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A Flocculation Phenomenon with Human Sera and Suspensions of the Virus of Epidemic Influenza.

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Neutralization and complement-fixation tests with the virus of epidemic influenza have been described and studied rather extensively but no agglutination, precipitation, or flocculation reaction has as yet been reported. Theoretically such reactions should be demonstrable, provided a sufficient quantity of virus is contained in the antigen.[1] Attempts to purify and concentrate the virus by ultracentrifugation in this laboratory have not in general yielded suitably reactive antigens. During the course of investigation it was observed, however, that a flocculative phenomenon could be obtained consistently with certain human sera and crude suspensions of lungs of mice infected with influenza virus, the reaction being especially marked with sera of patients convalescent from epidemic influenza. It is the purpose of this report to describe the conditions under which the reaction occurs and to discuss briefly some of the results.

Methods. Mice of the Swiss strain are inoculated intranasally with 1 to 2% suspensions of lungs of mice infected with PR8 strain of influenza virus. At the time of appearance of visible areas of pulmonary consolidation—approximately 40 to 50 hours after inoculation—the mice are sacrificed and the lungs ground with alundum in meat-infusion broth (pH 7.8) to form a 10% suspension by weight. After light centrifugation equal volumes of supernatant fluid and serum are mixed in small tubes and incubated at 37°C. After 1 hour's incubation the tubes are shaken lightly and the degree of flocculation is recorded in the usual manner. Frequently flocculation does not occur until the tubes are shaken but once the reaction takes place a precipitate settles to the bottom of the tubes in large coarse floccules. Meat-infusion broth is not essential in the preparation of the antigen and can be replaced by M/150 phosphate buffer pH 7.2 or 0.4% NaCl solutions. However, antigens prepared with broth are more stable than those prepared with either buffer or 0.4% saline.

Results. The majority of sera from patients convalescent from epidemic influenza produced strong flocculative reactions with infected-mouse-lung antigen while sera from the same individuals

[1] Merrill, M. H., *J. Immunol.*, 1936, **30**, 169.

during the acute stage of the disease, on the whole, failed to react. On the other hand, convalescent sera from an epidemic of non-influenzal upper respiratory infection which occurred in the western United States during 1936[2] failed to produce the reaction as did all but one of a number of sera from convalescent cases of pneumococcal pneumonia. Furthermore, antigens prepared in a similar manner from normal mouse-lung failed to react with any of the sera.

Strong sera induce good flocculation in dilution of 1:8 or 1:16, but with undiluted serum the antigen is effective in dilution not greater than 1:2 or 1:4.

Heating of serum or antigen at 56°C for 1 hour destroys the ability of either to induce the reaction.

Table I summarizes some of the results obtained with sera from several respiratory infections. It is to be noted that the convalescent serum from one case of streptococcal infection of the throat showed no increased neutralizing or complement-fixing antibodies for influenza virus, yet evoked a fairly good flocculative reaction, whereas, none of the 3 reactions was positive in the presence of "acute" serum from the same individual. The results of the neutralization-tests with sera from the California epidemic of 1936 are recorded as "no change," indicating no increase in antibody-titer in convalescent as compared with acute serum. The technic of the neutralization test employed with these sera differed somewhat from that usually employed and so the serum-titers are not strictly comparable with the other tests.

Specificity of the reaction. There are several indications that the reaction is a specific one between the influenza virus and its antiserum. These are (1) the occurrence of the reaction in the presence of serum from convalescent influenzal patients and not in the presence of serum from the same individuals during acute stages of the disease; (2) the failure of convalescent sera from cases of pneumococcal pneumonia and other respiratory infections to induce the reaction; (3) the occurrence of the reaction with the serum of convalescent influenzal patients in the presence of antigen prepared from lungs of mice infected with influenza virus and lack of the reaction in the presence of antigens prepared from normal mouse-lung. There is, however, some reason to doubt the specificity of the reaction. First, it occurs occasionally in the presence of convalescent serum from individuals who were considered not to have had influenza, as in the one case of hemolytic-streptococcal infection of the throat. Second, the intensity of the reaction obtained with

[2] Francis, T., Jr., *Am. J. Pub. Health*, 1937, **27**, 211.

TABLE I.
Flocculative Reaction with Suspensions of Influenza Virus and Different Sera.

Serum		Disease	Highest dilution of serum giving positive reaction: Flocculation with suspensions of: Infected lung	Flocculation with suspensions of: Normal lung	Neutralization	Complement-fixation
RM	Acute	Influenza	0	0	1-30	0
	Conv.		1-2	0	1-240	1-128
EW	A	"	±	0	1-6	1-16
	C		1-8	0	1-50	1-64
MW	A	"	0	0	1-10	1-8
	C		1-8	0	1-60	1-512
VH	A	Strept. throat	0	0	1-30	1-8
	C		1-4	0	1-30	1-8
LH	A	" "	1-4	0	1-100	1-32
	C		1-8	0	1-100	1-32
EB	A	Sinusitis	0	0	0	0
	C		0	0	0	0
RA	A	Pneumococcal	0	0		
	C	pneumonia	0	0		
LR	A	Pneumococcal	0	0		
	C	pneumonia	0	0		
JS	A	Pneumococcal	0	0		
	C	pneumonia	0	0		
AM	A	Pneumococcal	0	0		
	C	pneumonia	1-2	0		
WJ	A	Non-influenzal	0	0	No change*	
	C	upper	0	0		
LR	A	respiratory	0	0	" "	
	C	infection	0	0		
RE	A	California	0	0	" "	
	C	1936	0	0		
RG	A		0	0	" "	
	C		0	0		
HF		No recent	1-2	0	1-10	1-16
TM		infection	1-8	±	1-10	1-8
RD			1-8	±	1-2	1-8
AM			0	0	1-10	1-32
LV89			1-8	0	1-2	0
LV132			1-2	0	1-2	1-8

* Indicates no increase in neutralizing antibodies of convalescent serum as compared with acute serum.

various antigens does not parallel the viral content of the antigen as determined by infectiousness for mice. The intensity of the reaction

does parallel, however, within limits, the increased weight of the lung which takes place during infection with influenza virus. Third, in certain fractionation-experiments reactions of a somewhat different character have been observed in the absence of virus.

It may be that the antigen is a combination of active and altered virus, and that infectivity for mice is not an accurate measurement of the amount of virus that can react with the serum. On the other hand, it may be that the flocculating antigen is some substance other than the virus which appears in the mouse-lung during infection, similar perhaps to the nonspecific precipitinogen in yellow fever described by Hughes.[8] The problem is being investigated further.

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Effect of Sulfanilamide upon the Viability of Meningococci in Spinal Fluid.

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The investigations of Branham and Rosenthal,[1] Buttle, Gray, and Stephenson,[2] McPherson Brown,[3] Proom,[4] Rosenthal, Bauer, and Branham,[5] have established the curative value of sulfanilamide and related compounds in experimental meningococcal infections of animals. In man, favorable results in the treatment of meningococcal meningitis by means of sulfanilamide (either alone or in conjunction with antimeningococcal serum) were reported by numerous observers.[6-17] In a recent communication,[18] it was reported that sul-

[8] Hughes, T. P., *J. Immunol.*, 1933, **25**, 275.

[1] Branham, S. E., and Rosenthal, S. M., *Publ. Health Rep.*, 1937, **52**, 685.

[2] Buttle, G. A., Gray, W. R., and Stephenson, D., *Lancet*, 1936, 1286.

[3] McPherson Brown, Th., *Bull. Johns Hopkins Hosp.*, 1937, **61**, 272.

[4] Proom, H., *Lancet*, 1937, 16.

[5] Rosenthal, S. M., Bauer, H., and Branham, S. E., *Publ. Health Rep.*, 1937, **52**, 662.

[6] Schmidt, W., *Deutsche med. Wchnschr.*, 1936, **62**, 881.

[7] Schwentker, F. F., Gelman, S., and Long, P. H., *J. Am. Med. Assn.*, 1937, **108**, 1407.

[8] McIntosh, R., Wilcox, D. A., and Wright, F. H., *J. Pediat.*, 1937, **11**, 167.

[9] Mitchell, A. G., and Trachsler, W. H., *J. Pediat.*, 1937, **11**, 183.

[10] Brennemann, J., *J. Pediat.*, 1937, **11**, 238.

[11] Bernstein, S. S., *J. Pediat.*, 1937, **11**, 198.