Immunologic Response of Hamsters to Influenza Virus Strains.* (17483)

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(Introduced by W. Henle)

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Studies on strains of influenza virus isolated from the 1947 epidemic[1] were complicated by irregular reactions obtained in hemagglutination-inhibition tests with chicken sera (*e.g.*, nonspecific inhibition). In view of these difficulties, it was deemed desirable to supplement the antigenic studies with complement-fixation tests. Since chicken sera are unsatisfactory for this purpose, antiserum had to be prepared in another species. The hamster was selected because of its ability to develop antibodies to influenza virus following inoculation by the intranasal route.[2] The results obtained with hamster sera are the subject matter of this paper.

Methods and Materials. Viruses. The following virus strains were used:

PR8 and Lee
$L_3$47, $L_7$47, and $L_8$47
FM_1[3]
$L_4$49 and $L_8$49[4]
FJS (type A) and Warner (type B)†

Antigens. Allantoic fluids harvested 48 hours after intraallantoic inoculation of 10^{-3} or 10^{-4} dilution of seed virus which were centrifuged at 2,000 rpm for 10 minutes and dialyzed overnight against 20 parts of phosphate buffer saline were used as the "virus-bound" antigen. Obviously these preparations contained some soluble antigen. No attempt was made to separate the two antigens from the fluids. Ten or 20% suspensions of allantoic membrane were centrifuged at 2,000 rpm for 10 minutes, and following removal of the virus (as determined by absence of hemagglutination) by either centrifugation at 13,000 rpm for one hour or absorption with red cells, or a combination of both procedures, the supernatants were used as the soluble antigen. Suspensions of uninfected membranes were used as control antigens.

Sera. Hamsters were inoculated by the intranasal route with 0.1-0.2 cc of active infected allantoic fluids containing virus. Fifteen days later they were bled either with needle and syringe from the heart or by incision of the thoracic cavity and heart, allowing blood to run through funnel into tube. The sera were inactivated by heating at 56°C for 30 minutes. Before being used in the hemagglutination-inhibition test, they were absorbed with human or chicken erythrocytes, depending on which red cells were to be used in the test.

Hemagglutination-inhibition Test. The procedure for performing the hemagglutination-inhibition test has been described previously.[5] The tests were read after 30-45 minutes of incubation at room temperature. In the presence of hamster sera—especially in the lower dilutions—there was a tendency on the part of the cells to settle out quickly, so that the tubes with agglutinated cells showed patterns consisting of fuzzy, serrated rings, or clumps of cells against a background of a more or less smooth film of cells (the kind of film usually observed on the bottom of tubes showing agglutination of cells in the presence of human or chicken sera).

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† Obtained in 1948 from Australia through the courtesy of Dr. S. G. Anderson.

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TABLE I.
Cross-hemagglutination-inhibition Reactions with Hamster Antisera.

Antigen	Serum						
	PR8	FJS	FM_1	$L_3$47	$L_7$47	$L_8$47	Lee
PR8	*2560*	20	20	<15	<15	15	<15
FJS	<15	*320*	20	15	15	<15	<15
FM_1	<15	60	*320*	240	80	80	<15
$L_3$47	<15	40	240	*320*	120	60	<15
$L_7$47	<15	<15	60	60	*120*	30	<15
$L_8$47	<15	15	30	30	40	*240*	<15
Lee	<15	<15	<15	<15	<15	<15	*80*

TABLE II.
Cross-complement-fixation Test with Allantoic Fluid Antigens and Hamster Sera.

Antigen	Dilution	Serum			
		PR8	$L_7$47	$L_4$49	$L_8$49
PR8	und.	192*	<12	<12	<12
	1:2	192	<12	<12	<12
	1:4	96	<12	<12	<12
	1:8	192	<12	<12	<12
	1:16	24	<12	<12	<12
	1:32	<12	<12	<12	<12
$L_7$47	und.	12	48	24	24
	1:2	12	96	48	24
	1:4	24	96	48	48
	1:8	24	96	48	48
	1:16	12	48	48	24
	1:32	<12	24	<12	<12
$L_4$49	und.	12	48	48	48
	1:2	24	24	48	24
	1:4	24	48	48	48
	1:8	12	48	48	48
	1:16	12	24	24	48
	1:32	<12	12	<12	<12
$L_8$49	und.	12	nt	24	48
	1:2	24	nt	48	48
	1:4	24	nt	48	96
	1:8	12	nt	48	48
	1:16	12	nt	48	24
	1:32	<12	nt	<12	<12

* Serum titer expressed as the reciprocal of serum dilution.

Complement Fixation Test. The procedure of the complement fixation test was as follows: Serial, two-fold dilutions of serum in 0.1 cc amounts were mixed with 0.1 cc amounts of progressively diluted antigen and with 0.2 cc of complement diluted to contain 1.5-2.0 units. The tubes were kept overnight in the refrigerator. The binding of complement was determined by the addition of 0.2 cc of sensitized cells (consisting of 0.1 cc of 2% sheep cells plus 0.1 cc of optimally diluted hemolysin) and incubation at 37°C for 30 minutes. The titers represent initial dilution of serum, and are based on 3+ or 4+ readings.

Results. As shown in Table I, hamster sera gave highly specific reactions in the hemagglutination-inhibition test. Not only was there no reaction between the strains from 1947-1948 and the anti-Lee serum, but there was very little or no cross-relationship between these strains and the PR8 virus. Moreover, the recent strains exhibited antigenic differences among themselves. It appears that the $L_3$47 and FM_1 viruses are probably identical strains.

TABLE III.
Cross-complement-fixation Test with Soluble Antigens and Hamster Sera.

Antigen	Dilution	Serum			
		PR8	$L_7$47	$L_4$49	$L_8$49
PR8	1:2	48	12	24	12
	1:4	48	12	24	12
	1:8	24	12	24	24
	1:16	48	12	12	24
	1:32	48	<12	<12	<12
	1:64	12	<12	<12	<12
$L_7$47	1:2	12	24	48	24
	1:4	12	24	24	24
	1:8	12	24	24	24
	1:16	<12	12	<12	<12
	1:32	<12	<12	<12	<12
	1:64	<12	<12	<12	<12
$L_4$49	1:2	12	12	24	24
	1:4	12	12	24	24
	1:8	12	12	24	24
	1:16	<12	12	12	24
	1:32	<12	<12	<12	<12
	1:64	<12	<12	<12	<12
$L_8$49	1:2	12	nt	24	24
	1:4	12	nt	24	24
	1:8	12	nt	24	24
	1:16	<12	nt	12	12
	1:32	<12	nt	<12	<12

Using the complement-fixing allantoic fluid antigen of influenza virus, the 1947 and 1949 strains were distinctly set off from the PR8 virus by the lack of reaction—in the dilutions used—between their sera and the antigen of the PR8 virus (Table II). The anti-PR8 serum with a higher homologous titer than the other antisera reacted with the recent strains. Although when tested with the PR8 antigen in suboptimal dilution (1:16) the homologous and heterologous titers of this serum were the same, with higher concentrations of the PR8 antigen the homologous titer was 4 to 16 times greater than the heterologous titers.

As shown in Table III, the complement-fixation test with the soluble antigens brought out the kinship of these strains. The results with the PR8 antiserum resembled the corresponding findings with the "allantoic fluid" antigen, but the results with the other sera differed from those obtained with the allantoic fluid antigen in that these sera reacted with the PR8 soluble antigen to a titer of about the same magnitude as the homologous titers. This reaction was type-specific. There was no reaction between any of these sera shown in Tables II and III and the Lee virus antigens.

Discussion. The fact that hamster antisera are active in complement fixation as well as hemagglutination tests should make them very useful in the typing of newly isolated strains.

Taylor[6] has employed hamster sera in antigenic analyses of strains of influenza virus. In our laboratory it was possible to type the viruses isolated from the outbreak of influenza of 1949[4] by such a procedure on primary isolation. The membranes of embryos used for primary isolation served as source of the soluble antigen. Once the type of virus has been established, strain differentiation can be accomplished by agglutination-inhibition tests using hamster sera. A general idea about the relationship of the isolated virus to other newly identified strains (subtype specificity) may be obtained from the complement-fixation reaction with al-

[6] Taylor, R. M., *Am. J. Pub. Health*, 1949, **39**, 171.

lantoic fluid containing virus antigen. Another advantage in using hamsters is the ability of these animals to produce antibodies in response to low concentrations of virus. This makes it possible to prepare antisera to viruses in first or second egg passage. This is not always feasible with chickens because more virus is required to induce antibody formation in these animals. The value of hamster sera is slightly affected by a shortcoming; the hemagglutination-inhibition test is difficult to read because of the rapid settling of cells, giving rise to fuzzy or serrated discs of agglutinated cells.

Summary. Antisera prepared in hamsters by the intranasal instillation of allantoic fluids containing influenza virus were type-specific when used in the complement-fixation test with the soluble antigens; group- or subtype-specific (*e.g.* A prime) when used in the complement fixation test with the allantoic fluid antigen, and strain-specific in hemagglutination-inhibition tests.

It appears that the hamster is an animal best suited for quick identification of influenza viruses. It is suggested that their sera be used for systematic classification of strains.

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Effect of Epinephrine, Antihistaminics and Adrenergic Blockade on Egg White Edema in Adrenal Insufficient Rats.* (17484)

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Recently we reported a marked protection by 0.1 mg/kg epinephrine hydrochloride against the acute edema induced by the intraperitoneal injection of egg white in rats.[1] Selye[2] and Leger and Masson[3] showed that adrenalectomy greatly sensitized rats to this edema and led to death in a large percentage of the animals. Adrenal cortical extract protected such animals from dying *but not from the edema,* nor did it confer protection to animals with intact adrenals. Kellaway,[4] Wyman,[5] and Perla and Marmorston-Gottesman,[6] Wyman[7] and Ingle, *et al.*[8,9] showed that epinephrine antagonized histamine toxicity in adrenalectomized rats. The egg white edema syndrome could conceivably be caused by the local release of histamine or a histamine-like substance, since antihistaminics effectively combat the edema, as demonstrated by Leger and Masson,[10] Brown and Werner,[11] and ourselves.[1] The doses of antihistaminics required are considerable, however, which may also suggest that the protection results from precapillary arteriolar vasoconstriction[12] and decreased capillary filtration rather than "anti-

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