	3	1	0	0	0	0	240+	6
		C	3	1	0			0	4	4	4	4	4	0	240+	120
	L	A	4	4	1	0		0	2	1	0	0	0	0	240+	0
		C	4	4	1	0		0	4	4	4	4	1	0	240+	200
	S	A	0	0	0			0	0	0	0			0	240+	0
		C	2	0	0			0	0	0	0			0	240+	140

0 = no fixation of complement.
1, 2, 3, 4 = increasing degrees of fixation.
*A = acute, C = convalescent.

influenza in 1940 showed a sharp rise in antibodies to the Type B antigen but not to the antigen of Type A. In the case of patient Sq., no rise in complement-fixing antibodies was detected.

These observations, while limited in number, serve to emphasize the antigenic differences between the 2 viruses and to indicate that the complement-fixation reaction can be employed for the differential diagnosis of epidemic influenza due to virus of Types A and B.

11863

The Diurnal Levels of Blood Leukocytes in the Normal Rabbit.

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Extensive studies on the blood of normal rabbits,* which were made for another purpose,[1, 2] seemed to offer an unique opportunity to determine whether a definite diurnal cycle in the leukocyte level actually exists in this species. The animal material used had been acclimated to the laboratory for months or years before the counts were made. The exact age of the animals, as well as their breed and physical condition, was known in most instances. Counts were not made oftener than 3 times a week, and usually only once a week. The exact time of day at which the blood was taken had been recorded for each determination. Finally, the material was sufficiently extensive to make statistical analysis possible.

Materials and Methods. Although blood counts on normal rabbits were available for all months of the year, the November and December counts were selected for analysis because in these months the largest samples were available for each hour of the day from 9 a.m. to 5 p.m. The variation in the formula of the blood between the 2 months was not significant.

The animal material consisted of 204 young adult male rabbits examined in the months of November and December in the years

* These studies were done at the Rockefeller Institute for Medical Research, in the laboratory of Dr. Wade H. Brown. They were carried out by the author, in coöperation with Drs. Louise Pearce, Paul D. Rosahn, and C. K. Hu. The technical assistance of Mr. Leslie Kish was invaluable.

1 Pearce, L., and Casey, A. E., *J. Exp. Med.*, 1930, **51**, 83.

2 Casey, A. E., Rosahn, P. D., Hu, C. K., and Pearce, L., *J. Exp. Med.*, 1936, **64**, 453.