Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1959, **20**, 445-454

PROPERTIES OF INFLUENZA VIRUSES A/ASIA/57 AND A-EQUI/PRAHA/56

1. Agglutination of Red Blood Cells

B. TŮMOVÁ
O. FIŠEROVÁ-SOVINOVÁ

Institute of Epidemiology and Microbiology, Prague, Czechoslovakia

SYNOPSIS

Studies of the biological properties of the A/Asia/57 influenza viruses and of the strain A-equi/Praha/56 have shown that these agglutinate not only chicken, guinea-pig and human erythrocytes but also erythrocytes of swine, calves and horses. The ability to agglutinate horse erythrocytes, in particular, is a characteristic of these strains and has not been observed in other influenza strains of type A, A1, B, C or Sendai or in strains of mumps or Newcastle disease viruses.

In 1956 the authors isolated from a severe epizootic of a respiratory influenza-like disease of horses a virus (A-equi/Praha/56) which in most respects corresponded to the viruses of type A influenza, and had a common soluble antigen with the latter (Sovinová et al., 1957). Closer study of the strain, however, revealed that there were differences in its ability to agglutinate erythrocytes of several animal species.

Further, since 1 June 1957, 117 strains of influenza type A/Asia/57 have been isolated on the territory of Czechoslovakia. As is now generally known (Jensen, 1957; Meyer et al., 1957; Mulder et al., 1958), and as we ourselves have been able to confirm, this type of virus is distinct from the influenza strains previously encountered. In our present paper we should like to draw attention to the interesting ability of these influenza strains to agglutinate horse and calf erythrocytes and the red blood cells of swine; this property has likewise been observed in the strain A-equi, but has not been found in any other strains of the group mumps/Newcastle disease/influenza used in our laboratory.

Material and Methods

Strains

The following virus strains were used in our experiments:

A WS (28 egg passages)
A PR8 (198 ferret, 93 mouse and 112 egg passages)
A/Singapore/1/57 (from World Influenza Centre, London)
A/Netherlands/57 (6 egg passages; obtained from World Influenza Centre, London)
A-Swine (Shope) (15 ferret, 38 mouse and 38 egg passages)
A1 FM1 (33 egg passages)
A-equi/Praha/56 (8 egg passages)
B Lee (8 ferret, 37 mouse, 155 egg passages)
C/Hungary/RM (15 egg passages)
Sendai [1] (8 egg passages)
Mumps (Henle) (39 egg passages)
Newcastle disease virus (45 egg passages)

In addition, the strains A/Praha/2/57 (9 egg passages), A/Ústi/1/57 (5 egg passages), A/Bratislava/1/57 (5 egg passages), A/Ostrava/11/57 (4 egg passages), and A/Hradec/1/57 (8 egg passages), isolated in various towns of Czechoslovakia, A/USSR/1/57 (8 egg passages), and A/Romania/1/57 (6 egg passages), isolated from Soviet and Romanian tourists who had fallen ill after their arrival in Prague, were also used.

Two strains—the first and A1 designated A1/Ostrava/1/57 and the other B/Jihlava/1/57—were isolated from local epidemics in Czechoslovakia in February 1957 and served as control strains, having undergone only a small number (8) of passages.

Erythrocytes

Blood cells were all prepared in the same way, with the use of sodium citrate, washed three times in saline, centrifuged at 1500 revolutions per minute for 10 minutes, and diluted to a 10% suspension for storage. They were diluted again for use in our experiments.

Testing

For adsorption and elution, the method of Koziński and co-workers (1953) was employed, with the following modification in the final experiment: Into a set of tubes a uniform amount of virus and 2% blood cells was put, and the content was mixed thoroughly and adsorbed at 4°C for 3 hours. Samples of one tube at a time were taken for centrifugation and titration of virus from the supernatant fluid at 10-minute intervals at the beginning of the adsorption process and twice later at hourly intervals.

[1] The Virus Sub-committee of the International Nomenclature Committee is considering renaming the Sendai virus *Myxovirus para-influenzae* type 1. The use of the term influenza D is being specifically discouraged.—ED.

Another set of tubes was subjected to elution, after adsorption at 4°C, in a water-bath at 37°C, the liquid above the blood cells having been removed and further saline added.

Results

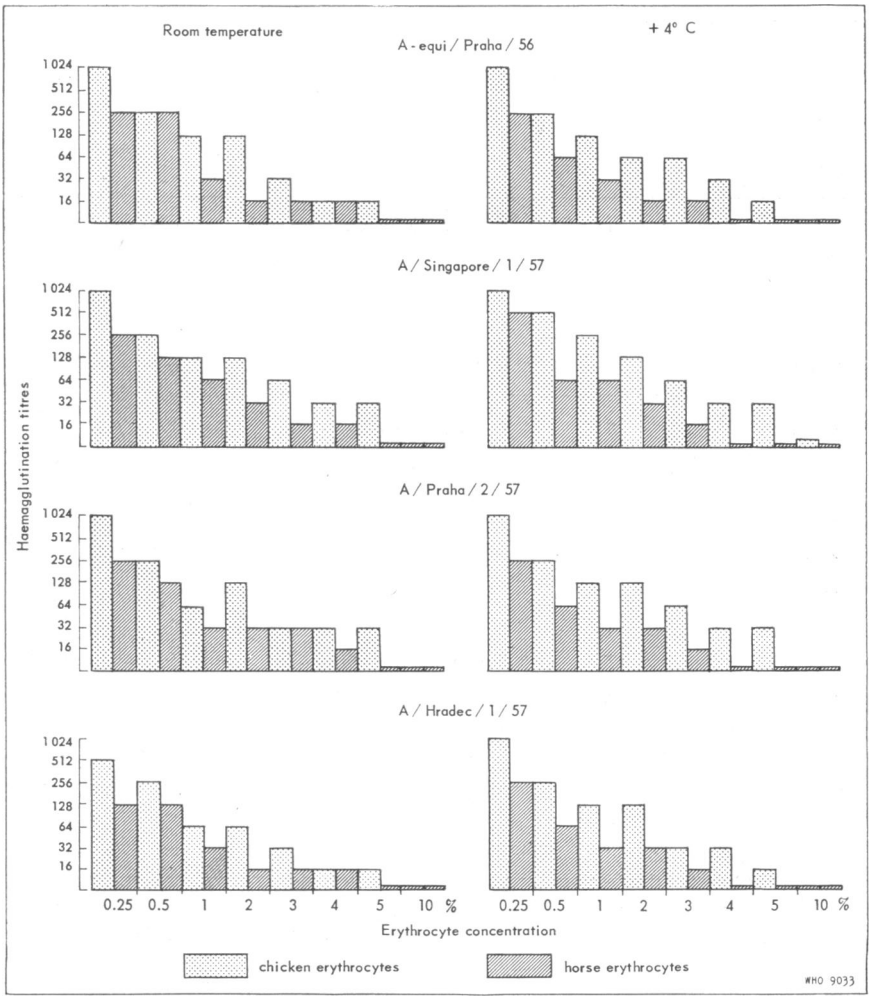
As may be seen in Table 1, all the strains of the group mumps/Newcastle disease/influenza agglutinated chicken, guinea-pig and human erythrocytes. The A/Asia/57 strains and the strain A-equi also agglutinated horse, calf and swine erythrocytes. The ability to agglutinate the latter group of red cells

TABLE 1. ERYTHROCYTE AGGLUTINATION WITH VARIOUS INFLUENZA, MUMPS AND NEWCASTLE DISEASE VIRUSES

Virus strain	Erythrocyte					
	chicken	guinea-pig	human	horse	pig	calf
Mumps	+	+	+	—	—	—
Newcastle disease	+	+	+	—	—	—
A WS	+	+	+	—	—	—
A-Swine (Shope)	+	+	+	—	—	—
A PR8	+	+	+	—	—	—
A1 FM1	+	+	+	—	—	—
A1/Ostrava/1/57	+	+	+	—	—	—
A-equi/Praha/56	+	+	+	+	+	+
A/Singapore/1/57	+	+	+	+	+	+
A/Netherlands/57	+	+	+	+	+	+
A/Praha/2/57	+	+	+	+	+	+
A/Ústí/1/57	+	+	+	+	+	+
A/Bratislava/1/57	+	+	+	+	+	+
A/Ostrava/11/57	+	+	+	+	+	+
A/Hradec/1/57	+	+	+	+	+	+
A/USSR/1/57	+	+	+	+	+	+
A/Romania/1/57	+	+	+	+	+	+
B Lee	+	+	+	—	—	—
B/Jihlava/1/57	+	+	+	—	—	—
C/Hungary/RM	+	+	+	—	—	—
Sendai	+	+	+	—	—	—

was never encountered in influenza strains of the types A, A1, B or C, nor was it observed in the Sendai, mumps and Newcastle disease strains. On the other hand, it was repeatedly verified in practically all the A/Asia/57 strains isolated. Various concentrations of red blood cells were used and agglutination was clear in those of 0.25% and 0.5%; higher concentrations gave less clear results. There were no differences at 0°C, + 4°C and room temperature (Fig. 1). The experiments were repeated several times, and for control red cells of other specimens (of the same species) were also used every time. Let it be stated merely as a point of interest that the

FIG. 1. AGGLUTINATION OF CHICKEN AND HORSE ERYTHROCYTES BY A/ASIA/57 AND A-EQUI/PRAHA/56 STRAINS AT DIFFERENT TEMPERATURES



titres were the same in horses which had been immunized intravenously with A/Asia/57 strains. Both the A-equi and the A/Asia/57 strains agglutinated erythrocytes of immunized and non-immunized horses in practically equally high titres (Table 2).

TABLE 2. HAEMAGGLUTINATION TITRES OF A/ASIA/57 AND [E-EQUI/PRAHA/56 STRAINS WITH ERYTHROCYTES OF NON-IMMUNIZED HORSES AND OF HORSES IMMUNIZED WITH A/ASIA/57 ANTIGEN

Virus strain	Horse erythrocytes	
	immunized	non-immunized
A/Singapore/1/57	>512	128
A/Netherlands/57	>16	64
A/Praha/2/57	128	32
A/Ústí/1/57	64	64
A/Bratislava/1/57	128	64
A/Ostrava/11/57	128	128
A/USSR/1/57	32	64
A/Hradec/1/57	>128	64
A-equi/Praha/56	>128	64

Haemagglutination titres of the A/Asia/57 and A-equi strains with horse and calf red cells as compared with titre levels observed in chicken and pig cells are presented in Fig. 2. The titres were very low, especially in the case of calf red cells, where they were as much as 32 times lower than those with chicken cells. The highest titres were obtained, in almost all the strains, with guinea-pig erythrocytes. It is interesting that the titres obtained with guinea-pig and horse red cells were not equal, although both represent titres with mammalian cells. This discrepancy may also be seen on the curve showing adsorption of the viruses.

The process of adsorption and the maintaining of the viruses adsorbed on to the blood cells is given in Fig. 3 and 4; the elution of the A/Asia/57 virus is shown in Fig. 5. As stated earlier, adsorption was followed up for three hours at + 4°C. Whereas in the case of guinea-pig erythrocytes the virus separated from the blood cells, in that of chicken and horse erythrocytes it remained bound for the whole three hours. Elution of the A/Asia/57 virus followed practically the same course in both chicken and horse erythrocytes. It was not possible to test adsorption on, and elution from, swine and calf red cells because of technical difficulties in procuring the material necessary for such trials.

FIG. 2. AGGLUTINATION OF VARIOUS ANIMAL ERYTHROCYTES BY A/ASIA/57 AND A-EQUI/PRAHA/56 STRAINS

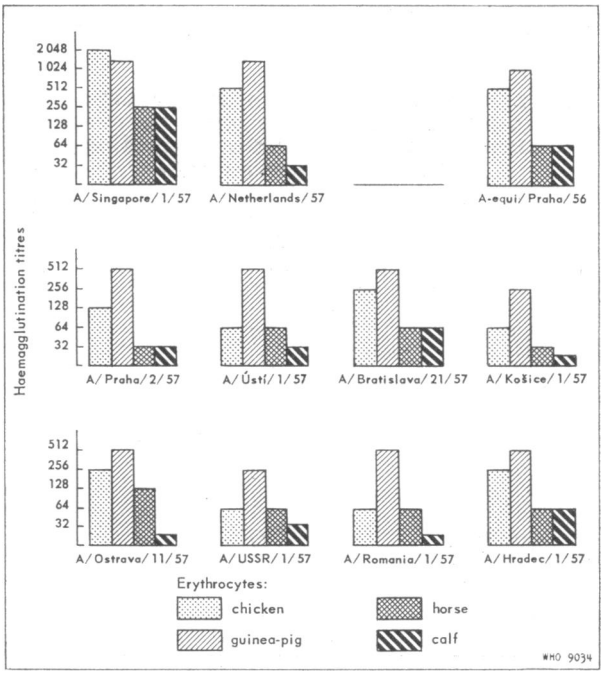


FIG. 3. ADSORPTION OF A/HRADEC/1/57 STRAIN ON GUINEA-PIG, HORSE AND CHICKEN ERYTHROCYTES

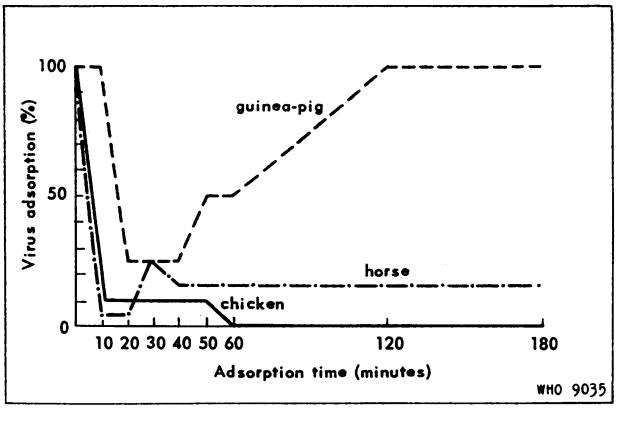


FIG. 4. ADSORPTION OF A-EQUI/PRAHA/56 STRAIN ON GUINEA-PIG, HORSE AND CHICKEN ERYTHROCYTES

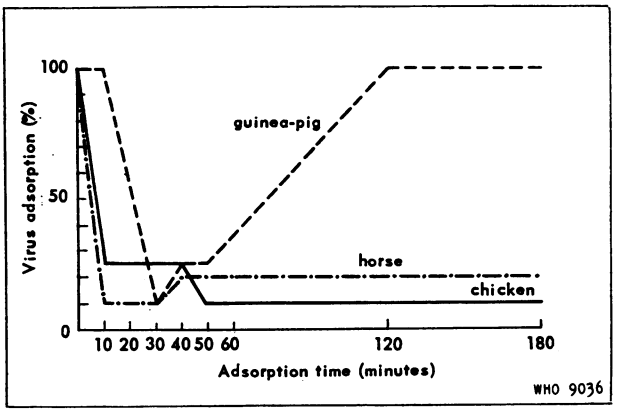
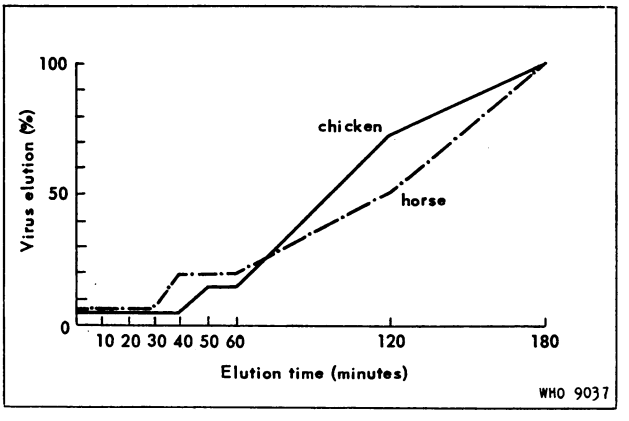


FIG. 5 ELUTION OF A/HRADEC/1/57 FROM CHICKEN AND HORSE ERYTHROCYTES



Discussion

It has been the object of this paper to draw attention to certain interesting findings on the agglutination of horse, swine and calf erythrocytes by A/Asia/57 influenza viruses and by the virus A-equi/Praha/56, which was isolated a year before the Asian influenza pandemic from an outbreak of respiratory disease in horses. This agglutination was not observed in any other type of influenza virus or in the viruses of mumps or Newcastle disease. We do not think that negative haemagglutination in these strains was due to their having sustained a larger number of passages through the chicken embryo. We deliberately used two strains, influenza types A1

and B, isolated three months before the epidemic occurred, as controls; these strains also gave negative haemagglutination.

It is, rather, our opinion that the phenomenon is one of " selective haemagglutinability " as described by Tamm (1954) in the Lee virus, with cat erythrocytes, by Horváth et al. (1951) likewise in the Lee virus, with hamster red cells, and by Borecký (1956) in his study of the agglutination of suslik erythrocytes by influenza viruses B and C and by the Newcastle disease virus. Borecký also drew attention to the possible diagnostic use of this phenomenon.

Naturally, we cannot exclude the possibility of there being other strains that would also give positive results in the haemagglutination reaction with horse erythrocytes. But we have tested, besides trying a large number of A/Asia/57 strains, haemagglutination not only in a large number of A/Asia/57 strains, but also in 16 strains of types A, A1 and B, isolated in the period 1949-57, repeatedly obtaining negative results.

After we had finished the experiments described above, we obtained a certain number of other strains from the World Influenza Centre in London. Among these, we found two which produced the same phenomenon as the A/Asia/57 and the A-equi/Praha/56 strains; these were both of animal origin—one a duck influenza virus and the other a fowl plague virus. Agglutinability was found repeatedly in tests carried out both with the British duck influenza strain and the A-anatis/Košice/1956 strain which had

TABLE 3. HAEMAGGLUTINATION-INHIBITION REACTIONS WITH HORSE AND CALF ERYTHROCYTES *

Immune rat sera	Horse erythrocytes							Calf erythrocytes					
	A-equi/Praha/56	A/Singapore/1/57	A/Praha/2/57	A Iksha **	A/Ústí/1/57	A/Hradec/1/57	A/Ostrava/11/57	A-equi/Praha/56	A/Praha/2/57	A Iksha **	A/Ústí/1/57	A/Hradec/1/57	A/Ostrava/11/57
A-equi/Praha/56	1024	0	0	0	0	0	0	256	0	0	0	0	0
A/Singapore/1/57	0	2048	2048	512	2048	1024	512	0	256	64	64	128	128
A/Praha/2/57	0	2048	2048	512	512	1024	512	0	512	256	256	256	256
A Iksha **	0	2048	2048	1024	1024	2048	1024	0	512	256	64	256	256
Control	0	0	0	0	0	0	0	0	0	0	0	0	0

* Non-specific inhibitors were removed from all the sera with potassium periodate. 0 = haemagglutination same as in control of virus.

** A Iksha strain was isolated in the USSR (Zhdanov et al., 1957).

been isolated in Czechoslovakia from a respiratory disease of ducks. In the fowl plague strain, this ability had been ascertained previously, and has been described by Brandly et al. (1946) among others.

The agglutination of red blood cells, as performed by us, is so characteristic of the A/Asia/57 viruses that we made use of it during the Asian influenza epidemic, which was observed in Czechoslovakia from May 1957 (Raška et al., 1957), as an auxiliary test for identification of the strains isolated (Table 3).

The questions why this phenomenon is common to the A/Asia/57 strains, isolated in May 1957 or later, and to the strain A-equi/Praha/56, isolated in October 1956, and what is the relationship between these strains remain open for the time being.

RÉSUMÉ

En 1956, les auteurs ont isolé, au cours d'une épizootie du cheval, grave et d'allure grippale, un virus ressemblant sur plusieurs points à un virus A, avec lequel il a un composant antigénique commun. Il a été désigné comme virus A/Equi/Praha/56. Il se distinguait cependant des virus A connus jusqu'alors par des différences de pouvoir agglutinant vis-à-vis des hématies de diverses espèces animales.

En 1957, 117 souches du virus A/Asia/57 ont été isolées en Tchécoslovaquie. Les auteurs appellent l'attention sur la faculté que possèdent les virus de ce groupe d'agglutiner les érythrocytes de cheval, de veau et de porc. Ils partagent cette propriété avec le virus A/Equi et se distinguent des virus du groupe oreillons/maladie de Newcastle/grippe, qui ne la possèdent pas. Un tableau synoptique indique que ce dernier groupe de virus agglutine les hématies de poulet, de cobaye et d'homme. Presque toutes les souches A/Asia/57 et la souche A/Equi agglutinent en outre les hématies de cheval, de veau et de porc. Selon les auteurs, il s'agirait d'une « hémagglutination sélective » telle que l'ont décrite certains auteurs pour le virus Lee avec les hématies de chat et de furet, pour les virus B, C et Newcastle, avec les hématies d'écureuil (suslik).

Seize souches de types A, A1, et B, isolées de 1949 à 1957 ont donné des résultats négatifs avec les hématies de cheval. Deux des souches reçues du Centre mondial de la Grippe, en revanche, produisaient les même réactions que la souche A/Asia/57. Il s'agissait d'un virus du canard et d'un virus de la peste aviaire. Le pouvoir agglutinant particulier des souches A/Asia/57 était si caractéristique qu'il a servi de test auxiliaire de diagnostic au cours de l'épidémie de grippe asiatique. Les raisons de l'analogie du pouvoir agglutinant des souches A/Asia/57 et de la souche A/Equi/Praha/56 sont inconnues.

REFERENCES

Borecký, L. (1956) *Čsl. Mikrobiol.*, **1**, 282
Brandly, C. A., Moses, H. E., Jungherr, E. L. & Jones, E. E. (1946) *Amer. J. vet. Res.*, **7**, 289
Horváth, S. Szölösy, E. & Ivánovics, G. (1951) *Acta physiol. Acad. Sci. hung.*, **2**, 77
Jensen, K. E. (1957) *J. Amer. med. Ass.*, **164**, 2026
Koziński, A. W., Mikulaszek, E. & Sitek, K. (1953) *Med. dośw. Mikrobiol.*, **5**, 457
Meyer, H. M. et al. (1957) *Proc. Soc. exp. Biol. (N.Y.)*, **95**, 609
Mulder, J. et al. (1958) *Lancet*, **1**, 810

Raška, K., Pečenka, J. & Tůmová, B. (1957) *Čas. Lék. čes.*, **96**, 1383
Sovinová, O., Tůmová, B., Pouska, F. & Němec, J. (1957) *Čsl. epidem.*, **6**, 213 (Also in *Acta virol. (Praha)*, 1958, **2**, 52, English edition)
Tamm, I. (1954) *J. Immunol.*, **73**, 180
Zhdanov, V. M. et al. (1957) *Lancet*, **2**, 735