STUDIES ON VACCINIA HAEMAGGLUTININ

I. SOME PHYSICO-CHEMICAL PROPERTIES

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(With 1 Figure in the Text)

Haemagglutination by vaccinia virus preparations was first described by Nagler (1942, 1944, Clark & Nagler, 1943) who found that the red cells of approximately 50 % of fowls are agglutinated by saline suspensions of vaccinia virus prepared from the chorioallantoic membrane of chick embryos, that calf lymph lacks this property and that human subjects following vaccination produce specific haemagglutinin-inhibiting substances in their sera which can be quantitatively measured by a modified Hirst test technique. Later work by Burnet and his coworkers revealed several important facts which placed vaccinia haemagglutinin (hereafter abbreviated as v.H.) in a separate category distinct from other virus haemagglutinins. Briefly, the main findings of these workers may be summarized as follows:

(1) Rabbit skin virus preparations also possess haemagglutinating activity (Stone & Burnet, 1946).

(2) The haemagglutinating titre can be disassociated from the infectivity titre by centrifugation or absorption with susceptible fowl red cells (Burnet & Stone, 1946).

(3) Phospholipids like lecithin, cephalin and cardiolipin agglutinate the same range of susceptible cells (Stone, 1946a). Furthermore, the v.H. can apparently be destroyed by the α-toxin of type A of *Cl. welchii* and by cobra snake venom, both known to contain the enzyme lecithinase (Stone, 1946b).

(4) On these findings Burnet (1946) suggested that v.H. probably represents a phospholipid-protein complex in the nature of a soluble antigen.

Haemagglutinins apparently similar to that of vaccinia have been reported in variola (North, 1944) and ectromelia viruses (Burnet & Stone, 1946). The haemagglutinin of the latter virus has been shown to be immunologically related to vaccinia, but agglutinates mouse red cells as well as susceptible fowl cells.

In an investigation on the problem of virus haemagglutination, the present author reached the conclusion already reported by Burnet & Stone (1946) on the duality of vaccinia haemagglutinin and virus. It appeared that further studies on the nature of v.H. would be desirable. Consequently experiments have been performed to investigate some of its physico-chemical properties and it is the purpose of this paper to record the results of these experiments.

Strains of virus. Three strains of vaccinia virus have been employed: (1) Egg or testicular materials prepared from a testicular strain of the Cambridge Pathology Laboratory kept by passage in rabbit testis and recently adapted to the chorioallantoic membrane of chick embryos. (2) Rabbit materials prepared from Salaman's rabbit skin adapted strain. (3) 'Sheep lymph', namely, obtained from the Lister Institute, in the form of wet pulp.

Vaccinia haemagglutinin preparations. Chorioallantoic membranes with confluent lesions were collected 48–72 hr. after inoculation and ground in saline to 20 % (by weight) suspension. After clarification by low-speed centrifugation, the extract retained its haemagglutinin titre for at least 3 months at 4° C. Sheep lymph suspensions were prepared in a similar manner. Most experiments with rabbit materials were performed with the supernatant fluid obtained from the first angle centrifugation in the process of preparation of elementary body suspension (hereafter abbreviated as E.B.S.), about 2 % ether being added as a preservative. Besides being rich in v.H., the supernatant fluid also contains L-S antigen and some undeposited virus. In this form, the haemagglutinin titre tends to drop considerably after keeping for some weeks at 4° C., and an opacity and small floccules appear in the solution. The fluid medium contained very low salt content (m/250 McIlvaine's buffer); it was found that addition of strong NaCl solution to a final 0·9 % concentration served to stabilize v.H. in solution. In later experiments, such materials have also been kept frozen at −20° C. without preservatives with satisfactory results.

Elementary body suspension and soluble antigens. These were prepared according to the technique of Craigie (1932, as modified by Parker & Rivers, 1935) with the important modification that vigorous scraping was used to harvest the virus from rabbit skin. Stone & Burnet (1946) had already reported that most of the v.H. occurred in the deeper skin

layers. The elementary bodies were washed at least three times.

Anti-vaccinia sera. As specified later, both convalescent and hyperimmune sera from rabbits were used. The hyperimmune sera were prepared by further immunizing convalescent rabbits with a course of three to four intravenous injections of E.B.S.

Red blood cell suspensions. Susceptible fowl red cells were regularly obtained from two fowls by venepuncture. Citrated blood cells were washed three times in saline. Packed cells stored at 4° C. are satisfactory to the end of 1 week. A 0·5 % suspension was diluted out on the day of test.

Infectivity titration. The infectivity tests were performed either by intradermal inoculation of the shaved backs of rabbits or on the chorioallantoic membrane of 12-day old chick embryos, by pock counting according to the technique of Burnet (Beveridge & Burnet, 1946).

Haemagglutination test. The test was performed in round-bottom tubes of 0·8 × 7·0 cm. size. Serial twofold dilutions of v.h. were made in saline containing M/250 McIlvaine's buffer at pH 7·0, the volume in each tube being 0·25 c.c. An equal amount of 0·5 % fowl red cells was added. The tubes were shaken thoroughly and allowed to stand at room temperature for 1 hr. by which time the cell column had just completely settled. The patterns of the red cells at the bottoms of the tubes were taken as an indication of the degree of agglutination.

+ +indicates complete agglutination, when the bottom is covered by a shield of agglutinated cells, the edges of which tend to roll down in tubes with strong concentrations of v.h. In such tubes, large clumps can be tapped up after settling.

+indicates partial agglutination with a ring of red cells at the bottom and aggregated cells around it.

±indicates slight agglutination with a central button-shaped mass and fine aggregates around it.

−indicates no agglutination with a small round, smooth central 'button'.

The tube showing + agglutination was taken as the end-point; this is usually clearcut. The end-point was interpolated between two dilutions when the change of pattern was either too abrupt or too gradual.

At this point, certain characteristics of vaccinia haemagglutination may be mentioned.

(1) The reaction is essentially slow and progressive and increases with time.

(2) Agglutination is accelerated at 37° C. and markedly inhibited at 4° C.

(3) The agglutination titre varies with the pH of the medium, being progressively lower on the acid side and higher on the alkaline side of neutrality.

(4) The presence of much normal serum tends to cause rapid slipping of agglutinated cells. This difficulty can be obviated by taking a preliminary reading 20–30 min. after set-up. The titre read at this time is usually one tube lower than at the final reading.

(5) Within the limits of technical error, variation of red cell concentration from 0·25 to 2 % showed that the titre obtained is inversely proportional to the cell concentration.

(6) Whenever there is any doubt of the specificity of the haemagglutinin in question, titrations were always performed in parallel in saline, and in the presence of normal rabbit serum and of anti-vaccinia serum. The presence of non-specific haemagglutinin in certain chorioallantoic membrane preparations has been reported by Stone & Burnet (1946).

The result of day to day titration indicates that provided these factors are controlled and the same fowl blood is used, the maximum error does not exceed one dilution.

All titres in this and the next paper are expressed in terms of the initial dilution of the materials tested.

THE OCCURRENCE OF VACCINIA HAEMAGGLUTININ IN VACCINIA PREPARATIONS FROM DIFFERENT SOURCES

As already mentioned, v.h. has been found in vaccinia materials from chorioallantoic membranes and rabbit skin, but not in calf lymph. In an attempt to demonstrate the specific occurrence of this haemagglutinin, several vaccinia preparations from different sources were examined, namely, (1) 20 % saline suspension of chorioallantoic membranes; (2) 20 % saline suspension of rabbit testis; (3) crude virus suspension from rabbit skin; (4) purified E.B.S. from rabbit skin; (5) 20 % saline suspension of sheep lymph. The results of such tests performed in parallel in saline, and in the presence of normal rabbit and of anti-vaccinia serum are shown in Table 1.

Table 1. *Titration of vaccinia haemagglutinin in virus preparations from different sources*

Materials	Saline	Normal rabbit serum 1/10	Anti-vaccinia serum 1/10
Chorioallantoic membranes	128	128	< 2
Rabbit testis	128	96	< 2
Rabbit skin	64	64	< 2
Purified E.B.S.	24	16	< 2
Sheep lymph	32	24	< 2

< Used here and in subsequent tables indicates negative result with the lowest dilution tested.

These experiments demonstrated that specific haemagglutinin was present in all the vaccinia materials tested. Only susceptible fowl cells were agglutinated and the agglutination could be

inhibited by convalescent rabbit serum. Suspensions of normal chorioallantoic membrane, rabbit skin or testis had no haemagglutinating activity. Unfortunately, calf lymph was not available for testing.

THE RELATIONSHIP OF VACCINIA HAEMAGGLUTININ TO VIRUS

The report of Burnet & Stone (1946) indicates that when a vaccinia virus suspension was centrifuged at 15,000 r.p.m. for 1 hr., most of the haemagglutinin remained in the supernatant fluid, whereas most of the virus was found in the deposit. The fact that purified E.B.S. even after repeated washings still retains some haemagglutinin activity prompted us to examine in greater detail the relationship between these two entities.

titre of the original suspension, the first angle centrifuge supernatant fluid and the final purified virus were titrated on the chorioallantoic membrane of chick embryos. The result of this experiment is shown in Table 2.

The data in Table 2 confirm the finding of Burnet & Stone that v.H. and virus are distinct entities. Both virus and haemagglutinin were more abundant in the deeper skin layers, but whereas the v.H. ratio between the two layers was 1 : 32, the virus ratio was only 1 : 3. The first angle centrifugation of the deep layer material reduced the virus in the supernatant to approximately 2 % of the original suspension, whereas the v.H. titre remained unchanged. With repeated washings, there was a progressive drop in haemagglutinin titre and the final purified E.B.S. retained only 1/128 of the activity of the

Table 2. *Haemagglutinin and infectivity titrations of vaccinia virus from rabbit skin in the process of purification*

Differential centrifugation	Materials tested	Superficial layers		Deep layers	
		Haemagglutinin	Infectivity*	Haemagglutinin	Infectivity*
	Crude	8	$1\cdot1 \times 10^8$	256	$3\cdot4 \times 10^8$
First 'Angle'	Supernatant	4	—	256	$5\cdot9 \times 10^6$
	Deposit	2	—	64	—
Second 'Angle'	Supernatant	4	—	32	—
	Deposit	2	—	32	—
Third 'Angle'	Supernatant	1	—	16	—
	Deposit	1	—	8	—
Fourth 'Angle'	Supernatant	1	—	4	—
	Deposit	1†	—	2	—
Final 'International'	Supernatant purified virus	1†	$1\cdot2 \times 10^8$	2	$3\cdot1 \times 10^8$
	Deposit	1	—	1	—

* Average of pock counts from two dilutions, 3–4 eggs per dilution, inoculum 0·1 c.c.

† Used here and in subsequent tables indicates just detectable agglutination.

The preparation and purification of vaccinia virus from rabbit skin was carried out according to Craigie's method as modified by Parker & Rivers (1935). Rabbit skin virus was harvested 72 hr. after infection into M/250 McIlvaine's buffer at pH 7·0. A first portion of 20 c.c. was used to collect material obtained from the more superficial layers of skin by very gentle scraping. The material from deeper layers was then separately collected into a second 20 c.c. portion of buffer by vigorous scraping with the sharp edge of a scalpel. Both preparations after preliminary clarification on the International Centrifuge were spun four times on a Swedish angle centrifuge run at 5000 r.p.m. for 1 hr. each time. The supernatant fluid was removed after each run and the last drop drained off with filter paper. The deposit was resuspended in its original volume. Samples of all the supernatant fluids and resuspended deposits were kept for haemagglutinin titration. The virus

original suspension, although the virus titre was practically the same as that of the crude suspension. The association of v.H. with virus particles, however, appears to be closer than one would expect if v.H. were a soluble component that could be readily washed away. Thus, the ratio v.H. in purified virus to v.H. in crude suspension was 1 : 6 for material from superficial layers and 1 : 128 for material from deep layers. The dilution involved in the washing process in both cases was at least 625,000-fold (assuming a 50-fold dilution in each washing) so that simple mechanical carrying over could not be held responsible for the residual haemagglutinin associated with the purified virus. Experiments of this nature have been repeated several times with essentially similar results. The specificity of the v.H. present in the final suspension was confirmed. These results were at first interpreted as an indication that the haemagglutinin was either elaborated by the virus or adsorbed on to

it and reliberated during washings. However, following up titration of two batches of E.B.S. in the course of storage either at room temperature or at 4° C. failed to reveal any appreciable increase of haemagglutinin titre.

ESTIMATION OF PARTICLE SIZE AND STATE OF DISPERSION

Filtration experiment. Filtration of chorioallantoic membrane preparations through Seitz E.K. disks usually resulted in total loss of haemagglutinin activity. Rabbit material after sedimentation of virus could be filtered through a Seitz disk with 4–16-fold reduction of titre. In order to determine more precisely the usefulness of filtration methods in the separation of v.H. from virus, two batches of supernatant fluid from rabbit skin extract spun in the angle centrifuge were filtered through Gradocol membranes according to the method of Elford (1938). Equal amounts of the fluid containing v.H. and Hartley's broth of pH 7·6 were mixed and 10 c.c. portions filtered through collodion membranes of graded pore size. The haemagglutinin and infectivity titre of the original material and of the filtrates were determined. The result of these experiments are shown in Table 3.

Table 3. *Haemagglutinin and infectivity titrations of Gradocol membrane filtrates*

Pore diameter of membrane	Exp. I Haemag- glutinin	Exp. II Haemag- glutinin	Exp. II Infectivity*
Original	16	64	$3·6 \times 10^6$
450 mμ filtrate	12	32	$< 10^3$
310 mμ filtrate	—	2	$< 10^2$
230 mμ filtrate	< 2	< 2	0
112 mμ filtrate	< 2	< 2	0
56 mμ filtrate	< 2	—	—

* Average of pock count from two dilutions, 2 eggs per dilution, inoculum 0·1 c.c.

The filtration end-point was between 230 and 310 mμ. The haemagglutinin in the filtrate was found to be specifically inhibited by immune serum. The results of two experiments corroborate each other. A reliable figure for the size of v.H. particles could not be inferred from these results since the haemagglutinin was available only in low titre and strong adsorption by the filter appeared to be involved. Very little haemagglutinin activity could be recovered by backward washing of 230 mμ membranes which had retained all detectable v.H. Further experience with larger quantities of material showed that a trace of haemagglutinin did occasionally pass through 200 mμ membranes.

Centrifugation experiment. A centrifugation experiment was performed according to the method of Schlesinger (1934). The same batch of material used for the second filtration experiment was employed. Four flat-bottomed tubes, each with a piece of filter paper lying closely fitting against the bottom, were filled to 1 cm. height with the material to be analysed. One tube was kept standing as a control. The other three tubes were centrifuged in an Ecco centrifuge at 10,250 r.p.m. for 1, 2 and 3 hr. respectively. The supernatant fluids were carefully pipetted out and titrated for haemagglutinin activity. The result of such an experiment with virus from rabbit skin is shown in Table 4a.

Table 4a. *Haemagglutinin titrations on supernatant fluid after successive centrifugations of vaccinia haemagglutinin (rabbit skin preparation) at 10,250 r.p.m. for 1 hr. at a time*

Centrifugation	Haemagglutinin titre
None (original suspension)	64
After 1st hr.	32
After 2nd hr.	16
After 3rd hr.	8

Viscosity in c.g.s. units	$\eta = 0·0105$
Height of the fluid column in cm.	$h = 1$
Initial/final concentration	$co/ct = 2$
Density of medium	$\rho_m = 1·002$
Distance from bottom of tube to the axis of rotation in cm.	$R = 7·5$
Time in sec.	$t = 3600$
R.p.m.	$N = 10,250$

In order to confirm the above result, and to gain additional data for estimating the particle density of v.H., a second centrifugation experiment was performed with two preparations of vaccinia material derived from the rabbit skin and chorioallantoic membrane of chick embryos respectively. Both preparations had been spun on the angle centrifuge to remove the bulk of the virus. The supernatant fluids were then divided into two portions to one of which 40 % sucrose solution was added to bring the final sugar concentration to 10 %, while to the other, physiological saline was added as a control. The four final preparations were spun at 10,250 r.p.m. for 2 hr. again using Schlesinger's method with $h = 0·5$ cm. Both the initial and final haemagglutinin titres were determined. The data of this experiment are shown in Table 4b.

Comparing the results of the two centrifugation experiments, it will be noted that by halving the height of the fluid column and doubling the time of centrifugation in the second experiment, the values co/ct for rabbit material $= 12$ and co/ct for chick embryo material $= 8$, are nearly the cube of the value $co/ct = 2$ obtained in the first experiment. This confirms the essential findings of the first experiment.

From the data obtained by centrifugation in saline and 10 % sucrose respectively, it is calculated that the particle density ρ_p for rabbit v.H. $= 1\cdot11$ and ρ_p for chick embryo v.H. $= 1\cdot09$. Taking $1\cdot1$ as the value of average particle density, and assuming that the v.H. particles are approximately spherical, then by applying the equation of Schlesinger:

$$d = 6\cdot15 \times 10^8 \cdot \sqrt{\frac{\eta h \log co/ct}{(\rho_p - \rho_m)\, RtN^2}},$$

the calculated particle diameter of v.H. in both

particles actually represent a specific macro-molecular substance.

THERMOSTABILITY

Tests on the thermostability of v.H. are summarized in Table 5. The pH of the preparations before heating varied from $7\cdot20$ to $7\cdot34$. The maximum pH change after heating in any case did not exceed $0\cdot4$ pH unit.

These results show that v.H. is a remarkably thermostable substance, resisting boiling bath temperature (98–100° C.) for at least 10 min. but

Table 4*b*. *Determination of density of vaccinia haemagglutinin by centrifugation at 10,250 r.p.m. in saline and in 10 % sucrose*

		Haemagglutinin titre				
Materials	Suspending medium	Initial	Final supernatant	co/ct	ρ_m	η
Rabbit skin	Saline	24	2	12	1·004	0·0108
	10 % sucrose	24	8	3	1·050	0·0140
Chick embryo	Saline	128	16	8	1·006	0·0153
	10 % sucrose	128	64	2	1·053	0·0203
		$h = 0\cdot5$	$t = 7200$ sec.			
		$R = 7\cdot5$	$N = 10,250$ r.p.m.			

Table 5. *Effect of heating on different vaccinia haemagglutinin preparations*

			After heating at			
Materials	Titrated in presence of	Unheated	56° C. for 30 min.	70° C. for 30 min.	98–100° C. for 10 min.	Autoclave 15 lb. for 20 min.
Chorioallantoic	Saline	512	256	256	256	< 2
	Normal rabbit serum 1/10	512	256	256	256	—
	Anti-vaccinia serum 1/10	< 4	< 2	< 2	< 2	—
Rabbit skin crude suspension	Saline	64	48	48	64	< 2
	Normal rabbit serum 1/10	64	32	48	64	—
	Anti-vaccinia serum 1/10	< 2	< 2	< 2	< 2	—
Rabbit skin purified E.B.S.	Saline	16	32	32	48	< 2
	Normal rabbit serum 1/10	16	16	32	32	—
	Anti-vaccinia serum 1/10	< 2	< 2	< 2	< 2	—

preparations is found to be 65 mμ. The finding that a regular fraction was deposited after each spin suggests the relative homogeneity of the particles present. It is not possible to say whether these particles represent actual molecules or aggregates of molecules or some active substance adsorbed on to tissue particles. However, the homogeneity of particle size in any one preparation and of particle size and density in two preparations derived from entirely different sources strongly suggest that v.H.

being destroyed by autoclaving (15 lb. for 20 min.). The heated v.H. agglutinated only the susceptible fowl cells and was inhibited by anti-vaccinia serum, but not by normal rabbit serum. Thus, it appeared to have retained both its specific activity and its capacity of combining with anti-haemagglutinin in the immune serum.

There are, however, certain anomalies to be noted in these heating experiments. The first anomaly was encountered with chorioallantoic membrane pre-

parations, of which all batches tested showed some variable impairment of haemagglutinin titre after heating to 56° C. Heating to higher temperatures did not further reduce the titre. There is, however, no adequate evidence for the presence of thermolabile and thermostable components. Heated chorioallantoic membrane suspensions acquire a property of causing the slipping of red cells which may be partially responsible for the lower apparent titre. Some change in the state of aggregation of v.h. or state of combination with some tissue components may also have occurred during the heating process. The second anomaly was encountered when purified E.B.S. was heated. Heating to 70° C. or boiling bath temperature usually resulted in a slight increase of haemagglutinin titre (usually a 2-fold increase) of E.B.S. above the titre of unheated E.B.S. The reason for this is obscure, since the increase is usually too slight to permit any accurate experimental manipulation.

The thermo-stable character of v.h. has been utilized in recovering haemagglutinating activity from artificially neutralized haemagglutinin-antihaemagglutinin mixture.

pH STABILITY

The pH stability tests were conducted by diluting 0·1 c.c. of crude haemagglutinin preparations from the chorioallantoic membrane in 0·9 c.c. of buffer solutions ranging in pH from 1·0 to 12·0. The
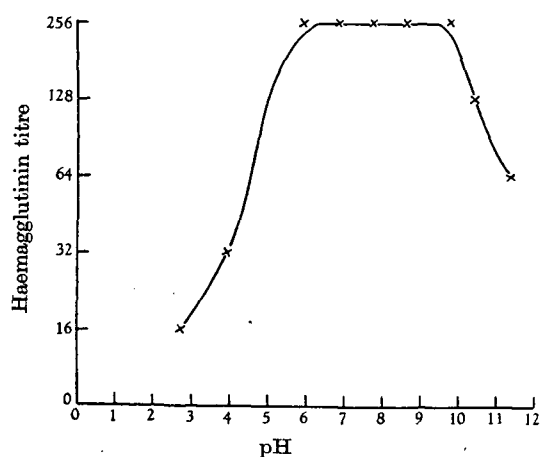

Fig. 1. pH stability of v.h.

following buffer solutions were employed: pH 1·0–3·0, Sørensen's glycine-HCl buffer; pH 4·0–8·0, McIlvaine's citric acid-phosphate buffer; pH 9·0–12·0, Sørensen's glycine-NaCl-NaOH buffer. The actual pH was measured by glass electrode. The mixtures were allowed to stand at room temperature for 2 hr. and then at 4° C. for a further 16 hr. Precipitates appeared in tubes of pH range 3·0–5·0.

These precipitates were separated and resuspended in McIlvaine's buffer at pH 7·0 and tested together with the supernatant fluids. All tubes were approximately neutralized using phenol red as indicator and the final volume was brought up to 1·6 c.c., thus making a final haemagglutinin dilution of 1/16. The results of haemagglutinin titration are shown graphically in Fig. 1.

The data presented show that v.h. is stable between pH 5·92–9·79. Progressive impairment of titre occurred on the acid side of the range and a slight loss occurred at pH 10·46. Only the titres in the supernatant fluid are represented in Fig. 1, although at pH 2·73, 3·92 and 4·97, an appreciable amount of haemagglutinin was found in the precipitate. This amount found in the precipitate could not adequately account for the loss in the supernatant fluid.

ATTEMPTS TO PURIFY VACCINIA HAEMAGGLUTININ

Several attempts have been made to obtain partially purified v.h. for the purpose of immunological studies without much success. The main difficulties appear to lie in the following facts: first, the quantity of active substance in question is probably extremely small; secondly, v.h. appears to aggregate readily and is difficult to redisperse after aggregation, thus seriously interfering with any reasonable assay of activity. In the course of such studies, several properties have been revealed which might aid in further studies on this subject:

The effect of ether extraction. v.h. could not be extracted by simple shaking with ether. No loss of titre occurred when rabbit skin material was thoroughly shaken with several changes of ether. A large excess of lecithin added to such vaccinia preparations so as to produce additional non-specific haemagglutinin titre could be readily removed by such extractions. A 20 % suspension of chorioallantoic membranes when similarly treated formed a bad emulsion. After breaking up the emulsion on standing or centrifugation, three layers separated out: a top ether layer, an intermediate emulsified layer and a bottom aqueous layer. The clarified aqueous layer usually retained only ¼–⅛ of the original titre. However, no specific haemagglutinin could be recovered from the ether soluble fraction (which contains high titre non-specific haemagglutinin due to tissue lipids) nor could any appreciable activity be demonstrated in the emulsified layer. The residual v.h. after one such extraction was not further reduced by subsequent repetition of the procedure. Furthermore, a 2 % chorioallantoic membrane suspension when similarly treated did not lose much of its titre. This seems to indicate that the loss of v.h. is not due to its ether solubility but more

Table 6. *Some properties of non-specific haemagglutinins*

| Haemagglutinating agent | Non-susceptible fowl R.B.C. | Susceptible fowl R.B.C. | | Titre in normal rabbit serum 1 : 10 | Titre in anti-vaccinia serum 1 : 10 |
| | | Titre in saline | | | |
		Unheated	Boiled 5 min.		
v.h., chorioallantoic	< 2	256	64	128	< 2
Lecithin emulsion 0·01 %	< 2	1024	1024	< 2	—
Cholera filtrate	< 2	64	2†	< 2	—
Trypsin 1 %	< 2	64	< 2	4	—

likely to aggregation and adsorption during the emulsifying process. Ether extraction combined with freezing of one sample of 20 % chorioallantoic preparation according to McFarlane & Kekwick's technique (1942) gave essentially the same result.

Ammonium sulphate precipitation and dialysis. As mentioned by Burnet (1946), v.h. could be precipitated by 50 % saturation with ammonium sulphate. It is also precipitated from solution by dialysis of a crude suspension against distilled water. The precipitated material however did not redissolve well in sodium chloride solutions varying from 0·9 to 6 % salt strength at pH 6·0–10·0. Only a small fraction of haemagglutinating titre could be recovered from the precipitate.

Adsorption and elution from kaolin. v.h. could be adsorbed on to kaolin at pH 6·0 and eluted at pH 10·0. In one such experiment, 5 c.c. of 20 % chorioallantoic membrane suspension with a titre 1/512 was diluted with 15 c.c. McIlvaine's buffer at pH 6·0 and 1 g. of kaolin (B.D.H., acid washed) was added. v.h. was completely adsorbed in 2 hr. The kaolin was then separated and eluted with 5 c.c. glycine-NaOH buffer at pH 10·0 for 5 min. and again with a second 5 c.c. portion at 4° C. overnight. The first eluate yielded a titre of 1/64, the second eluate a titre of 1/16. The recovered haemagglutinin was specific.

NON-SPECIFIC HAEMAGGLUTININS RESEMBLING VACCINIA HAEMAGGLUTININ

Various lipids agglutinating the same range of fowl red cells susceptible to v.h. have been reported by

Stone (1946a). Several other agents have been encountered displaying the same activity: commercial trypsin preparations (Fairchild, B.D.H.) and some Seitz filtrates of peptone water cultures of *V. cholerae*. They resembled the lipid haemagglutinins in being inhibited by normal rabbit serum, though to a much less extent in the case of trypsin haemagglutinin, but differed from them in being completely or nearly completely destroyed after heating to 70° C. or higher.

Table 6 shows some properties of these non-specific haemagglutinins and their differentiation from v.h. The occurrence of these non-specific haemagglutinins emphasizes the importance of controlling the specificity of all haemagglutinin preparations.

SUMMARY

1. Specific vaccinia haemagglutinin has been identified in vaccinia materials derived from chick embryo, rabbit skin, rabbit testis and sheep skin.

2. The haemagglutinin is distinct from, and apparently unassociated with, the elementary bodies.

3. Centrifugation studies indicate that the haemagglutinin activity is associated with particles of the order of 65 mμ in diameter and having an approximate density value of 1·1.

4. Vaccinia haemagglutinin retains its haemagglutinating activity after boiling for 10 min. It is stable within the pH range 5·92–9·79.

5. The behaviour of vaccinia haemagglutinin to various chemical procedures and an attempt at its purification are described.

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