for certain stocks of albino mice are susceptible and others, even though of related lines, are relatively resistant. 2. In 19% of the mice which reacted to homologous brain tissue-Freund type adjuvant mixtures, lesions characteristic of disseminated encephalomyelitis can be found in the central nervous system in the absence of any demonstrable objective symptoms of illness. 3. A diagnosis of the experimental encephalomyelitis should therefore be based on the results of histological examination of the nervous system as well as on typical symptomatology. The question is discussed whether reactors might not have been overlooked among animals of other species hitherto employed, when the basis for the diagnosis of the encephalomyelitis was only the presence of outward signs of illness.

Received September 20, 1950. P.S.E.B.M., 1950, v75.

Role of the Erythrocyte in Inhibition by Allantoic Fluid of Mumps Virus Hemagglutination. (18170)

ALFRED L. FLORMAN. (Introduced by G. Shwartzman.)

From the Division of Bacteriology and Pediatric Service of Mount Sinai Hospital, New York.

It is known that virus hemagglutination is the result of a reaction between the virus and the red blood cell, and that this reaction may be specifically inhibited by sera containing antibodies for the virus. A variety of nonspecific substances may also interefere with this reaction(1). A substance of this sort present in allantoic fluid has been studied most intensively for its effect upon the agglutination of red blood cells by influenza viruses(2,3). From these investigations, it appears that normal allantoic fluid (NAF) contains an inhibitor which reacts with and is inactivated by these viruses. A filtrate of *Cl. welchii* and an enzyme from *Vibrio cholera* similarly inactivate this inhibitor. It has been postulated that a coenzyme exists for the hemagglutinating virus-inhibitor system. However, it has not been clear whether this hypothetical substance comes from the virus or from the red blood cell(2).

In the course of comparative studies on agglutination of chicken and human erythrocytes by a strain of mumps virus (Enders), observations were made which seem to furnish information regarding this question. These observations are the subject of the present communication.

Materials and methods. Viruses (a) *Mumps.* The Enders strain was used. For passage 0.1 ml of a 10^{-1} dilution of virus in 10% rabbit serum-saline was inoculated into the allantoic sac of 7-day-old chick embryos. These embryos were incubated further at 37°C for 4 days, chilled and the allantoic fluid collected. (b) *Influenza.* The PR-8 and Lee strains were used. For passage 0.1 ml of a 10^{-3} dilution of virus in 10% rabbit serum-saline was inoculated into the allantoic sac of 10- to 11-day-old chick embryos. The allantoic fluid was collected after 2 days at 37°C and a period of chilling.

Normal allantoic fluid (NAF) was obtained from chilled 11-day-old embryos. All the fluids were stored after collection in lusteroid tubes at approximately −72°C.

Chicken red blood cells were obtained by bleeding chickens from the wing vein and allowing the blood to flow into an excess of 2% sodium citrate or sodium oxalate.

Human group O cells were obtained from the Hospital Blood Bank where they had been collected in ACD (acid citrate dextrose) solution. The cells were washed in normal saline 3 times and used as 1% or 25% suspensions as indicated. In some experiments other anticoagulants were used without modifying the results. Cells were not used if

1. Anderson, S. G., *Fed. Proc.*, 1949, v8, 631.
2. Svedmyr, A., *Brit. J. Exp. Path.*, 1948, v29, 309.
3. Hardy, P. H., Jr., and Horsfall, F. L., Jr., *J. Exp. Med.*, 1948, v88, 463.

they were more than 3 days old.

Hemagglutinin titrations. Serial two-fold dilutions of virus containing allantoic fluid were made in saline in a volume of 0.4 ml. To each dilution, 0.4 ml of a 1% cell suspension was added. The tubes containing these mixtures were shaken and examined after being permitted to settle at room temperature for one hour. The pattern at the bottom of each tube was noted and graded from a 4+ shield-like pattern to 0, which represented a button of freely movable cells. The highest dilution of virus giving definitely positive agglutination (+1), was considered as the end point, representing 1 hemagglutinating unit of virus. *Inhibitor titrations.* Serial two-fold dilutions of allantoic fluid were made in saline in a volume of 0.2 ml. To each dilution, a specified number of hemagglutinating units of virus were added in a volume of 0.2 ml. Finally 0.4 ml of a 1% cell suspension was added. The tubes were shaken and the precipitated cell pattern observed after 1 hour at room temperature. These were graded as in the hemagglutinin titrations.

Absorptions of virus. Equal volumes of virus containing allantoic fluid and 25% cell suspensions (usually 0.5 ml of each) were mixed in a series of tubes. These were shaken frequently while at 37°C and after a specified period, quickly centrifuged, so that the supernatant fluid could be readily removed. The fluid was then titrated for residual hemagglutinin content using a 1% suspension of chicken cells. The period of contact between 25% cell suspension and allantoic fluid usually varied between 2 and 62 minutes, including the period of centrifugation.

Experimental. Agglutination of chicken and human erythrocytes by mumps virus. The Enders strain of mumps virus agglutinates both chicken and human red blood cells. Other strains (*e.g.* Habel) appear to agglutinate only chicken cells(4). It is known that allantoic fluids from different eggs contain varying amounts of hemagglutination inhibitor(3,5,6). However, from our present studies it seems clear that the inhibitor for mumps (Enders) hemagglutination in any particular sample of infected allantoic fluid is more apparent when human rather than chicken cells are used. This is illustrated in Table I which gives the results of a representative experiment in which fluids from embryos with titers with chicken cells which are comparable are also examined with human cells. Among 11 serial passages of mumps virus in which undiluted fluid from 78 individual embryos were tested, it was found that only 48% of those which agglutinated chicken cells also agglutinated human red blood cells. Yet many of the remaining 52% did agglutinate human cells almost as well as they did chicken erythrocytes when they were diluted beyond the effective range of the inhibitor (Table I).

TABLE I. Titration of Allantoic Fluid Infected with Mumps Virus Using Chicken and Human Red Blood Cells.

Fluid	Cells	Final dilution of fluid								
		1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
151–1	C	4	4	4	4	4	4	4	3	0
	H	3	4	4	4	4	2	0	0	0
2	C	4	4	4	4	4	4	4	0	0
	H	0	0	0	0	0	0	0	0	0
3	C	4	4	4	4	4	4	4	±	0
	H	0	0	±	1	1	0	0	0	0
4	C	4	4	4	4	4	4	4	4	0
	H	3	3	4	4	4	4	2	0	0
5	C	4	4	4	4	4	4	3	2	0
	H	0	2	3	4	4	2	0	0	0

C = Chicken.
H = Human.

Absorption of mumps virus by chicken and human erythrocytes. Further evidence of the greater activity of this inhibitor in the presence of human cells than in the presence of chicken cells was obtained from absorption studies. Fig. 1 presents results of representative experiments done with fluids which did not appear to have excess inhibitor, since when used undiluted they agglutinated human cells. Nevertheless, clear evidence of absorption of virus was obtained only with

4. Florman, A. L., Unpublished experiments.

5. Beveridge, W. B., and Lind, P. E., *Austral. J. Exp. Biol. and Med. Sci.,* 1946, v24, 127.

6. Lind, P. E., *Austral. J. Exp. Biol. and Med. Sci.,* 1948, v26, 93.

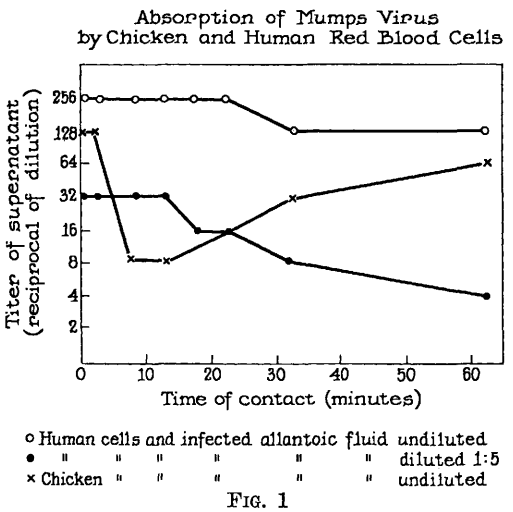

FIG. 1

chicken cells. When human cells were used there was no apparent absorption, unless the allantoic fluid was first diluted. Even then the absorption was definitely slower than with chicken cells. In other experiments, using similar suspensions of chicken and human cells with influenza virus, it was easy to demonstrate absorption of virus from undiluted infected allantoic fluids by both species of cells.

Effect of NAF and mumps infected allantoic fluid on small quantities of mumps virus in presence of human cells. Normal allantoic fluids diluted as high as 1:64 and 1:128 were capable of preventing agglutination of human cells by two and four units of mumps virus. This effect was less apparent as the amount of virus was increased. However, it was significant that doubling the amount of virus did not give a proportionate decrease in inhibitor activity. Allantoic fluids from several mumps infected embryos acted in a similar fashion though quantitatively much less effectively. The inhibitor effect was not noted beyond a dilution of 1:8 and 1:16. It seemed that a considerable amount of the inhibitor had been inactivated by the virus already present in this infected fluid.

Comparative inhibiting effect of NAF on agglutination by mumps virus of chicken and human cells. In order to determine whether the apparently greater inhibiting effect of allantoic fluid on mumps virus agglutination of human red blood cells is not merely a reflection of lesser sensitivity of human cells for the virus, the experiment summarized in Table II was carried out. A single pool of mumps virus was diluted as indicated. Different amounts of NAF were added to aliquots of each dilution. To one set of each pair there was added a suspension of chicken cells and to the other set a similar suspension of human cells. The linear reduction of titer when chicken cells are employed stands in striking contrast to the precipitous reduction when human cells are used. A comparison of the effect in the presence of 2 and 4 units of virus in each set is especially noteworthy. It is consequently clear that the inhibitor is more active in the presence of human cells, and that the relative sensitivity of these cells for the virus did not determine the observed results.

Attempts to modify the mumps virus-inhibitor reaction in presence of human cells. The use of infected allantoic fluids of varying ages (2 to 105 days), failed to modify the nature of the mumps virus-human red blood cell absorption curve. Variations in pH from 6.8 to 8.4 did not change this reaction, nor did the use of undiluted allantoic fluid which had been previously exposed for 1 hour to a 25% suspension of human cells.

Comparison of effect of NAF on agglutination by mumps and influenza viruses of chicken and human erythrocytes. In Table III there are presented the results obtained when small quantities (2 agglutinating units) of mumps and influenza viruses were mixed

TABLE II. Inhibitory Effect of NAF on Agglutination by Mumps Virus of Chicken and Human Cells.

		Final dilution of virus								
Cells	NAF	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	Saline
C	0	4	4	4	4	*4*	*4*	4	0	0
C	1:4	4	4	3	1	*0*	*0*	0	0	0
C	1:8	4	4	4	4	*1*	*0*	0	0	
C	1:16	4	4	4	4	*4*	*2*	0	0	
H	0	3	4	*4*	*4*	3	0	0	0	0
H	1:4	0	0	*0*	*0*	0	0	0	0	0
H	1:8	0	0	*0*	*0*	0	0	0	0	
H	1:16	0	0	*0*	*0*	0	0	0	0	

Italic figures = 2 and 4 units of virus present.

TABLE III. Effect of NAF on Agglutination of Chicken and Human Red Blood Cells by Mumps and Influenza Viruses.

Virus*	Cells	Final dilution of NAF 1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	Saline
Mumps	C	0	0	0	±	2	3	3	2	3
	H	0	0	0	0	±	1	2	3	4
Lee	C	0	±	3	4	4	4	4	4	4
	H	4	4	4	4	4	4	4	4	4
PR-8	C	0	0	1	2	3	3	3	3	3
	H	0	4	4	4	4	4	4	4	4
Saline Controls	C	0								0
	H	0								0

* 2 hemagglutinating units present in each instance.

with varying amounts of NAF and tested with chicken and human erythrocyte suspensions. Although the results with mumps virus are most striking, they are not essentially different from those obtained with PR-8 and Lee viruses. However, in contrast to the findings with mumps virus, with the influenza viruses the effect is more obvious with chicken cells than with human cells.

Discussion. The results of these experiments suggest that with mumps virus the equation introduced by Hardy and Horsfall (3) to explain the virus-inhibitor reaction:

$$\underset{\text{(Virus)}}{V} + \underset{\text{(Inhibitor)}}{I} \rightleftharpoons \underset{\text{(Virus—Inhibitor combination)}}{VI}$$

is influenced in the presence of human cells to move in the direction of combined virus (VI). Consequently, agglutination which depends upon the presence of free virus may not be apparent until the fluid is diluted beyond the zone of effective activity of the inhibitor. Since this same fluid before dilution may show clear agglutination of chicken cells, it would seem that in the presence of chicken cells the equation is influenced in the reverse direction (in the direction of free virus). With PR-8 and Lee viruses the same type of activity can be presumed to take place, with the exception that in those systems the chicken cells furnish something which influences the equation in the direction of combined virus.

Summary. It is shown that the inhibitor present in allantoic fluid for hemagglutination by and absorption of mumps virus is more active when human erythrocytes rather than when chicken red blood cells are used. It appears that the species of erythrocyte present influences the reaction between mumps virus and inhibitor in the direction of more or less combined (non-hemagglutinating) virus. A similar influence, though to a less striking degree, is also shown for the red blood cell in the influenza virus-inhibitor reaction.

Received August 11, 1950. P.S.E.B.M., 1950, v75.

Development of Fatty Livers in Fasted Male Mice Bearing a Transplantable Lymphosarcoma.* (18171)

ELIJAH ADAMS.† (Introduced by Abraham White.)

From the Department of Physiological Chemistry, School of Medicine, University of California, Los Angeles, Calif.

A variety of experimental conditions including dietary alterations, endocrine treatment, and the administration of toxic substances, are known to result in the development of fatty livers in laboratory animals (1). During the course of an investigation

* This investigation was aided by a grant (to Abraham White) from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

† American Cancer Society Postdoctoral Fellow. Present address: School of Medicine, University of Utah, Salt Lake City.

1. McHenry, E. W., and Patterson, J. M., *Physiol. Rev.*, 1944, v24, 128.