THE HEMAGGLUTINATING ACTIVITY OF THE VARIOLA VIRUS

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Among the properties of the variola virus studied only slightly is its hemagglutinating activity. Information on this is practically limited to the communication of North, who noted the ability of the variola virus to produce agglutination of hen and chick embryo erythrocytes. We considered it expedient to check the hemagglutinating activity of the variola virus with respect to erythrocytes of various species of animals. The present work is aimed at augmenting the available data concerning the variola virus and its properties, as well as at attempting to establish differences between the smallpox virus and the closely related vaccine virus, using hemagglutination as an index. This attempt is important for differentiation of the viruses mentioned.

Variola virus (strain "Narz") grown in developing chick embryos was used in this work; it had undergone 18 passages by the time of the investigation. Parallel experiments were staged with vaccine virus adapted to chick embryos (ovovaccine).

Virus-containing suspensions were prepared with physiologic solution 1:10 from chorio-allantoic membranes of chick embryos infected with variola virus and ovovaccine after appropriate incubation (120 hours at 34.5° for variola virus and 72 hours at 34.5° for ovovaccine virus).* The virus suspensions so obtained were centrifuged for 10 minutes at 2500 rpm, the supernatant liquid was pipetted off and, after checking for bacterial contamination and infective titer, used for hemagglutination reaction.

The hemagglutination reaction was carried out using 1 ml volumes with two-fold descending dilutions of virus and 1% suspension of thrice washed erythrocytes. Parallel controls were set up to check for spontaneous agglutination of erythrocytes. The intensity of the reaction was designated by plusses (++++, +++, ++, +, ±, –). The hemagglutination titer was taken to be the final dilution of the virus which elicited visually perceptible hemagglutination (±).

Erythrocytes of the following animals were used in the experiments: cocks, chicks, developing chick embryos, pigeons, white mice, white rats, guinea pigs, rabbits, cats, dogs, rams, hamsters, piglets, frogs. Human erythrocytes were also used. Taking into account the available data (Masiukova, Le Khe Min[5]) concerning the effect of temperature on the rate and intensity of hemagglutination reaction of vaccine viruses, the experiments were staged at various temperatures (4°, 20°, 35°). The experimental results are presented in Table 1.

As Table 1 shows, of the 15 species of erythrocytes tested variola virus elicited agglutination only in cock erythrocytes. The spectrum of hemagglutinating activity of vaccine virus proved to be wider: it produced agglutination of cock, pigeon, white rat, white mouse, chick and cat erythrocytes; with the exception of the first two cases the titer and intensity of hemagglutination were very low. The highest titer and intensity of the reaction was observed in the case of cock erythrocytes. Comparison of the hemagglutinating titers of variola virus and vaccine at various temperatures showed that, as a rule, the titers rose to some extent with a rise in temperature. It must be stressed that although the vaccine virus and the variola virus possessed the ability of agglutinat-

*Infection of chick embryos with variola virus was effected by application to the chorio-allantoic membrane, with ovovaccine by introduction into the allantoic sac.

TABLE 1

Hemagglutinating Activity of Variola Viruses and Ovovaccine

Erythrocyte donors	Ovovaccine virus						Variola virus					
	reaction at temperature of											
	4°		20°		35°		4°		20°		35°	
	hemagglutination reaction titer	intensity of reaction	hemagglutination reaction titer	intensity of reaction	hemagglutination reaction titer	intensity of reaction	hemagglutination reaction titer	intensity of reaction	hemagglutination reaction titer	intensity of reaction	hemagglutination reaction titer	intensity of reaction
Cocks	320	++++	320	++++	640	++++	10	±	20	+	40	+
Chicks	0	—	20	±	10	±	0	—	0	—	0	—
Chick embryos	0	—	0	—	0	—	0	—	0	—	0	—
Pigeons	40	+	80	+	80	+	0	—	0	—	0	—
White mice	0	—	10	±	20	+	0	—	0	—	0	—
White rats	0	—	0	—	20	+	0	—	0	—	0	—
Cats	0	—	0	—	20	±	0	—	0	—	0	—
Dogs	0	—	0	—	0	—	0	—	0	—	0	—
Ram	0	—	0	—	0	—	0	—	0	—	0	—
Guinea pigs	0	—	0	—	0	—	0	—	0	—	0	—
Rabbits	0	—	0	—	0	—	0	—	0	—	0	—
Hamsters	0	—	0	—	0	—	0	—	0	—	0	—
Piglet	0	—	0	—	0	—	0	—	0	—	0	—
Frog	0	—	0	—	0	—	0	—	0	—	0	—
Man	0	—	0	—	0	—	0	—	0	—	0	—

ing cock erythrocytes the hemagglutinating titer and intensity of the reaction were considerably lower in the case of the variola virus.

The differences found in the hemagglutinating activity of the two viruses for cock erythrocytes could be determined by either the individual sensitivity of the erythrocytes used or the particular properties of the strain of variola virus used in the investigation.

In order to clarify these questions we staged special experiments in which erythrocytes from 20 cock-donors were used, the erythrocytes themselves differing in their sensitivity to the agglutinating action of the vaccine virus. The investigation was made with 10 strains of variola virus (3-5 passages through chick embryos) simultaneously.

Data presented in Table 2 confirmed our conclusion that the variola virus possessed weak hemagglutinating activity with respect to cock erythrocytes. None of the 10 strains tested gave a hemagglutination titer exceeding 80. In some strains no hemagglutinating ability could be detected under our experimental conditions. At the same time, a clearly consistent feature must be mentioned: hemagglutinating ability of variola virus could only be detected when erythrocytes highly sensitive to the agglutinating action of ovovaccine virus were used. Thus, erythrocytes which gave a titer of 0 to 320 with a standard suspension of ovovaccine virus did not agglutinate with variola virus. Agglutination appeared when erythrocytes which gave titers of 640 and higher with the same suspension of ovovaccine virus were used. The greatest hemagglutinating activity of variola virus was noted in those experiments in which the erythrocytes most sensitive with respect to ovovaccine virus were used.

Unlike the vaccine virus which, beginning with the first passages through chick embryos, gives consistently high hemagglutinating titers, the hemagglutinating activity of variola virus remains weak and fluctuates from passage to passage, sometimes being completely absent (traced through 26 passages).

North has noted that variola virus can elicit (very weakly, it is true) agglutination of chick embryo erythrocytes. In contrast to these data we did not obtain any positive results with chick embryo erythrocytes which may

TABLE 2

Relation of Hemagglutinating Activity of Variola Virus to the Sensitivity of Cock Erythrocytes to the Agglutinating Action of Ovovaccine Virus

Hemagglutination reaction titer with ovovaccine virus	Strains of variola virus									
	"Narz"	"Dzh"	"NKh"	"Khal"	"Ali"	"Um"	"Bur"	"Dus 11"	"ND"	"Utag"
	number of passages									
	5	5	4	4	5	4	3	5	4	5
	hemagglutination reaction titer									
0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0
160	0	0	0	0	0	0	0	0	0	0
320	0	0	0	0	0	0	0	0	0	0
320	0	0	0	0	0	0	0	0	0	0
320	0	0	0	0	0	0	0	0	0	0
320	0	0	0	0	0	0	0	0	0	0
320	0	0	0	0	0	0	0	0	0	0
320	0	0	0	0	0	0	0	0	0	0
320	0	0	0	0	0	0	0	0	0	0
640	0	0	0	0	0	0	0	0	0	0
640	0	0	0	0	0	0	0	0	0	0
640	20	0	0	0	0	0	0	20	0	0
640	20	0	0	0	0	0	0	20	0	0
640	20	0	0	0	0	0	0	20	0	0
640	20	20	0	0	20	0	0	20	0	0
640	20	20	0	0	20	0	0	20	0	0
1280	40	0	0	0	0	0	0	20	0	0
1280	40	20	0	0	20	0	20	20	20	0
1280	80	20	0	0	40	0	20	20	0	0

be explained by the fact that in our experiments less concentrated virus suspensions were used and by differences in individual sensitivity of erythrocytes.

Thus, we have found that the variola virus has weak hemagglutinating activity which becomes apparent only with cock erythrocytes highly sensitive to the ovovaccine virus.

SUMMARY

Data on hemagglutinating activity of variola virus are presented in this paper. It was demonstrated that out of 15 types of erythrocytes obtained from various species of animals the virus of smallpox caused mild agglutination only in case of cock erythrocytes. It was also shown that the ability of the smallpox virus to cause hemagglutination appeared only with cock erythrocytes which were highly sensitive to the virus of this vaccine. This was established by experiments with 10 different strains of smallpox virus.

LITERATURE CITED

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*In Russian.