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Hemagglutination with Polyhedrosis Virus of Tobacco Caterpillar, *Spodoptera litura* F.

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ABSTRACT

The polyhedrosis virus of tobacco caterpillar *Spodoptera litura* F. agglutinated 0.25 per cent chicken and Guinea pig erythrocytes and 0.5 per cent monkey erythrocytes, but did not agglutinate the bovine and sheep erythrocytes. Incubation temperature and also length of storage period of virus in refrigerator had no effect on the hemagglutinating property of the virus.

THE pioneering study of Hirst (1941) on the hemagglutination with influenza virus provided a new tool for study of certain basic properties of viruses. Serology is frequently used to characterize insect viruses. Krywienczyk and coworkers demonstrated that complement fixation (Krywienczyk and Bergold, 1960, 1961 ; Krywienczyk, 1962), gel diffusion (Krywienczyk and Bergold, 1960b; Krywienczyk *et al.*, 1969), immunoelectrophoresis (Krywienczyk, 1963 ; Krywienczyk and Sohi, 1967), could be successfully employed on insect viruses. Hemagglutination, however, was generally unsuccessful with insect viruses (Cunningham and Tinsley 1964), until Miyajuma and Kawase(1969) reported the agglutination by the cytoplasmic and nuclear polyhedrosis virus of silkworm, of certain vertebrate erythrocytes. Shapiro and Ignoffo (1969) also reported the agglutination of chicken erythrocytes by the nuclear polyhedrosis virus of cotton bollworm, *Heliothis zea.* Recently, Norton and Discapua (1975) described the agglutination of chicken erythrocytes by nuclear polyhedrosis virus of *Porthetria dispar.* In the present study the polyhedrosis virus of *Spodoptera litura* F. was used to study its hemagglutination properties with different mammalian and avian erythrocytes.

MATERIAL AND METHODS

Polyhedrosis virus preparation : The virus was obtained from diseased *S. litura* larvae. Diseased larvae (10 g) were blended in 50 ml of normal saline (0.85 per cent) and virus was purified by differential centrifugation. The fluid was clarified at 2000 rpm and the virus was pelletized at 15,000 rpm. The purity of the pellatige virus was examined by microscopy. Thd pellet was collected, diluted, in normae saline and standardized by taking the l count with hemocytometer. The suspension containing 2.13×10^6 per ml. polyhedral bodies was used for the test.

Hemagglutination: For hemagglutination test, erythrocytes of sheep, chicken, monkey, guinea pig and cattle were used at 0.25, 0.5 and 1.0 per cent concentrations. The effect of incubation at 40°C, 23±2°C or 37°C was also examined. The effect of storage of virus on hemagglutination was

TABLE I

Response of different erythrocytes and their concentration on hemagglutination by Spodoptera litura polyhedrosis virus

Source and concentration of erythrocytes	%	Reciprocal of viral dilutions								
		2	4	8	16	32	64	128	256	512
Cattle ..	0.25	−	−	−	−	−	−	−	−	+
	0.50	−	−	−	−	−	−	−	−	−
	1.00	−	−	−	−	−	−	−	−	−
Chicken ..	0.25	+	+	+	+	+	+	+	+	±
	0.50	+	+	+	+	+	+	+	+	±
	1.00	+	+	+	+	+	+	+	+	±
Monkey ..	0.25	−	−	−	−	−	−	−	−	−
	0.50	+	+	+	±	−	−	−	−	−
	1.00	−	−	−	−	−	−	−	−	−
Guinea pig ..	0.25	+	+	+	+	+	+	+	+	±
	1.00	+	+	+	+	+	+	+	+	±
Sheep ..	0.25	−	−	−	−	−	−	−	−	−
	0.50	−	−	−	−	−	−	−	−	−
	1.00	−	−	−	−	−	−	−	−	−

Note : +=Agglutination ; ±=Partial agglutination ; −=No agglutination

examined by storing the virus in refrigerator (4°C) for 12, 24 or 36 hr before using for the test and was compared with the freshly harvested virus.

Two-fold serial dilutions of the virus suspension were prepared in normal saline in perspex plates (WHO type) starting from 1:2. To each well 0.25 ml (equal quantity of erythrocyte suspension was added and incubated at test temperatures). The highest dilution of virus producing at 100 per cent hemagglutination was considered as the titre. The results were read at an interval of 30 min. At the end of two hours, the plates were kept at 4°C overnight. The normal saline solution and erythrocyte suspension mixture were kept as controls.

RESULTS

Hemagglutination with different erythrocytes : From the results of hemagglutination test recorded in Table I, it was observed that chicken, guinea pig and monkey erythrocytes were agglutinated by the polyhedrosis virus of *S. litura*, whereas, the bovine and sheep erythrocytes were not agglutinated. The highest hemagglutination titre was observed in chicken and guinea pig erythrocytes. Although the monkey erythrocytes were agglutinated, the titre obtained was very low (1 : 8 only). In none of the cases hemagglutination was observed up to one hour of incubation. It occurred with chicken erythrocytes in 2 hour and with guinea pig erythrocytes on overnight incubation at 4°C.

Effect of erythrocyte concentration : The different erythrocyte concentrations (0.25, 0.5 and 1.0 per cent) did not influence the agglutination of chicken and guinea pig erythrocytes by the virus. With monkey erythrocytes agglutination was observed only at 0.5 per cent concentration. However, there was no effect of erythrocyte concentration on hemagglutination titre.

Effect of incubation temperature: It was noted that the incubation temperature of 4°C, 23±2°C or 37°C had no effect on hemagglutination with different erythrocytes used, the hemagglutination titre being the same at all the temperatures employed in the studies. No elution was seen with this virus.

Effect
nation: The storage of virus for 12, 24 or 36 hr at 4°C had no influence on hemagglutination property of the virus as the titre was the same with fresh or stored virus using chicken and guinea pig erythrocytes.

Discussion

The studies on hemagglutination by insect viruses are of recent interest and in the present work the hemagglutinating property of polyhedrosis virus of *Spodoptera litura*, is being reported for the first time. The agglutination of vertebrate erythrocytes by insect viruses has been reported earlier with polyhedrosis virus of *Bombyx mori* (Miyajima and Kawase, 1969), nuclear polyhedrosis virus of *Heliothis zea* (Shapiro and Ignoffo, 1970) and *Porthetria dispar* (Norton and Discapua, 1975). The chicken and guinea pig erythrocytes are known to be agglutinated by orthomyxo and paramyxo viruses (Rosen, 1964). The chicken erythrocytes have also been used with insect viruses (Miyajima and Kawase, 1969; Shapiro and Ignoffo, 1969; and Norton and Dicapua, 1975). Although storage of the polyhedrosis virus at 4°C up to 36 hr did not affect the hemagglutinating property, but in our preliminary studies prolonged storage resulted in reduction in the hemagglutination titre by this virus, which might probably be due to autolytic changes. Variation in the hemagglutination at different temperatures has been reported with animal viruses (Rosen, 1964) but no such effect was seen with polyhedrosis virus of *S. litura*.

The presence of the property of hemagglutination in this polyhedrosis virus, can be usefully exploited to assay the virus and also to understand the cell-virus interactions. The lack of elution, is suggestive of the absence of the enzyme responsible for the receptor destruction, which has been known to be absent in pox and toga viruses of the vertebrates though they show hemagglutination. However, work on the specificity of this hemagglutination by inhibiting with the antiserum, and further standardisation of the test to identify the nature of the receptors is in progress.

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